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Haemolytic *E. coli* Associated with the Outbreaks of Avian Influenza [H5N1] in Nigeria

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Abstract: Avian Influenza (AI) strain H5N1 outbreak with very high mortality in 2 commercial poultry farms in Nigeria was subjected to further laboratory investigations to document all contributory etiological factors. Tissues from flocks on the farms located over 200 km apart were sampled for bacteriology. Haemolytic *E. coli* and an unidentified Gram variable rod were isolated from the first farm; *Pasteurella haemolytica* and haemolytic *E. coli* were isolated from the second farm. Antibiotic susceptibility test showed haemolytic *E. coli* was resistant to 6, partially to 3 and fully susceptible to *Enrofloxacin* (*Tarivid*®). *Pasteurella haemolytica* was resistant to 5 and susceptible to 3 antibiotics. The unidentified Gram variable pleomorph was sensitive to 10 antibiotics used. The isolation of haemolytic *E. coli*, in avian influenza outbreaks with high degree of antibiotic resistance is hereby documented.

Key words: Outbreaks, avian influenza, haemolytic *E. coli*, bacteria, antibiotics, resistance

INTRODUCTION

The current H5N1 avian influenza (AI) epidemic which started in Southeast Asia late in 2003 was diagnosed in Nigeria in January and confirmed in February, 2006 (Anonymous, 2006). As at April 2006, the epidemic had spread to poultry in 12 of 36 States and the Federal Capital Territory of Nigeria. The severity in affected individual flocks has varied from moderate to very high with a ceiling of between 75 and 100% mortality in chickens and turkeys, respectively. It is generally known that secondary or concurrent bacterial infections tend to intensify the clinical pictures and other effects of primary viral diseases (Swayne, 2003). The low pathogenic avian influenza (LPAI) viruses which ordinarily induce inapparent infections to drop in egg production and mild respiratory disease in poultry, when complicated with bacteria either as concurrent or secondary infections cause severe respiratory disease with high mortality (Paul and Schrier, 2001).

The aim of this study was to investigate the presence of bacterial infections in two outbreaks of AI and assess effect and course of the disease in the two farms.

Farm history: History obtained from farm 1 included multiple species, breeds and ages of birds (turkeys, geese, ducks, ostriches and chickens from day old to layers) before the outbreak of AI. Some clinical signs observed during the outbreak were misshapen, soft-shell to shell-less eggs, dyspnoea, rales, sneezing, drooling salivation, cyanosis of the comb, swollen wattles and combs, decumbency, greenish yellow diarrhea, anorexia, bleeding from nears. Stretching of the neck, gasping, somnolence, coughing and oedema of the face; depression, prostration, convulsion and torticollis. The medication employed at various time by the farm was *Terramycin* LA®, *Triple sulphur PO*® (sulphadimidine, sulphadiazine, sulphaguanidine, vitamins A & K), *Conflox*®, *Neoceryl*® and *Vitalyte PO*®. Routine vaccination schedule against Newcastle (ND) and Gumboro diseases were strictly observed and carried out.

In farm 2, chicken was the only bird stocked in the farm. Clinical signs observed were greenish yellow diarrhea sometimes with urates, excess mucous discharges from the nostrils and sometimes bloody, weakness, somnolence, cyanotic combs and wattles that are shrunken, anorexia and decrease in egg

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production. Medication included *Furaltandone®*, *Chlortetracycline®*, *Oxytetracyclin LA®* and *Neocloxin®*. The farm equally observed regular vaccination against ND and Gumboro.

MATERIALS AND METHODS

Samples of unclothed blood, cloacae and tracheal swabs taken from sick birds were taken for bacteriology. These samples were cultured on appropriate media involving Selenite broth, Blood Agar (BA) and MacConkey Agar (MA) and incubated for 24-48 hunder aerobic conditions. Isolates were observed, stained, examined, sub-cultured on Eosin Methylene Blue (EMB) and subjected to biochemical test using standard techniques for proper identification of the isolates. The isolates were further subjected to antibiotic susceptibility test using disc diffusion method of Bauer and his co workers. The antibiotics used for *E. coli* as a Gram negative organism were *Tarivid* 10 µg; *Peflacine* 10 µg; *Ciproflox* 10 µg; *Augumentin* 30 µg; *Gentamycin* 10 µg; *Streptomycin* 30 µg; *Ceporex* 10 µg; *Nalidixic acid* 30 µg; *Septrin* 30 µg and *Ampicilin* 30 µg. Those used for the unidentified Gram variable bacterium were *Ciproflox* 10 µg; *Norfloxacin* 30 µg; *Gentamycin* 10 µg; *Lincocin* 30 µg; *Streptomycin* 30 µg; *Rifampin* 10 µg; *Floxapen* 30 µg; *Erythromycin* 30 µg; *Chloramphenicol* 20 µg and *Ampiclox* 30 µg.

