

BACTERIAL AND PARASITIC AGENTS ASSOCIATED WITH OUTBREAKS OF HIGHLY PATHOGENIC AVIAN INFLUENZA (H5N1) IN NIGERIA.

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SUMMARY

The Highly pathogenic avian influenza (H5N1) was reported in Nigeria in early January 2006. A total of 430 suspected samples were submitted to the National Veterinary Research Institute, Vom for analysis. 149 of the analysed samples were positive for highly pathogenic avian influenza, out of which 89 were found to have different bacterial organisms. 60 had parasitic agents and 22 had both bacterial and parasitic agents associated with the outbreaks. Cases associated with *E. coli* alone had a mortality of 9.15%, *Klebsiella* spp alone had a percentage mortality of 26.86%. Combined infections with *E. coli* and *Klebsiella* spp resulted in mortality of 66.41%, 28 cases were negative for any bacterial growth and had mortality of 13.12%.

Some parasites found to be associated with the outbreak of HPAI are; *Eimeria* spp (45 cases) and *Ascaridia galli* (10 cases) found in cases with mortalities of 18.34% and 58.33% respectively. Mortalities in positive cases that had concurrent bacteria and parasite complications were found to be progressively increasing; *E. coli* and *Eimeria* spp-4.57%; *Klebsiella* spp/*Eimeria* spp-10.23% and *E. coli*/*Klebsiella*/*Eimeria* spp-19.25%. It is not clear how far these infections

contributed to mortalities caused by the HPAI.

KEY WORDS: Bacterial; Parasitic; Agents, Highly pathogenic avian influenza, Outbreak; Nigeria.

INTRODUCTION

Highly Pathogenic Avian Influenza (HPAI) is a viral disease affecting the digestive, nervous and respiratory systems of all domestic and wild birds. It is characterised by respiratory, reproductive, digestive and/or nervous signs, high morbidity and mortality with an incubation period of few hours to 3 days (1). It is highly contagious and may be fatal in humans. Avian influenza viruses (AIVs) are members of the genus Influenza A and family Orthomyxoviridae. The influenza viruses that constitute this family are classified into types A, B or C based on differences between their nucleoprotein and matrix protein antigens. Avian Influenza viruses are further categorised into subtypes according to the haemagglutinin (H) and neuraminidase (N) antigens. There are 15 haemagglutinin and 9 neuraminidase subtypes of influenza A viruses (1).

AI virus is infective for most commercial, domestic and wild avian species. Chickens and turkeys are highly susceptible to infection and clinical

disease, while ducks and geese although susceptible to infection, suffer clinical disease from only highly virulent strains (2). Ostriches, emus, quails, ducks, guinea fowls, pigeon are susceptible to AI in varying degrees.

Birds that die of per acute disease may show minimal clinical signs and gross lesions (2). However, some of the clinical signs most commonly encountered during the outbreak include; ruffled feathers, soft shell to shell-less eggs and droopiness. In Nigeria however, affected birds sometimes remain active with little or no effect on feed intake and production. Other clinical signs are cyanosis of combs and wattles, oedema and/or swelling of head, combs, wattles, and cervical regions, yellowish-green diarrhoea, seromucous drooling nasal discharges, respiratory rales, sneezing coughing and dyspnoea, incoordination torticollis, leg paralysis, hyperaemia of the shanks and swollen foot pads (8; 12; 10).

Not only is infection with avian influenza potentially deadly by itself but it also weakens the host immune system thus allowing opportunistic microbes to invade and cause disease. The intestines of birds are known to be colonized by a large number of microbes; most of which are non-pathogenic or even beneficial. Others though non-pathogenic in healthy individuals can produce disease in those with compromised immune status. About half of the death from influenza outbreaks is not caused by the influenza virus directly but from secondary bacterial pneumonia infections (1, 2 & 3) reported that many of those who died in the 1918 human epidemic succumbed to secondary bacterial infections such as pneumonia and bacteraemia (3). (3; 8) reported the outbreak of avian influenza in two Fraser valley turkey farms where bacterial culture from tissues of dead

birds yielded *Escherichia coli*, which contributed to 8-9% of the mortality.

