Investigation of Highly Pathogenic Avian Influenza H5N8 Outbreak in Kano, Kano State, Nigeria

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Abstract: Highly Pathogenic Avian Influenza (HPAI) virus (H5N8) has been in circulation in Europe, parts of Asia and Sub-Saharan Africa for some time now but was recently identified for the first time in Nigeria in November, 2016. Evidence from epidemiologic disease investigation conducted at the index farm (mixed species back yard poultry farm located at Danbare) indicated that the new clade of the virus might have been introduced through some local chickens bought from a poultry vendor in a nearby Live Bird Market (LBM) at Janguza and introduced into the farm without observance of basic biosecurity measures, a week before the outbreak occured. Unfortunately, the primary source of the pullets and possible extend of spread of the virus could not be traced due to lack of bird movement records system that could have enabled the tracing of the actual source of the local chickens introduced into the affected farm beyond the Janguza LBM where the pullets were purchased from an unknown hawker. Participatory disease surveillance (PDS) at the Janguza LBM revealed Newcastle disease (ND) to be one of the major diseases observed at the LBM which was further corroborated by the laboratory result of samples collected from the LBM (37.5% of the oropharygeal samples were positive for NDV). Also, the cold season ('*Sanyi*') was identified by PDS as the season in which 72% of diseases occured as compared to other seasons of the year, indicating the need for application of more precautionary/preventive measures to diseases at this period. Pooled samples from apparently healthy ducks in Sabon Gari LBM, which apparently had no epidemiological link with the index farm were positive for HPAI H5N8 virus, further revealing the possibilities of circulation of this clades of the HPAI viruses in Kano State and possibly beyond without being detected. Some of the major risk factors believed to have facilitated the introduction, establishment and spread of HPAI viruses in Nigeria include: poor regulation of the poultry industry, weak disease surveillance network, clustering of poultry farms without adhrence to basic biosecurity measures by poultry value chain operators, poor implementation of biosecurity measures in LBMs and weak movement control during outbreaks. The investigation revealed the need for enhancement of continual disease surveillance activities in Kano State and the entire country. In conclusion, the cofirmation of H5N8 in the country along with the existing H5N1 outbrekas that resulted in the culling of over 3.6 million birds so far, posses severe threat to the poultry industry, economic stability and food security of Nigeria considering the current ongoing economic recession in the country. The use of PDS has proved to be a useful tool in this outbreak investigation and the tool may be used in subsiquent disease outbreak investigations.

Key words: HPAI H5N8, Outbreak Investigation, Index Farm, Live Bird Markets, Participatory Disease Surveillance, Kano State, Nigeria

1.0 Introduction

Avian influenza A virus (H5N8) is an emergent, highly pathogenic avian influenza (HPAI) virus affecting birds which was initially reported in January 2014 in England (PHE, 2016; Hanna *et al.,* 2015), and other

countries such as the People's Republic of China, Japan and the Republic of Korea who reported outbreaks of the novel Eurasian (EA) H5N8-reassortant clade 2.3.4.4 viruses in migratory birds and domestic poultry. By November 2014, there were multiple reports of the H5N8 clade 2.3.4.4 in wild birds from South Korea, Japan, Russia, Germany, Netherlands, and North America (OFFLU, 2016; Ip *et al*., 2016, Kwon *et al*., 2016; Verhagen *et al*., 2015; Lee *et al*., 2014;) and by 2015, H5N8 was identified in the United States of America (USA) and Canada. In 2016, cases of a new H5N8 reassortant were reported in wild birds in Republic of Korea and Russia, as well as India and ten other countries of Europe (Austria, Croatia, Denmark, Germany, Hungary, Poland, Switzerland, Sweden, Finland and Netherlands) and from the middle east, Israel and Iran (OFFLU, 2016; Ip *et al*., 2016). This H5N8 lineage of viruses to date has not been reported to cause disease in humans or other mammalian species (OFFLU, 2016). So far in Sub-Saharan Africa, outbreaks of HPAI H5N8 has been confirmed in Uganda and subsequently in Nigeria (FAO, 2017).

Nigeria is endowed with a population of 170 million people and 160 million poultry. The poultry sector in Nigeria accounts for about 58% of the total livestock production in the country (Amos, 2006) and the poultry sub-sector offers the quickest returns to investment, outlays in livestock enterprise being one of the cheapest, most common and best sources of animal protein in the country (Ojo, 2002). In Nigeria, the outbreak of Highly Pathogenic Avian Influenza (HPAI) H5N1 was first confirmed in a commercial poultry farm in February, 2006 making it the first country in Africa to report the presence of HPAI H5N1 and within months, the disease spread very fast and by 2008, 25 out of the 36 States of the Federation had reported the disease in poultry with one confirmed fatal human case being a potential threat to the health of the public (Bird Flu Watch, 2009). Characterization of viral isolates and phylogenetic analysis revealed three separate introductions of the virus into the country through either migratory wild birds, illegal trade in infected poultry and poultry products (Vakuru *et al*., 2010). The disease posed serious threat to the poultry industry, food security and the nation's economy as over 1.2 million birds were culled (Vakuru *et al*., 2010). The disease was controlled through concerted efforts and with the support of Development Partners and other Donor Agencies, in 2008. In January 15, 2013, Nigeria expressed self-declaration of disease freedom to the OIE (FMARD, 2013).

