CASE REPORT

Sokoto Journal of Veterinary Sciences

(P-ISSN 1595-093X: E-ISSN 2315-6201)

http://dx.doi.org/10.4314/sokjvs.v23i2.7

Sati *et al.*/*Sokoto Journal of Veterinary Sciences*, **23**(2): 108 – 112.

Co-infection of *Escherichia coli* and *Aspergillus fumigatus* in a Noiler flock in Jos, Plateau State, Nigeria

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Publication History:
Received: 29-11-2024
Revised: 10-03-2025
Accepted: 13-03-2025

Abstract

Infections in poultry settings are a major setback for farmers, and they lead to economic losses, reduced protein availability, poor productivity and in cases of zoonotic infections, may constitute public health risks. Noiler breed is a hybrid of Nigerian indigenous chicken and the White Plymouth Rock chicken, which was introduced into the Nigerian meat and egg production market, especially for rural dwellers, because they require minimal management to thrive. A case of depression, respiratory distress, increased mortality and drop in egg production in a 33-week-old Noiler flock is hereby reported. Investigation carried out involved history taking, observation of clinical signs, postmortem examination of carcasses, culture and identification as well as antimicrobial and antifungal susceptibility testing using standard procedures. *Escherichia coli* and *Aspergillus fumigatus* were isolated. Antimicrobial susceptibility testing revealed that the *Escherichia coli* was sensitive to enrofloxacin, ceftriaxone and sulphamethoxazole/trimethoprim but resistant to ampicillin, amoxicillin, tetracycline and chloramphenicol. The antifungal susceptibility test showed that the *Aspergillus fumigatus* was susceptible to amphotericin B, voriconazole, itraconazole and econazole nitrate but resistant to anidulafungin, nystatin and fluconazole. A 7-day treatment was instituted accordingly, and a follow-up visit 10 days afterwards showed the mortality had dropped to zero and egg production had significantly risen. This case has shown the urgent need to provide veterinary services to farmers to increase productivity to meet the protein needs of the public, secure the farmers' sources of livelihood and protect public health as both pathogens have zoonotic implications.

Keywords: Antifungal susceptibility, Antimicrobial susceptibility, *Aspergillus fumigatus, Escherichia coli*, Noiler breed

Introduction

Poultry is one of the most common types of animals raised due to its relatively low production cost and near absence of religious and cultural taboos associated with its consumption (Melesse, 2014). The Noiler is a dual-purpose breed of chicken resulting from a cross between Nigerian indigenous chicken and the White Plymouth Rock chicken that was developed by Amo Farm Sieberer Hatchery Limited, Ibadan Nigeria and aimed at addressing the challenges of food and financial insecurities especially among women and farmers who are mostly rural dwellers (Tiough *et al.,* 2022). Noiler chickens are known to be hardy, surviving on low-quality feedstuffs to provide good quality meat and eggs (Tiough *et al.,* 2022).

One of the major challenges of poultry production in Nigeria is diseases (Adene & Oguntade, 2006). These diseases could be viral, fungal, parasitic and bacterial, which could occur as mixed infections (Fang *et al.,* 2021). Avian pathogenic *E. coli* (APEC) can occur as a primary or secondary infection, and depending on the virulence of the strain, host immune status and other predisposing factors, the infection can manifest in the form of high morbidity, mortality, and production losses (Guabiraba & Schouler, 2015). Aspergillosis is commonly found in poultry, especially if the birds are immunocompromised or stressed (Carrasco *et al.,* 2001).

Bacterial and fungal infections have traditionally and preferentially been controlled using a large array of antimicrobials. However, the emergence of antimicrobial resistance in these organisms has led to ineffective antimicrobial therapy and, hence, veterinary treatment failure. This has resulted in monitoring of antimicrobial resistance profiles of pathogenic bacteria/fungi to ensure effective antimicrobial treatments in poultry and reduce the development of drug resistance.

In this report, a case of increased mortality and a drop in egg production in a 33-week-old Noiler flock was investigated. Findings from clinical signs, pathological lesions with culture and sensitivity testing showed a mixed infection. The use of appropriate treatment yielded satisfactory results. These results also provide information on the need for timely veterinary services to be provided for rural poultry farmers to enhance productivity and secure livelihoods.