(14) in the investigation of outbreak of highly pathogenic avian influenza in water fowls and wild birds in Hong Kong reported that fresh tissues (lung, liver, spleen and small intestines) from birds showing gross lesions were cultured aerobically and anaerobically on blood agar (BA), selective agar and broth for *Mycoplasma* spp using standard procedures. Sera from geese were tested for mouse toxicity for botulinum toxin. Bacteriology revealed moderate growth of *Pasteurella* spp from tissues of Canada goose. Tissues from Little egret had moderate growth of *Salmonella typhimurium*. Moderate growth of *Aeromonas hydrophila* was recovered from a chestnut breasted teal. Serum from Canada geese gave negative result in mouse toxicity test.

This paper presents a report of the bacteria and parasitic agents found to be associated with the outbreak of H5N1 in Nigeria and their involvement in exacerbating the effect of the disease.

MATERIALS AND METHODS

Outbreaks of HPAI in Nigeria:

The first outbreak was detected in early January 2006 and it occurred in farm at Jaji, (latitude 10° 48'N; longitude 7° 34'), which is a small village situated along Kaduna-Kano road and about 20km from Kaduna city. The case was first reported on 16th January 2006 and was further investigated on 17th January 2006. The farm (100 hectares) is fenced from the major highway and the surrounding households. It has a running stream and has a lot of economic trees and plantation, which provides haven for a variety of wild local birds. The farm had a total of 46,000 commercial layers, 200 ostriches, 200 turkeys, few geese, 4000 layer breeders, 2000 broiler chicks and about 150 heads of cattle and 50 sheep which are of White Fulani and

Yankasa breeds respectively. Death of these different poultry species were said to have started 2 weeks prior to the date of reporting and had 100% morbidity.

Similarly another suspected outbreak was reported on 16th January 2006 from M & D farm located within a residential area in Bukuru town (latitude 9° 47'N; longitude 8° 51'E) about 10km from Jos city. The farm had a flock size of 1000 commercial layers kept on deep litter and recorded 70% mortality within 2 days while morbidity was 100%.

By 27th January 2006, another suspected outbreak was reported in Janguza farm (latitude 12° 00'N; longitude 8° 30'E) in Kano metropolis. Kano is in North-western part of Nigeria and is located along the same axis with Jaji- Kaduna. It is 200 km from where the first outbreak was reported. The farm has an area covering about 3 hectares with a flock size of 3000 commercial layers and 2000 cockerels, and it is fenced but located within a densely populated residential quarters. The closest poultry farm is less than 1km away. There were little or no biosecurity measures put in place prior to this outbreak. Morbidity was 100% for the layers with 80% mortality while the cockerel had 30% morbidity and had not recorded any mortality as at the time of the visit.

By now, multiple outbreaks were being reported in other farms in Kaduna, Kano, Jos and the disease was spreading fast to other States in Nigeria. As of June 2006, 15 States and the Federal Capital Territory (FCT) had outbreaks of the highly pathogenic avian influenza caused by H5N1 subtype confirmed by the Reference Laboratory in Padova, Italy. The outbreak started in commercial laying chickens but later spread to other types and breeds such as broilers, cockerels, ostriches, pigeons, guinea fowl, turkey, geese, duck and

local/backyard poultry as well as wild birds

Submission of samples from suspected flocks

During this outbreak, in the case of suspicion of a HPAI virus infection based on acute or extensive mortality, depression, apathy, decrease in feed and/or water intake, diarrhoea, lack of coordination, drop in egg production e.t.c carcass and/ or live birds from such flocks were submitted to the National Veterinary Research Institute (NVRI), Vom for post mortem examination and collection of tissues depending on the gross pathological lesions for laboratory analysis.

Post mortem Examination (PME)

Complete post mortem examination was carried out on all carcasses submitted and depending on the presence and nature of the gross lesions, additional organ samples were aseptically taken for bacteriological and parasitological analysis.