However, in December 2014, H5N1 reoccurred in yet another commercial poultry farm and a Live Bird Market (LBM) in Kano and Lagos States, respectively. The current H5N1 virus in circulation was proven to be distinctive from the previous one that spread between 2006 and 2008. From December 2014 to date, HPAI H5N1 has spread to 26 States and the Federal Capital Territory (FCT) (73%) out of 36 States and the Federal Capital Territory (FCT) of the country. So far, 761 poultry farms and a Zoological garden have been affected with over 3.6 million birds culled.

In November, 2016, an outbreak of a new strain of HPAI virus (H5N8) in a mixed poultry farm as well as other species of animals at *Unguwan Danbare* in Kano, was confirmed by the country's Central Veterinary Diagnostic Laboratory and Research Institue, Vom on the 16th of December, 2016. This triggered a new wave of concern in the country with the fact that the H5N1 strain is still circulating in the country and is yet to be eliminated with diverse economic effect and a threat to the food security in the nation with poultry as the main source of meat in the country. To this end, the decision to carry out an investigation of the outbreak was made by the Federal Ministry of Agriculture and Rural Development (FMARD) in order to find out the possible source of introduction and the extent of spread of the virus and recommend critical measures that needs to be taken to restrict and control the outbreak.

2.0 Materials and Methods

The affected farm, the State veterinary clinic that handled the index case, the LBM that had epidemiologic link with the affected farm and a randomly selected LBM (with no direct link to the farm) were visited by the disease investigation team. Standard outbreak investigation questionnaire was administered to the affected poultry farm's workers to find out details of the outbreak that occurred on the farm. Data and information from operators of the affected farm and from the veterinary clinic on: the species of birds involved, onset of clinical signs, mortalities within species, action taken during and after

the outbreak, level of biosecurity measures, sources and movement of poultry and poultry products into and out of the affected farm was collected and collated.

2.1 Sample collection

Using simple random sampling technique, swabs (oropharyngeal and cloacal) as well as environmental samples were collected from birds in cages from the LBMs that were visited. Each sample was systematically assigned a unique identification number at the time of collection to facilitate matching of tracheal (oropharyngeal) and cloacal swabs collected from the same bird while other samples were pooled and labelled accordingly. A record sheet was created to track the samples. The information captured on the specimen records included the sample identification number, species sampled (Guinea fowls, Mallard ducks, Muscovy ducks and local chickens), and type of sample collected (cloacal, oropharyngeal and environmental swab). Samples were placed in viral transport media, labeled and packaged to ensure the maintenance of cold chain before being transported to the laboratory within 24 hours of collection. Samples were analyzed at the BSL3 Central Veterinary Diagnostic Laboratory at the National Veterinary Research Institute Vom, Plateau State, Nigeria using Real-Time Reverse-Transcription Polymerase Chain Reaction (RRT-PCR). The samples were stored at–20◦C pending RNA extraction, which typically occurred within 1 week of sample arrival.

2.2 Viruses

Avian Influenza Virus reference strains with different H and N subtypes representative of HPAIV isolates and the vaccine strain of Newcastle Disease Vaccine (NDV) was used to evaluate the RRT-PCR assay.

2.3 RNA extraction

Viral RNA was extracted from stock viruses and field samples using an RNeasy Mini kit (Qiagen, Hilden, Germany) according to manufacturer's instructions. Extracted nucleic acids were stored at -20^{0}C until further use.

2.4 Primers and probes for RRT-PCR

Primers and probes based on the studies of Kim *et al*. (2013) and Kim and Park (2015) were used for the analysis.

2.5 Real-time Reverse—Transcription Polymerase Chain Reaction (RRT-PCR)

The RRT-PCR was carried out according to the method described by Kim and Park (2015). The RRT-PCR amplification was carried out using RealMOD Probe HiSenSriptq RT-PCR Mix (Intron Biotecnology, Seongnam, Korea) and a real-time PCR instrument (Applied Biosystems, Waltham, MA, USA). A total reaction volume of 20 µL, containing 10 µL of 2×qRT-PCR enzyme mix, 0.4 µM of each primer and probe and 5 µL RNA template was prepared according to the manufacturer's instruction. The RRT-PCR Programme consisted of 10 minutes at 45^{0}C for reverse transcription and 30 sec at 95^{0}C for the activation of Taq enzyme followed by 40 cycles of 95^{0}C for 15 sec and 55^{0}C for 30 sec for amplification. For interpretation of the RRT-PCR results, samples producing a Cycle thresh hold (Ct) of <37 were considered positive and a high Ct-value (>37) were considered negative.

2.6 Participatory Disease surveillance (PDS)

The PDS team for this study was composed of six Veterinarians. At the LBM, PDS was conducted to find out the common symptoms and diseases of the birds in Janguza LBM. Pre-advocacy visit at the Janguza LBM Kano was conducted the eve before the interview and the PDS took place the following day. The exercise was carried out on a day that was not the market day in order to get the full attention of the traders in the LBM. Efforts were made to include poultry traders of all age groups. The Geographical Positioning System (GPS) on our android handset (phone) was used to determine location of the market and various areas visited, cartons, counters (beans), biros, digital camera and others were used for the study. Each person was assigned a role before moving out. The team was composed of designated roles such as note taker, observer, tool applicator and the facilitator. In order to avoid bias, the PDS Team did not mention HPAI during the interview process. The following tools were used during the course of the participatory disease surveillance: (a) Check list consisting of the following items: mutual introduction,

identification of respondents, duration of the existence of the market, sources of birds, location of buyers that patronize them on their market days, livestock species kept, Where do you take processed chickens to?, poultry diseases, source of livelihood aside trading of live bird if any, Do you the fowl sellers also process the fowls?, questions and advice. (b) Scoring and ranking: proportional pilling**.** (c) Visualization: includes mapping, timelines, seasonal calendar and transect walk.