Case Presentation

Case history and clinical manifestations

A farmer presented six Noiler carcasses to the Central Diagnostic Laboratory in the National Veterinary Research Institute, Vom, Nigeria. The flock history received from the farmer showed the birds were 33 weeks old, and the flock size was 250. The birds were sourced from Amo Farm Sieberer Hatchery Limited, Awe, Oyo State, Nigeria, and they had been vaccinated at day old at the hatchery against Newcastle disease (intra-ocular) and Marek's disease. Gumboro disease vaccine was administered at two and four weeks, Newcastle disease vaccine (LaSota) at three weeks, while fowl pox disease and Newcastle disease vaccines (Komarov) were given at 6 weeks of age. The birds were also revaccinated against Newcastle disease (Komarov) at 16 weeks of age. Deworming was done at 14 weeks of age using levamisole hydrochloride. History of antimicrobial use from the farmer showed the birds were placed on AMOXY-COL WSPR (Kepro, Holland) for seven days from the day of arrival of the day-old Noilers, and this was repeated at week 19. The Noilers were on deep litter, the source of water was from a well, and the farmer used commercially produced layer feed to feed the birds. One week prior to presentation, the farmer noticed a drop in feed consumption, depression and a gradual decline in egg production from 83% to 57% as at the day the case was reported. Three days later, there was respiratory distress and a mortality pattern of 3, 9, 15 and 12 within the last 4 days before the case was presented.

Investigations

Postmortem examination*:* Upon postmortem examination, the lungs were congested, the air sacs were cloudy, and hepatic fibrosis (Plate I) as well as splenomegaly (Plate II) were also seen. The Liver, spleen, lungs and cloacal swab samples were collected for microbial culture and sensitivity testing.

Bacteriology: Samples were enriched in Muller-Haas tetrathionate broth and E. coli broth and plated on Xylose deoxycholate agar (XLD) and MacConkey agar and incubated at 37℃ for 24 hours. There was no characteristic Salmonella growth on XLD but pinkish colonies were seen on MacConkey agar (Plate III), which is presumptive for *E. coli*. These were further plated on sorbitol MacConkey agar, and pale colonies were seen (Plate IV), which is suggestive of pathogenic *E. coli.* Biochemical tests showed that the organism was citrate, Voges-Proskauer and urease negative and indole and methyl red positive. All reagents used were manufactured by Oxoid, UK.

The susceptibility of the isolate was tested against a panel of 8 antimicrobial discs (Oxoid, UK) using the disk diffusion method as described by Bauer *et al*. (1966). The different antimicrobials had zones of inhibition as evidenced by the inability of bacteria to grow within a circumscribed area on the plate.

The result revealed that *E. coli* was resistant to ampicillin, amoxicillin, chloramphenicol and tetracycline, intermediate for Streptomycin and sensitive to enrofloxacin, ceftriaxone and sulphamethoxazole-trimethoprim (SXT) (Plate V). The Clinical and Laboratory Standards Institute (CLSI) guidelines were used for testing, and the clinical breakpoints were employed as interpretive criteria for the antimicrobial agents to classify bacterial isolates as susceptible, intermediate, or resistant, as shown in Table 1.

Mycology: Harvested tissue samples were seeded on Sabouraud dextrose agar (SDA) and incubated at 26°C. After 72 hours, the lung tissue on SDA had growth that initially appeared velvety and white, which later turned blue-green as conidia (Plate VI). The isolate was stained with lactophenol cotton blue, and septate and hyaline (transparent) hyphae were seen. The conidiophore and vesicle were flask-shaped and uniseriate, producing phialides and small, round conidia, which is characteristic of *Aspergillus*

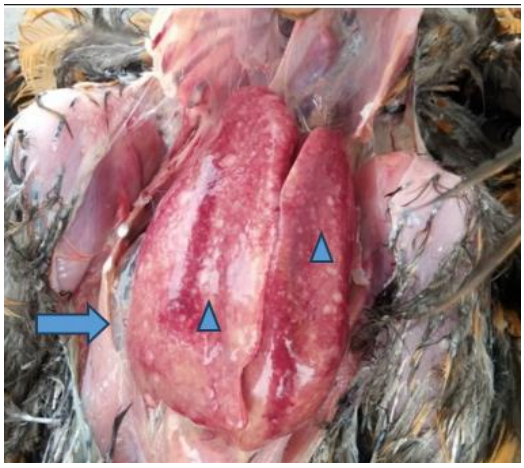

Plate I: Post-mortem lesion of the lungs showing cloudy air sacs (blue arrow), congested lungs and fibrosis in the liver (blue triangles)

Plate II) Post-mortem lesion of the spleen showing enlarged spleen (green star)

Plate III: *E. coli* on MacConkey agar as smooth, round colonies with lactose fermentation with a pinkish colour

Plate IV: Pathogenic *E. coli* appearance on Sorbitol MacConkey agar seen as pale colonies

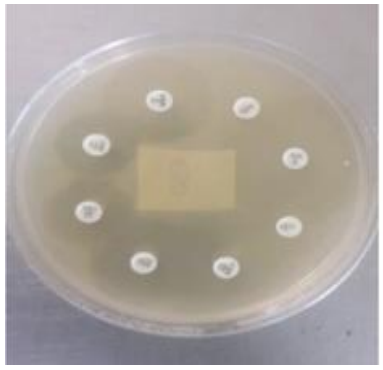

Plate V: Antimicrobial susceptibility test for *E. coli* plated on Muller Hinton agar with different antibiotics showing zones of inhibition