Virus Isolation

Virus culture was carried out as described by (OIE, 2005). Nine days old chicken (9 day-old) embryonated eggs obtained from specific antibody negative (SAN) flocks were used for viral isolation. 0.2 ml of the supernatant (of 20% w/v tissue homogenate) was inoculated into five viable embryonated chickens eggs via the Allantoic cavity. The eggs were incubated at 37°C in a humidified incubator. They were candled daily and those with dead embryos were chilled at 4°C for 12-18 hrs, after which a qualitative analysis was carried out on the dead embryos to check for haemagglutination (HA) activity using 10% washed chicken red blood cells from SAN flocks as indicator. Allantoic fluid from eggs that showed positive for HA activity were harvested aseptically and tested for sterility by plating on

blood agar and incubated at 37°C for 24 hrs (2, 6).

AGID Test

AGID test was carried out as described in OIE manual 2005. The supernatant was inactivated using 0.1% formaldehyde and used as the test antigen with standard avian influenza antigen and antisera obtained from national Veterinary Service Laboratory (NVSL) Iowa, USA. The agar gel plates were incubated at 37°C in a humidified incubator and examined after 24 hrs. Positive specimen showed Precipitating lines of identity to avian influenza type A group specific Ribonucleic Protein (RNP) antigen in the AGID test.

Haemagglutination Inhibition test (HI)

Alpha haemagglutination Inhibition test was carried with serially diluted positive Allantoic fluid using standard procedures (OIE, 2005). Newcastle disease monospecific antisera showed no inhibition of the test antigen.

Bacteriological Examination

Fresh tissues (lung, liver, spleen, intestine, heart and trachea) from suspected cases that showed gross lesions were cultured both aerobically and anaerobically on blood agar and McConkey agar using standard procedures.

Parasitological Examination

Faecal samples from suspected cases were also analysed by direct wet smear microscopy and/ or McMaster technique for intestinal parasitism.

RESULT

Bacteriology

A total of 89 tissues were collected from AI suspected cases and cultured for possible bacterial isolation, 28 (31.46%) yielded *E. coli*, 18 (20.22%) gave *Klebsiella* species. Other bacteria isolated included; *Pasteurella* species,

Streptococcus species, *Pseudomonas* species, *Salmonella* species, *Aeromonas* species, e.t.c (Table 1)

The percentage mortality of the flocks affected by each of the bacterial agents isolated is illustrated in part Table 2. The percentage mortality in the 28 flocks that gave no bacterial growth was found to be 13.12%, *E. coli* and *Klebsiella* Sp isolated in 13 cases, with a percentage mortality of 66.41% and *Klebsiella* Sp only, in 4 cases gave a percentage mortality of 26.86% while *E. coli* alone gave a percentage mortality of 9.15%.

Parasitology

Eimeria species (*E. necatrix*, *E. tenella*, *E. maxima*, *E. anatis*, *E. adenoids*, and *E. tropicalis*) were the most frequently encountered parasites in the cases analyzed 40 cases (82.19%) in 60 samples. *Ascaridia galli* was encountered in 10 cases (10.96%). Others included *Subulura brumpti*, *Iodamoeba nobell*, and *Trichomonas* species in ducks and *Railletina echinobothrida* in layers as showed in Table 3.

The percentage mortality of the flocks affected by parasitic agents is illustrated in Table 4. The percentage mortality in the 36 flocks where *Eimeria* species were identified was found to be 18.34%, *Ascaridia galli* gave a percentage mortality of 58.33%, *Eimeria* species and *Ascaridia galli* encountered in 9 cases gave a percentage mortality of 73.11%.

In part of Table 2 eight cases had both *E. coli* and *Eimeria* spp, four has *Klebsiella* and *Eimeria* spp and ten has *E. coli*, *Klebsiella* and *Eimeria* spp with mortalities of 4.57%, 10.23% and 19.25% respectively.

DISCUSSION

Secondary bacterial and parasitic complications have been a common finding in most viral infections. This is because most viral infections including avian influenza are not only potentially deadly by themselves, but also weakens the host immune system thus allowing opportunistic microbes to invade and cause disease. The findings of this study have established that *Escherichia coli*, *Pasteurella multocida*, *Pasteurella (Mannheimia) haemolytica*, *Klebsiella* species, *Streptococcus faecalis*, *Staphylococcus aureus*, *Staphylococcus albus*, *Pseudomonas aeruginosa*, *Aeromonas* species, *Moraxella* species, *Salmonella gallinarum* and *Proteus* species may be associated with outbreaks of highly pathogenic avian influenza (HPAI) in Nigeria.