2.7 Data analyses

The PDS data was analyzed based on the method of PDS result analysis as indicated in "A Manual for Participatory Disease Surveillance Practitioners: Introduction to participatory epidemiology and its application to highly pathogenic avian influenza participatory disease surveillance" by Mariner and Paskin (2009).

3.0 Results

3.1 Investigation at the index farm in Unguwan Dan'bare Kano State (Longitude: 8.2618 ^{0}E; Latitude: 11.5729 ^{0}N)

The outbreak occurred in an isolated mixed farm (the nearest farm being about 1/2-1 km away from the farm) with total flock size of 344 birds of different species and types. The farm had a bore hole as the means of water supply and people visited the area to fetch water and the tap area was marshy. On entry into the farm, there were various species of animals in the compound such as bovine (4) (Friesian cross breed, Sokoto Gudali, Red Bororo), caprine (West African dwarf goats) (16), ovine (14) (Sudanese, Uda and Balami breeds) and (26) rabbits.

A total of 344 birds (males and females) of different avian species were on the farm as briefed by the caretaker of the farm a (Fig. 1). The exotic and local pigeons (136) had the highest number whereas the least were the Indian ducks (6) and *Kwakwa* (6) as shown (Fig. 1).

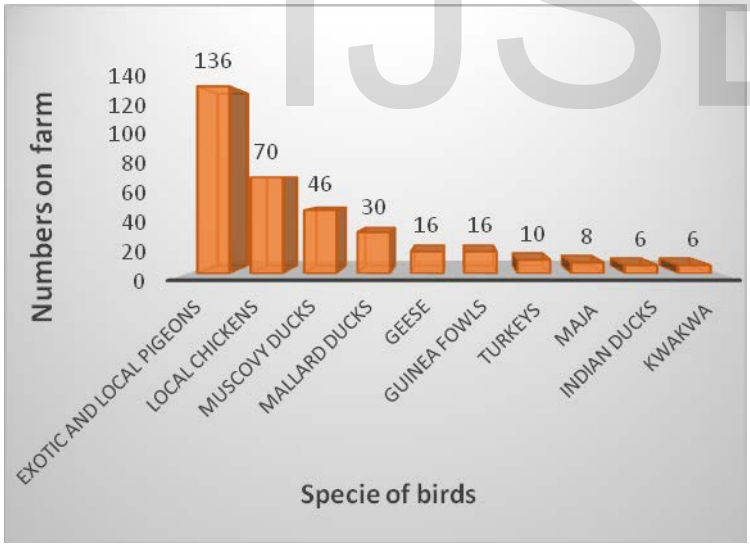

Fig 1: The species of birds at the index farm in *Unguwan Dan'bare* Kano State before the outbreak

Initially, carcasses of dead birds were thrown over the fence of the farm's premises but when advised by the consulting Veterinarian the dead carcasses were subsequently burnt inside a pit and buried. Clinical signs as observed by the care taker were: difficulty in breathing, struggling and sudden death of one of the six pullets (on the 21st of November) that were bought on the 13th, of November and introduced into the affected farm. By the 22nd of November, 4-5 exotic cocks, 3 pullets, 1 Mallard and 1 turkey died before the incident was reported to the Veterinary Clinic of the State Ministry of Agriculture and Natural Resources at Gwale on the same date. On the 22nd a pullet that was taken to the veterinary clinic died at the clinic while the remaining ones died on farm. So, by the 22nd of November, all the six local pullets

that were introduced into the farm had died and on the same day the turkeys, guinea fowls and Mallard ducks were showing signs of illness. There was 80% morbidity in the Muscovy ducks. On the farm, samples were collected from one exotic cock and Mallard duck. All the guinea fowls, Muscovy ducks and local chickens died before the day of the depopulation (17th of December, 2016). Thus, birds depopulated on the farm were the Mallard ducks, Indian ducks and pigeons. The pullets were bought from a trader at Janguza LBM, the trader had a bicycle which he used in transporting himself and the birds (local chickens).

3.2 Ministry of Agriculture and Natural Resources Gwale Veterinary Kano (Longitude: 8.3039^{0}E, Latitude: 11.5912^{0}N)

Information gathered from the consulting veterinarian revealed that; there was no biosecurity observed on the farm (index farm); a locally made incubator at the farm (incubating duck and chicken eggs together at the same time) indicating that hatch-able eggs were sourced from other places and brought to the farm for hatching; construction of poultry house was on-going on the farm, hence workers and visitors were seen on site. This scenario affirms the absence of biosecurity on the farm. Clinical signs observed were: sudden death, high mortality, ruffled feathers, depression, swollen head and neck, cyanosis of the comb and wattles, haemorrhages on the shanks and greenish diarrhoea. Necropsy was also conducted on carcasses of the pullets and the ducks and gross lesions observed were; congestion of the trachea, haemorrhages on the skin, thigh muscles, serosal surface of the gizzard, in the shanks, intestine and proventriculus-gizzard junction, greenish fluid in the intestine, enlarged spleen, kidneys and caecal tonsils as well as enlarged and friable liver.