Table 1: Antimicrobial susceptibility test result for *Escherichia coli*

Sensitive	Intermediate	Resistant
Enrofloxacin	Streptomycin	Ampicillin
Ceftriaxone		Amoxicillin
SXT		Chloramphenicol
		Tetracycline

fumigatus (Plate VII). Antifungal susceptibility test was conducted using the disc diffusion method using seven antifungal discs (Oxoid, UK) as described by Bauer *et al.* (1966) to ascertain the most effective antifungal(s) to be used. The result showed that the Aspergillus fumigatus was resistant to anidulafungin, nystatin and fluconazole but sensitive to amphotericin B, voriconazole, iatraconazole and econazole nitrate (Table 2). This was determined by the zone of inhibition of these antifungals to the A. fumigatus, shown by the inability of the fungus to grow within a circumscribed area on the plate (Plate VIII).

Case management

Based on the results obtained, the birds were placed on enrofloxacin and amphotericin B for 7 days. The farmer was also advised to improve hygiene and sanitation practices on the farm, along with the implementation and adherence to proper disinfection protocols and biosecurity measures. A follow-up visit 10 days later showed an increase in egg production (81% compared to 57% prior to treatment) and zero mortality.

Discussion

Noiler chickens have gained acceptance among rural farmers in Nigeria as they are very hardy, having the twin qualities of resilience found in indigenous breeds and meat/egg production of the White Plymouth Rock breed. These traits, however, do not protect them from endemic diseases such as avian pathogenic *E. coli* (APEC) and aspergillosis as seen in this case report. APEC can cause colibacillosis in poultry of all ages, acting as the primary or secondary pathogen and is associated with severe extraintestinal disease, as reported by (Shehata & Hafez, 2024), which was noted in the lungs, liver and spleen during postmortem. Reports of aspergillosis in Nigerian poultry are available (Kwanashie *et al.,* 2013: Bitrus *et al.,* 2024), but it has not been documented in Noiler chickens.

Mixed infection in Noiler chickens should be given necessary attention as the essence of introducing them is to have birds that yield good meat and eggs while being hardy. This is important because veterinary care is often scarce in rural areas where this group of poultry is mostly found. In addition to the diagnosis of a mixed infection in this study, different antimicrobial resistance profiles were detected for both APEC and *A. fumigatus*. This could indicate a broader incidence of antimicrobial resistance (AMR) in livestock populations, with far reaching public health implications.

This case report highlights the importance of timely and accurate laboratory diagnosis in achieving

Table 2: Antifungal susceptibility testing was done using a panel of seven antifungal drugs

Susceptible	Resistant
Amphotericin B	Anidulafungin
Voriconazole	Nystatin
Iatraconazole	Fluconazole
Econazole nitrate	

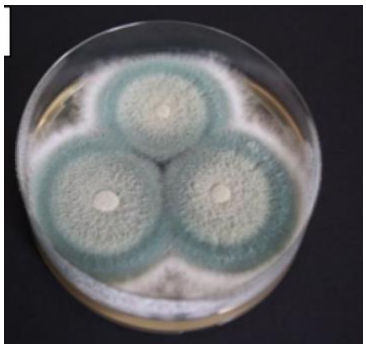

Plate VI: Growth of Aspergillus fumigatus on seen asSabouraud dextrose agar velvety or powdery, turned blue-green as conidia

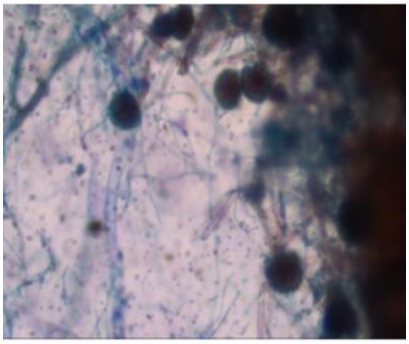

Plate VII: Aspergillus fumigatus stained using lactophenol blue stain

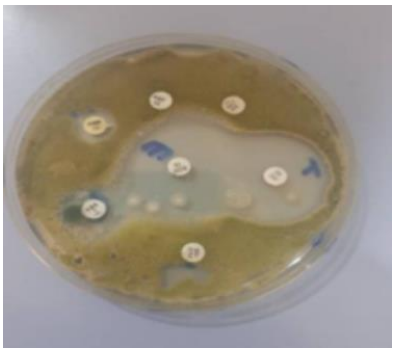

Plate VIII: Antifungal susceptibility test with different antifungal agents and zones of inhibition

efficient disease management to mitigate the impact of such diseases on poultry health and productivity and also to reduce losses associated with increased cost of treatment.

A notable limitation of this study is the absence of molecular characterization of the identified pathogens, which would have accurately identified the circulating strains of the pathogens in the flock.

Acknowledgements

The authors wish to acknowledge the management of the Pathology Faculty at the Postgraduate College of Veterinary Surgeons of Nigeria, NVRI Vom Study Centre, for their unwavering support.

Conflict of Interest

The authors declare that there is no conflict of interest.

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