E. coli had the highest frequency (28) followed by *Klebsiella* species (18) and *Streptococcus faecalis* (3). In addition it was observed that complication with *E. coli* alone caused a mortality of 9.15% while that by *Klebsiella* spp alone had a mortality of 26.86%. Whereas complication involving *E. coli* and *Klebsiella* spp, and *Klebsiella* spp/ *Streptococcus faecalis* gave a percentage mortality of 66.41% and 97.62% respectively. However, the percentage mortality in positive cases with no bacteria complication was found to be lower than those with multiple bacterial isolates.

The percentage mortality in cases where only *E. coli* was isolated (9.15%) is consistent with the findings (8-9%) of (7) and showed no significant difference with positive cases without bacterial complication. This could be as a result of the involvement of either non-pathogenic *E. coli* in some of the cases, or intervention with *E. coli* sensitive antibiotics prior to presentation and different management practices. One of

the hypotheses tallies with a case in one of the affected farms in Jos that had mortality of 400 in a laying flock of 1200 birds on the day it was first reported (fourth day of the onset of the disease). Based on the tentative diagnosis of fowl cholera, colibacillosis and fowl typhoid treatment was instituted using gentamycin intramuscularly (5 mg/kg body weight). However, 24 hours later *E. coli* was isolated from organs submitted for bacteriological culture and mortality was observed to have dropped by 200 (50%) after treatment.

(14) in an investigation of outbreaks of HPAI in water fowls and wild birds in Hong Kong reported that fresh tissues (lung, liver, spleen and small intestines) from birds showing gross lesions were cultured aerobically and anaerobically on blood agar (BA), selective agar and broth for *Mycoplasma* spp using standard procedures. Sera from geese were tested by mouse toxicity test for botulinum toxin. Bacteriology revealed moderate growth of *Pasteurella* spp from tissues of Canada goose. Tissues from Little egret had moderate growth of *Salmonella typhimurium*. Moderate growth of *Aeromonas hydrophila* was recovered from a chestnut breasted teal. This agrees with the isolation of *Salmonella* spp, *Pasteurella* spp and *Aeromonas* spp in this study.

The parasitic agents found to be associated with the outbreak of HPAI in Nigeria are *Eimeria* spp (45) and *Ascaridia galli* (10) in cases that had 8.34% and 58.33% mortalities respectively, whereas a combination of *Eimeria* spp and *Ascaridia galli* had a mortality of 73.10%. From the results obtained in this study, it is obvious that certain bacteria and parasitic agents were found to be associated with the outbreak of HPAI in Nigeria and contributed in exacerbating the effect of the disease to a level that is not clear.

A total of 22 cases had both bacterial and parasitic infections out of the 147 positive cases of HPAI representing 14.97%. However, eight of these samples had *E. coli* and *Eimeria* species concurrently representing 36.36% of the total samples with a mortality of 4.57%; 4 cases representing 18.18% and mortality of 10.23% had *Klebsiella* spp and *Eimeria* spp, while 10 cases representing 45.45% with a mortality of 19.25% had *Klebsiella* spp, *E. coli* and *Eimeria* spp.

This finding also tallies with what was earlier presented for bacterial and parasitic agents in separate cases. The percentage mortality for *E. coli* alone consistently remained low, which was explained to be probably due to the fact that the organism (*E. coli*) might be the non-pathogenic type (strain). The mortality increased in the cases of

Klebsiella spp/*Eimeria* spp and *Klebsiella* spp/*Eimeria* spp/*E. coli*, indicating that the mortality kept increasing as the number of complicating organisms increased. In normal situations, infection by a combination of these organisms/parasites might result in high mortality if not promptly controlled. Therefore, when they are found complicating a disease that already is characterized by high mortality, the effect will definitely be exacerbated.