Information provided by the care taker of the affected farm revealed that the onset of the disease was on the 19th of November 2016 but the incident was reported to the clinic on the 22nd of November, 2016. Mortality on date of report was observed in exotic cockerels (5), turkeys (3), ducks (4) and local chickens (5). Samples were sent to the laboratory on the 23rd of November, 2016 for testing and the results were positive for H5N8 strain of HPAI.

3.3 Interview at Janguza LBM, Kano (Longitude: 8.2411 ^{0}E, Latitude: 11.5830 ^{0}N)

The LBM is in the middle of the main market which is by the side of a trunk A road leading to neighboring Katsina State, Nigeria. The main market days are Thursdays and Sundays of the week. Birds were kept in cages made from wood and wire mash some of which have been reinforced by pieces of cloth were stacked on top of each other (Plate 1). The stalls are near a poultry processing area (Plate 2). Also, observed at the processing area were piles of slaughter wastes some of which had been bagged close to the evisceration and dressing tables made of wood (Plate 3 & Plate 4). The species of birds on sale during the teams visit were; turkeys, pigeons, Mallard ducks, guinea fowls, local chickens and broilers. Local chickens were mixed with guinea fowls in the same cage. On market days, poultry are being moved from the stalls in the LBM area to the road side where they are sold together with those from other sources (unknown) The Janguza LBM has been in existence for over 30 years. The species of birds and animals commonly sold at the market included; local chickens, broilers, geese, local ducks, pigeons, peacocks, parrots, rabbits, turkeys, and guinea fowls (Plate 1). Pictures of the exercise conducted (PDS) is indicated in Plates 5 & 6 below.


Plate 1


Plate 2

Plates 1 & 2: Sections of Janguza LBM, indicating birds in wooden cages, the slaughter, dressing and processing area with a pot on fire

Plates 3 & 4: Sections of Janguza LBM, showing a man packing the feathers to be transported to crop farms and the wooden tables used for dressing carcasses.

Plate 5 & 6: Interviews and PDS with the LBM traders at Janguza LBM, Kano State, Nigeria.

Birds for sale were bought from the following villages and towns; Lambu, Walawa, Unguwan Rimi, Kabo, Shanono, Garo, Rimin Gado, Tofa, Dawakin Tofa, Talata Kanya (Garki-Jigawa State), Manga, Azare (Bauchi State), Wudil, Ladin makoli, Kwanan Dangora, Karaye, Kwankaso, Tudun Wada, Jajaye, Lokoya, Kafur (Katsina State), Babara, Lapaya. Birds were also brought into the Janguza LBM for sale from nearby Langel village and nearby clusters of poultry farms as well as from other LBMs across the State and beyond. Traders that come to buy birds from Janguza LBM were mainly from: Abuja, Illela (Sokoto State), Lagos and Kalari (Borno State) towns. Others came from smaller LBMs located by/at Bayero University Kano, Sharada, Kurmi, Sabon Gari, Kasuwan Rimi and Tarauni. People bring their chickens from other surrounding villages for dressing in the live bird market. Also, Operators of Restaurants buy live chickens and get them processed at Janguza LBM. Fowl sellers interviewed indicated that frozen chickens were also sold in Janguza LBM. The fowl sellers at the market also engaged in arable farming, livestock production and local poultry production as sources of livelihood. The team further observed that the carcass dressing area was on a heap of feathers and decomposing fecal and other debris from carcasses which constituted a favorable media for disease pathogens, environmental and public health hazards. The feathers and the entire debris were packed in sacks and on enquiry the team was informed that they were taken to crop farms to be applied as manure.

3.3.1 Participatory Disease Surveillance (PDS)

Further information was acquired from the fowl sellers and processors PDS tools:

(a) Proportional pilling was used to find out the percentages of each species of birds that were brought to LBM and the results were indicated as depicted in Fig. 2:

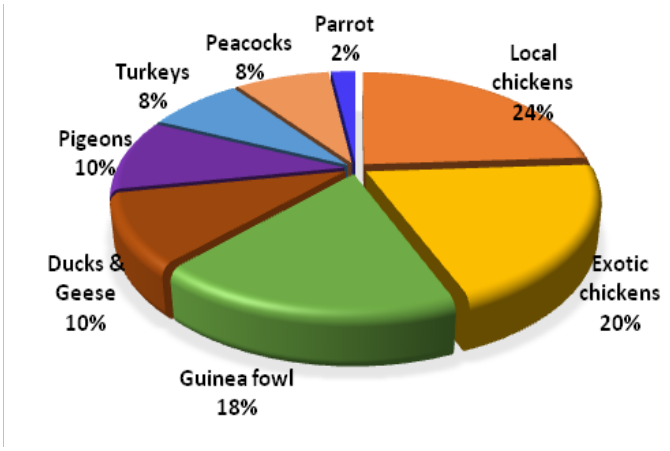

Fig 2: Percentage of different species of birds brought to the Janguza.