RESULTS

Two bacteria were isolated from farm 1. The first was hemolytic on BA, as shown in Fig. 1, a lactose fermenter on MA and when sub cultured on EMB, gave the greenish metallic sheen characteristic of *E. coli* (Fig. 2). The second isolate from this farm was an unidentified gram variable pleomorph (Fig. 3). This isolate has been sent to a reference laboratory for identification. Two isolates were obtained from the second farm, the first being a haemolytic organism, similar in growth and characteristics as in the first farm and confirmed to be a haemolytic *Escherichia coli*. The other isolate was a gram negative rod that produced opaque but small colonies that were haemolytic on BA. They were late lactose fermenters on MA. The isolate subjected to biochemical tests was confirmed to be *Pasteurella haemolytica*.

Antibiotics susceptibility test: The susceptibility of the isolates to the antibiotics used is summarized in Table 1. Haemolytic *E. coli* isolated from the 2 farms showed similar pattern in results to the antibiotics sensitivity tests. They were resistant to *Nalidixic acid*, *Peflacine*, *Augumentin*, *Septrin*, *Ceporex* and *Ampicilin*, partially sensitive to *Streptomycin*, *Ciprofloxacin* and *Gentamycin* but very sensitive to *Tarivid* (Fig. 4). The *P. haemolytica* was resistant to *Nalidixic acid*, *Nitrofurantoin*, *Chloramphenicol*, *Erythromycin*, *Ampicilin* and

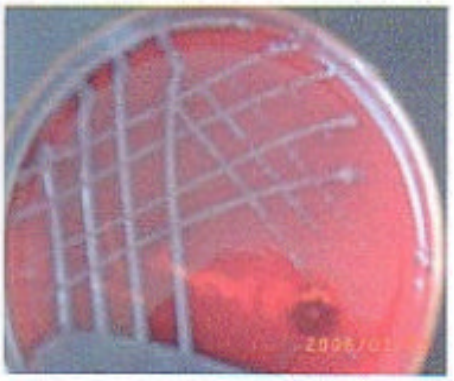

Fig. 1: Haemolytic *E. coli* on blood agar

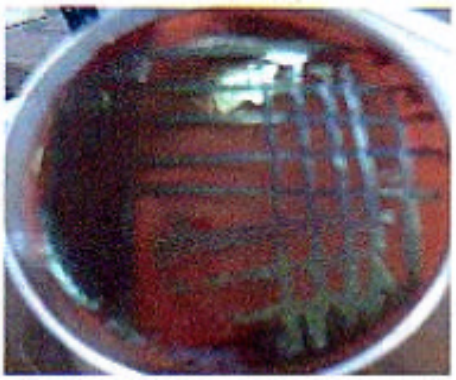

Fig. 2: Haemolytic *E. coli* on EMB

E. coli

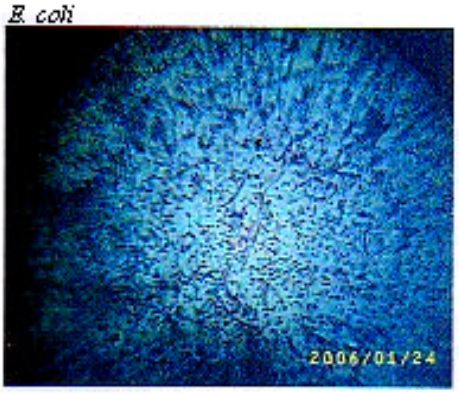

Fig. 3: Microscopic appearance of Gram variable pleomorph, x1000

Table 1: Antibiogram of the isolates from the two farms

Antibiotics	*E. coli*	*P. haemolytica*	Unidentified gram variable pleomorph
Nalidixic acid	R	R	-
Peflacine	R	-	-
Augumentin	R	-	-
Septrin	R	S++	-
Ceporex	R	-	-
Ampicilin	R	R	-
Streptomycin	S+	S+++	S+++
Ciprofloxacin	S+	-	S+++
Gentamycin	S+	S+++	S+++
Tarivid	S++	-	-
Norfloxacin	-	-	S+++
Lincocin	-	-	S+++
Rifampin	_	_	S++
Floxapen	-	-	S++
Erythromycin	-	R	S+++
Chloramphenicol	-	R	S++
Ampiclox	-	R	S++
Nitrofurantoin	-	R	-