Avian influenza (AI) affects the respiratory, digestive and/or nervous systems. *Klebsiella* spp, *E. coli*, and *Eimeria* spp also cause respiratory and digestive disorders respectively (11; 13; 15); It is obvious that they would have complementary roles on the mortality of affected birds when they occur concurrently in a flock.

TABLE 1
MORTALITY

ORGANISM	FLOCK SIZE	MORTALITY PERCENTAGE	MORTALITY (%)
<i>E. coli</i> & <i>Eimeria</i> spp (n=8)	3200	100%	4.57
<i>Klebsiella</i> spp & <i>Eimeria</i> spp (n=4)	3200	250%	10.23
<i>E. coli</i> & <i>Klebsiella</i> spp (n=13)	8673	2742%	19.25
<i>E. coli</i> & <i>E. coli</i> (n=1)	3200	300	
<i>Klebsiella</i> & <i>E. coli</i> (n=1)	128	123	
<i>Staphylococcus aureus</i> (n=1)	3200	300	
No bacterial growth (n=28)	17248	6560	
<i>E. coli</i> & <i>Eimeria</i> spp (n=8)	3200	100%	4.57
<i>Klebsiella</i> spp & <i>Eimeria</i> spp (n=4)	3200	440	10.23
<i>E. coli</i> & <i>Klebsiella</i> spp & <i>Eimeria</i> spp (n=10)	8673	1672	19.25

n = number of flocks affected

TABLE 1: BACTERIA ISOLATED WITH FREQUENCY OF OCCURRENCE.

BACTERIA ISOLATED	NUMBER OF CASES
<i>Pasteurella multocida</i>	1
<i>Pasteurella haemolytica</i>	1
<i>Escherichia coli</i>	28
<i>Klebsiella species</i>	18
<i>Streptococcus faecalis</i>	3
<i>Staphylococcus aureus</i>	1
<i>Staphylococcus albus</i>	1
<i>Pseudomonas aeruginosa</i>	2
<i>Aeromonas species</i>	2
<i>Moraxella species</i>	1
<i>Salmonella gallinarum</i>	1
<i>Proteus species</i>	2
No bacteria growth	28
Total	89

TABLE 2: FLOCK SIZE, DIFFERENT BACTERIAL ISOLATES AND % MORTALITY

ORGANISM	FLOCK SIZE	MORTALITY	PERCENTAGE MORTALITY (%)
<i>Escherichia coli</i> (n=14)	28844	2638	9.15
<i>Klebsiella</i> spp (n=4)	3500	940	26.86
<i>E. coli</i> & <i>Klebsiella</i> (n=13)	86513	57455	66.41
<i>E. coli</i> & <i>S. albus</i> (n=1)	23000	300	1.31
<i>Klebsiella</i> & <i>S. faecalis</i> (n=1)	126	123	97.62
<i>Staphylococcus aureus</i> (n=1)	2000	200	10.00
No bacterial growth (n=28)	47549	6240	13.12
<i>E. coli</i> & <i>Eimeria</i> spp (n=8)	23926	1094	4.57
<i>Klebsiella</i> spp & <i>Eimeria</i> spp (n=4)	4300	440	10.23
<i>E. coli</i> , <i>Klebsiella</i> spp & <i>Eimeria</i> spp (n=10)	86513	16655	19.25

N = number of flocks affected

TABLE 3: PARASITIC AGENTS IDENTIFIED IN POSITIVE CASES OF HPAI

PARASITES	NUMBER OF CASES
<i>Eimeria</i> species	45
<i>Ascaridia galli</i>	10
<i>Subulura brumpti</i> (duck)	1
<i>Trichimonas spp</i> (duck)	1
<i>Iodamoeba nobell</i>	1
<i>Rallietina echinobothrida</i>	2
Total	60

N = number of flocks affected

TABLE 4: FLOCK SIZE, THE PARASITIC AGENTS AND % MORTALITY

PARASITE	FLOCK SIZE	MORTALITY	Mortality (%)
<i>Eimeria</i> species (n=36)	114900	21073	18.34
<i>Ascaridia galli</i> (n=1)	1200	700	58.33
<i>Eimeria</i> & <i>A.galli</i> (n=9)	76861	56194	73.11

N = number of flocks affected

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