Ranking indicates that local chickens were the commonest type of birds brought to the market followed by exotic chickens, guinea fowl, ducks, geese and pigeons, turkeys, peacocks and the least common , parrots respectively (Fig. 2).

B) Weekly, about 16% of the birds that were brought to the LBM were observed sick on arrival birds and when asked ''what do you do with the sick birds?'', the operators said 'the birds are slaughtered and sold'' at cheaper rates. (Fig. 3). Also, they made mention that there are people that specifically buy the sick birds.

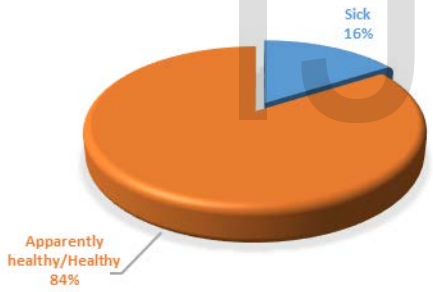

Fig. 3: Proportion of sick and healthy birds brought weekly to the Janguza LBM.

The cold season (*sanyi*) was associated with the period with the highest number of diseased birds (72%) followed by the hot period (*bazara*) amounting to (22%), then autumn, popularly known as *Kaka* (4%) and finally spring (*Damina*) (2%) as indicated in Table 1.

Table 1: Seasonal pattern of diseases of birds observed at Janguza LBM.

S/No	Seasons of the year	Seasons (in Hausa language)	Percentage (%)	Ranking
1	*Sanyi*	Winter (cold season)	72	1
2	*Bazara*	Summer (Hot season)	22	2
3	*Kaka*	Autumn (after harvest)	4	3
4	*Damina*	Spring (rainy season)	2	4
	Total		100	

Yellowish faeces ranked number one followed by whitish feces, tape worm, greenish feces, diseased lungs, ascites, ectoparasites (lice) and bloody faeces shown in Table 2.

Table 2: Proportion of clinical signs of diseases and postmortem lesions as observed by meat processors at Janguza LBM.

S/No	Sign/Symptom (in Hausa Language)	Sign/Symptom	Percentage (%)	Ranking
1	*Kashin ruwan dorawa (Chiwon Hanta/swollen liver)*	Yellowish faeces	27	1
2	*Farin Kashi*	Whitish faeces	17	2
3	*Tsusan chiki*	Tapeworms	15	3
4	*Koren Kashi*	Green faeces (ND)	11	4
5	*Chiwon Huhu*	Diseased lungs	9	5
6	*Rucoom chiki*	Ascities	8	6
7	*Kwarkwatan Kaji*	Ectoparasites (lice)	7	7
8	*Kashin jinni (Basir)*	Bloody stool	6	8
	Total		100	

Greenish faeces ranked first on the list followed by whitish faeces, yellowish faeces, gasping for air, torticollis and lastly swelling of comb and wattles (Table 3).

Table 3: Proportion of clinical Signs/symptoms of diseases as observed by fowl sellers at Janguza LBM.

S/No	Symptoms (in Hausa Language)	Symptoms	Percentage (%)	Ranking
1	*Koren kasha*	Greenish faeces	27	1
2	*Farin kasha*	Whitish faeces	22	2
3	*Yelon kashi*	Yellowish faeces	18	3
4	*Ciwon mashako*	Gasping for air	17	4
5	*Haukan kaji*	Torticollis	12	5
6	*Kumburin kai*	Swelling of the comb and wattles	6	6
	Total		100	

Whitish faeces and yellowish faeces ranked first followed by greenish faeces, tapeworms, diseased lungs, gasping for air, ascites, lice, torticollis, bloody faeces and swollen comb and wattles respectively (Table 4 belows).

Table 4: Indicating signs/symptoms of diseases, scores and ranking as demonstrated by meat processors and fowl sellers at Janguza LBM, Kano, Kano State, Nigeria.

S/No	Symptoms (in Hausa Language)	Symptoms	Scores	Ranking

1	*Farin kasha*	Whitish faeces	120	1
2	*Kashin ruwan dorawa*	Yellowish faeces	120	1
3	*Koren kasha*	Greenish faeces	110	2
4	*Tsusan chiki*	Endoparasites (tapeworms)	60	3
5	*Huhu*	Diseased lungs	40	4
6	*Ciwon mashako*	Gasping for air	30	5
7	*Rucoom chiki*	Ascites	30	5
8	*Kwarkwatan kaji*	Ectoparasites (lice)	20	6
9	*Haukan kaji*	Torticollis	20	6
10	*Kashin jinni*	Bloody faeces	10	7
11	*Kumburin kai*	Swollen comb and wattles	10	7
	Total		560	

3.4 Investigation at Sabon Gari LBM, Kano (Longitude: 8.3290^{0}E, Latitude: 12.0148^{0}N)

Sabon Gari LBM is a strategically located major LBM in Kano metropolis with a lot of commercial live bird activities taking place daily at the market, which makes the market relevant in this investigation. At the market, the team met with the Chairman, Secretary and other members of the live bird market operators in their office located within the market arena. They were briefed on the objectives of the visit by the team and the current trend of HPAI viruses (H5N1 and H5N8) in the country. They expressed their grievances on the situation of the disease and hoped for more assistance from the government. Subsequently tracheal and cloacal swabs (samples) were collected from some ducks and local chickens. The samples were preserved in viral transport media and, transported to laboratory at NVRI for testing.