Isolated on BA

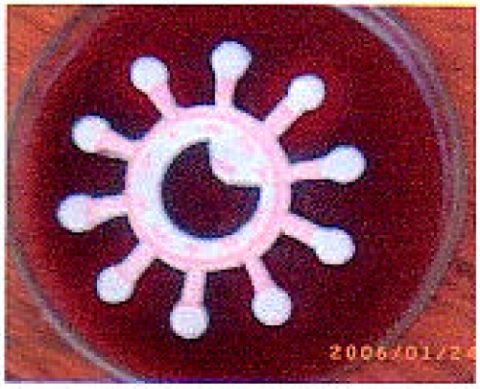

Fig. 4: Antibiogram of haemolytic *E. coli*

Fig. 5: Antibiogram of Gram variable pleomorph

Ampiclox but sensitive to *Septrin*, *Streptomycin* and *Gentamycin*. The unidentified gram variable pleomorph showed a remarkable susceptibility to all the antibiotics used (Fig. 5).

DISCUSSION

Highly pathogenic avian influenza viral infection such as H5N1 is an enough catastrophe in a poultry farm apart from the zoonotic importance which makes it more frightful. In a recent analysis of the several isolates obtained from Nigeria, It was concluded that the deadly virus arrived in the country from different sources (Ducatez *et al*., 2006). In all outbreaks of AI reported world wide in poultry, little attention is paid or any report made on the possible roles of intercurrent bacterial infections. The isolation of pathogenic bacteria with the attendant characteristic resistance to most antibiotics tested calls for a closer study on the roles, public health significance and clinical manifestation pattern of the disease during outbreaks. The consequences arising from the concurrent or secondary bacterial infections are usually towards producing increase severity of the clinical pictures observed in infections primarily initiated by other agents like viruses (Swayne, 2003). The clinical manifestations presented from the two outbreaks in this report were not exactly the same despite the confirmation of the H5NI infections in both cases. One common feature was the high mortality of over 90% recorded in both farms. Before the confirmation of avian influenza, caused by H5NI, workers in the two farms have handled the birds with minimal protections and some without any at all. The haemolytic *E. coli* and other isolates might have been disseminated to other parts of the farms and their homes. Luckily enough, no human casualty associated with the avian flu outbreaks have been reported among the poultry workers indicating perhaps that the circulating virus strain of H5NI in these cases is pathogenic to only birds.

Early reports of resistant strain of *E. coli* to Ampicilin, Tetracycline, Nitrofurantoin and Cefuroxime isolated from clinically healthy chicken were documented in the South-east of Nigeria (Chah *et al*., 2001; Okoli *et al*., 2002). A similar study conducted in the Northern Nigeria involving swabs of organs of poultry at post mortem, revealed resistance of *E. coli* to Cephalothin, Penicillin, Streptomycin and Sulphamethoxade-Trimethoprin (Kwaga *et al*., 2004). No report was made from these studies of any haemolytic *E. coli* been isolated and the high degree of resistance to most antibiotics tested as in this case. It is therefore important to initiate a detail study as to the roles of resistant strains of *E. coli* and other bacteria in our poultry industry and possibly the zoonotic implications of the agents.

Where layers are the main breeds, in a farm, a regular examination of the eggs should be carried out for purpose of public health significance involving resistant strains of *E. coli* from Colibacillosis in chicks. It will be a useful

epidemiologic approach to monitor possible secondary bacteriologic components of current AI epidemics in infection-prone and open sided poultry house and husbandry systems.

CONCLUSION

In conclusion, we found haemolytic *E. coli* resistant to a lot of antibiotics in 2 separate episodes of avian influenza out breaks in poultry in Nigeria.

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