3.4.1 Results of laboratory analyses

A total of 31 birds were sampled from the two LBMs. The samples from Janguza LBM (oropharyngeal and cloacal swabs) were negative for H5N8 but positive for NDV in oropharyngeal samples (37.5%) and 16.7% of cloacal samples. However, the pool of oropharyngeal samples from apparently healthy ducks sampled at Sabon Gari market tested positive for H5N8 (Table 5).

Table 5: Results of post H5N8 outbreak surveillance sample collection from Kano, Kano State, Nigeria.

	Janguza LBM					
Specie of poultry	Oropharygeal swabs tested	Samples positive for NDV by RRT-PCR (%)	Samples positive for AIV by RRT-PCR(%)	Cloacal swabs tested	Samples positive for NDV by RRT-PCR (%)	Samples positive for AIV by RRT-PCR
Guinea fowl	10	3	0	10	4	0
Mallard duck	3	3	0	3	0	0
Local duck	1	0	0	1	0	0

Local chicken	10	3	0	10	0	0
Total	24	9 (37.5)	0	24	4 (16.7)	0
Environmental samples	2		0			0
			Sabon Gari LBM			
Guinea fowl	2	2	0	2	2	0
Local duck	Pool of 4	Pool negative	Pool positive	Pool of 4	Pool negative	Pool negative
Local chicken	1	1	0	1	1	0
Total	7		4(57.1%)	3		

4.0 Discussion

In Nigeria, about 60% of the poultry population are in sector 3 (Backyard Production System). Since the reoccurrence of HPAI H5N1, in December 2014 in the country over 3.6million birds were culled. Thus, the detection of the new strain HPAI virus (H5N8) posed additional threats to the poultry industry, the nation's economy and food security. In effect, the outbreak of AI in the country has rendered a lot of youths jobless due to the collapse of some major commercial poultry farms that stand to employ a lot of workers, the same apply to minor commercial farms and backyard poultry owners. This investigation further confirms the presence of H5N8 circulating in Kano which has previously been detected in other countries such as England, China, South Korea and many other countries (OFFLU, 2016; Ip *et al*., 2016, Kwon *et al*., 2016; Verhagen *et al*., 2015; Lee *et al*., 2014).

Kano State is one of the worst hit States with the advent of the AI and it is also the State with the highest number of outbreaks of H5N1 and this is not surprising because it is a commercial Centre in the Nation and a lot of illegal imports of exotic birds (exotic pigeons, peacocks, sparrows and parrots) because they are kept as part of prestige for some people in Kano. Local birds and exotic birds are also brought in from different States of the federation for commercial purposes. In Kano State, disease surveillance and adherence to basic hygienic and biosecurity practices were at their lowest level due to poor regulation of the poultry industry and paucity of resources. Consequently, the confirmation of H5N8 in samples collected from apparently healthy duck in Sabon Gari LBM, which had no epidemiological link with the affected poultry farm might be an indication that the virus could be circulating in other LBMs in Kano State and beyond while remaining undetected due to poor surveillance activities in Kano State and beyond. Therefore, the national picture of the situation of HPAI viruses in circulation in Nigeria since the reoccurrence of the disease in December, 2014, remains largely unknown and calls for intensification of risk-based disease surveillance activities. The absence of disease surveillance activities in all major LBMs due to the limited capacity of the State to conduct epidemiological disease outbreak investigations, poor regulation of poultry industry, poor movement control, non-adherence to biosecurity measures, failure to report disease outbreak on time to Veterinary Authorities as well as influx of exotic birds into Kano without proper veterinary import certification were considered as the major risk factors associated with this outbreak.

The PDS revealed one of most notable sign of diseases mentioned as whitish diarrhoea which could be as a result of salmonellosis which is also known as bacillary white diarrhoea. The yellowish/greenish diarrhoea, torticollis, swollen comb and wattles could be as a result of ND. When the scores of these signs were combined and based on laboratory analysis, 37.5% of the oropharyngeal samples were positive for ND, our findings relate that the most common disease of birds to be expected in Janguza LBM is likely to be ND as indicated by our laboratory results from the LBM. The yellowish/greenish diarrhoea could be fowl typhoid or fowl cholera. This finding tallies with the studies by Vakuru (2010), Ndahi and Kwaghe (2011) and that by Kwaghe *et al*. (2012) revealing ND as major poultry disease affecting poultry in various villages in Plateau State, and likely other States in Nigeria. Likewise, the study of Anzaku *et al*., (2014) revealed ND as the major disease of poultry in Abuja with an overall mean score of 67.6 % compared to other poultry diseases. Also, the studies of Waziri *et al*., (2014), confirms ND as a disease with the most economic value in the poultry industry. This conveys the need for routine

vaccination of local as well as exotic chickens against the disease. Secondly, the use of seasonal calendar indicated the cold season (*Sanyi*) as the time when about 72% of disease outbreaks occur implying the need to be more proactive about that period on biosecurity and vaccination activities particularly for the local and exotic chickens in order to control morbidity and mortality rates thereby reducing losses in poultry industry. The presence of Tape worms and Coccidiosis based on this study will require the use of antihelmintics in treatment (with strict observance to withdrawal period) against these diseases in Janguza LBM even though they were not identified as major threats in the LBM. Ascites was mentioned as part of the symptoms observed and this is mostly seen in broilers between 4-5 weeks of age and usually diagnosed as pulmonary hypertension syndrome which seems to be a problem often encountered in this LBM. Furthermore, the study has proved that Participatory Epidemiology Surveillance could be a relevant and reliable tool in disease outbreak investigation, hence, the need to incorporate PDS in routine disease outbreak investigation.

In Nigeria, quite a number of the Local Government Areas in the country have participated in PDS activities (Babalobi *et al*., 2011). Major findings indicated that awareness of farmers on Avian Influenza was high and they were able to describe the clinical signs. Studies have indicated that Participatory Epidemiology (PE) can play a major role in determining diseases priorities, decision making in the control/prevention/eradication options. Participatory Epidemiology has led to greater interaction especially with rural farmers who showed willingness to cooperate in all Government disease control interventions. Farmers' knowledge on various channels of disease reporting has been improved using PDS which happens to be logistically inexpensive, flexible and will lead to timely control of diseases and when combined with conventional medical and veterinary diagnoses can assist both professionals gain a better understanding of Veterinary/Public health issues and dynamics (Babalobi *et al*., 2011).

4.1 Limitations of the study

The team was unable to trace the source of the local chickens (pullets) that were introduced into the affected farm beyond the Janguza LBM and the source/sources of the apparently healthy ducks that tested positive for H5N8 could not be traced due to lack of bird movement record system. Also, there was reluctance by the LBM operators at Sabon Gari LBM Kano to allow the team to collect adequate samples due to what they described as lack of incentives and support from government. Moreover, they seemed to have very low awareness on the importance of disease surveillance to their business. Perhaps, if the investigation team were given full cooperation, more samples could have been collected and clearer picture of the situation obtained. Also, the team was constrained by limitation of resources and as such could not extend the disease outbreak investigation to, villages, towns and other States that supplied live birds to major LBMs in Kano. This calls for more detailed and extended period of disease investigation activities in the future, in order to obtain clearer picture of disease events.

5.0 Conclusions

The virus was most probably introduced into the index farm through the introduction of apparently healthy local chickens (pullets) from a hawker in Janguza LBM, Kano State. The extent of spread of the H5N8 and the actual source of birds could not be determined due to the nature of the trader (hawker) from which the birds were purchased and introduced into the farm. It is most likely that the infected birds were from one of the surrounding villages not far from the LBM since the hawker was riding a bicycle, he probably was not from afar. This investigation has revealed that a lot still needs to be done in the areas of disease surveillance, basic hygienic practices, altitudinal and bad habits of poultry value chain operators, application of biosecurity measures and regulation of the poultry industry in Kano State.

The origin of the ducks that tested positive to H5N8 in samples collected from LBMs as well as extend of spread of HPAI viruses in circulation in Kano State and beyond were not determined due to lack of records of movement of poultry species and weak disease surveillance system. There is need for intensification of disease surveillance activities particularly at LBMs to ensure basic biosecurity measures in the LBMs and proper regulations of poultry industry in Kano State and Nigeria as a whole.

As part of the One Health Action, in contacts (staff of the affected farm and operators of LBMs) had been monitored by public health officials for symptoms of the disease in humans while joint Veterinary and Medical awareness creation activities on the zoonotic nature of HPAI and the need for good hygienic practices at all LBMs were conducted.

6.0 Recommendations

To regulate the poultry industry in Kano State and regularly conduct disease surveillance activities at all major LBMs, sampling birds at random on arrival and shipping of samples to the laboratory at NVRI for testing needs to be done routinely which should be supported by both State and Federal governments.

Every AI disease outbreak in the State should duly investigated in order for the State to device means of controlling the disease. The outbreak investigation forms (electronic copies) should be made available to all the States of the Federation for future investigations. Sensitization of farms around the market area to create awareness on the recent outbreak of H5N8.

In view of the challenges encountered in the disposal of waste, the team recommended to the Veterinary Authorities to collaborate with the State Ministry of Environment Kano State in order to resolve the problem. The movement of birds within and outside the State needs to be restricted and there is need to device a record for transportation or movement of birds to enable trace-back of disease outbreak.

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Conflict of interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

References

Amos, T. T. (2006). Analysis of backyard poultry production in Ondo State, Nigeria. *International Journal of Poultry Science*. 5: 247–50.

Anzaku S. A., Umoh J. U., Abdu P. A., Kabir J. and Bala A. (2014). Participatory Epidemiological Investigation of Newcastle Disease in Local Chickens in the Federal Capital Territory, Nigeria. *International Journal of Livestock Research*. 4(4):20-35.

Babalobi, O. O., Bolajoko, M. B. and Anzaku, S. A. (2011). Participatory animal disease surveillance, Panacea to the bane of animal disease under-reporting in Nigeria. *Epidemiol. et sante anim.* 59-60: 273-275

Bird Flu Watch (2009). A Publication of Nigeria's Avian Influenza Control and Pandemic Preparedness and Response Project (AICP). 1(1): 1-8.

Federal Ministry of Agriculture and Rural Development (FMARD) (2013). Self-declaration from Nigeria on its disease-free status from notifiable avian influenza: Submitted to the OIE on 25 January 2013 by Dr Joseph Nyager, delegate of Nigeria to OIE, Director, Department of Livestock, Ministry of Agriculture and Rural Development, Abuja, Nigeria. 1-2.

Food and Agriculture Organization of the United Nations (FAO). (2017). Sub-Saharan Africa HPAI Situation Update. *FAO Animal Health Service/ EMPRES*.1-7

Hanna, A., Banks, J., Marston, D. A., Ellis, R. J., Brookes, S. M. and Brown, I. H. (2015). Genetic

characterization of highly pathogenic avian influenza (H5N8) virus from domestic ducks, England, November 2014. *Emerg. Infect. Dis.* 21:879–882.

Ip, S. H., Dusek, R. J., Bodenstein, B., Torchetti, M. K., DeBruyn, P., Mansfield, K. G., DeLiberto, T and Sleeman, J. M. (2016). High Rates of Detection of Clade 2.3.4.4 Highly Pathogenic Avian Influenza H5 Viruses in Wild Birds in the Pacific Northwest During the Winter of 2014–15. *Avain Diseases*. 60:354–358.

Kim, E.M. and Park, C.K. (2015). Single-Step Real-Time Reverse Transcription-Polymerase Chain Reaction for simultaneous detection of H5N1 and H5N8 Highly Pathogenic Avian Influenza Viruses. Medwell Journals (Journal of Animal and Veterinary Advances). 14(6): 161-166.

Kim, H. R., Oem, J. K., Bae, Y. C., Kang, M. S. and Lee, H. S. and Kwon YK. (2013). Application of real time reverse transcription polymerase chain reaction to the detection of matrix, H5 and H7 genes of avian influenza virus in field samples from South Korea. *Virology Journal*. 10(85):1-5.

Kwaghe, A. V., Ndahi, M. D., Usman, J.G., Sambo, E., Waziri, I., El-Oji, A. A., Pam, E. G., Jost, C. C., Acho, I. O. and Tchangai, C. P. (2012). Highly pathogenic avian influenza participatory disease surveillance in Plateau State, Nigeria. *Archives des Sciences*. 65(5): 132-142.

Kwon, J. H., Lee, D. H., Swayne, D. E., Noh, J. Y., Yuk, S. S., Erdene-Ochir, T. O., Hong, W. T., Jeong, J. H., Jeong, S., Gwon, G. B. and Song, C. S. (2016). Highly pathogenic avian influenza A (H5N8) viruses reintroduced into South Korea by migratory waterfowl, 2014–2015. *Emerg. Infect. Dis.* 22:507–510.

Lee, Y. J., Kang, H. M., Lee, E. K., Song, B. M., Jeong, J., Kwon, Y. K., Kim, H. R., Lee, K. J., Hong, M. S., Jang, I., Choi, K. S., Kim, J. Y.., Lee, H. J., Kang, M. S., Jeong, O. M., Baek, J. H., Joo, Y. S., Park, Y. H. and Lee, H. S.(2014). Novel reassortant influenza A(H5N8) viruses, South Korea, 2014. *Emerg. Infect. Dis.* 20:1087–1089.

Mariner, J.C. and Paskin, R. (2009). Manual on participatory epidemiology: *Methods for the collection of action oriented epidemiological intelligence.* FAO Animal Health Manual No.10. Food and Agriculture Organization of the United Nations (FAO), Rome.

Ndahi, M. D. and Kwaghe, A. V. (2011). Participatory disease surveillance of highly pathogenic avian influenza in Mangu local government area of Plateau State, Nigeria. *Researcher*. 3(12): 8-14. http://www.sciencepub.net/researcher.

Ojo, S. O. (2002). Analysis of the three risk factors in commercial poultry production in Osun State, Nigeria. In: Proceedings of the 27th Annual Conference of Nigeria Society for Animal Production, 17–21 March 2002. Federal University of Technology, Akure, Nigeria.

Public Health England (PHE) (2016). Avian influenza A (H5N8) in the UK: risk assessment. 1-4. www.nationalarchives.gov.uk/doc/open-government-licence/version/2/

Vakuru, C.T. (2010). Situation-based survey of avian influenza viruses in possible 'bridge' species of wild and domestic birds in Nigeria. Institute of Tropical Medicine Antwerp (ITMA). *Master Thesis* in *Tropical Animal Health,* 122: 1 46.

Verhagen, J. H., van der Jeugd, H. P., Nolet, B. A., Slaterus, R., Kharitonov, S. P., de Vries, P. P., Vuong, O., Majoor, F.., Kuiken, T. and Fouchier. R. A. Wild bird surveillance around outbreaks of highly pathogenic avian influenza A (H5N8) virus in the Netherlands, 2014, within the context of global flyways. (2015). *Euro Surveill*. 20(12) :pii521069. Article DOI: http://dx.doi.org/10.2807/15607917.ES2015.20.12.21069

Waziri1, I. M. and Yunusa, B. K. (2014). Participatory methods in the profiling of livestock diseases in the Jos-Plateau, Nigeria .*Animal and Veterinary Sciences*. 2(5): 154-160. http://www.sciencepublishinggroup.com/j/avs

OFFLU (OIE and FAO) Statement. (2016). Situation Report and Guidance for H5N8 and other Eurasian H5 clade 2.3.4.4 Avian Influenza Viruses. 1-5.