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ANTIBIOTIC RESISTANT *SALMONELLA* AND *ESCHERICHIA COLI* ISOLATED FROM DAY-OLD CHICKS, VOM, NIGERIA.

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Abstract

Reports of large scale mortality of day-old-chicks were received at the National Veterinary Research Institute, Vom, Nigeria in 2007 to 2008. We investigated the cause of death using several virological and bacteriological techniques, isolated the pathogenic agents and carried out sensitivity tests. Our investigation revealed that *Escherichia coli* and *Salmonella* organisms were isolated in the outbreaks. A pattern of antibiotic resistance that seems to be increasing was also found. Considering the role of chickens and its products in the human food chain in Nigeria; and the close interaction between poultry and man, these resistant organisms may pose dangers to humans through the food chain or zoonotic infection and precipitate a similar pattern of resistance in man. We advocated for informed use of antibiotics in the food animals, especially poultry.

Keywords: *Escherichia coli*, *Salmonella paratyphi*, poultry, Nigeria

INTRODUCTION

Antibiotics have been used successfully in human and veterinary medicine in the past sixty years to turn many of the life threatening bacterial infections into treatable conditions. However, in recent times, antibiotic resistance has become an important health and food safety issue with the emergence of many drug-resistant species of microbial pathogens in human (1). The use of several veterinary antibiotics/conbiotics for therapeutic or prophylactic administration, especially in the poultry operations is particularly worrisome in view of the potential to extend such drug into the human food chain or the possibility of reduce efficacy

of such drugs sometimes administered by non qualified personnel (2, 3, 4, 5, 6).

Poultry production is an important income activity; its success is largely hinged on good management practices and sourcing of the stock from reputable hatchery. In recent times, there has been a gradual and documented increase (30-50%) in day-old-chicks mortality in many farms in parts of north central Nigeria (Plateau, Kaduna and Bauchi). This is linked most often to hatchery originated infections and efforts to treat using many of the commonly available veterinary antibiotics have met with little or no success.

It should be noted that the inability of many major hatchery to meet the national requirements for day-old-chicks has led to the establishment of many small scale hatchery facilities without the recourse to very strict and stringent but necessary conditions of hatchery operations.

A recent survey in Ekiti state, Nigeria indicated that there has been a gradual increase in the use of various antibiotics including quinolones, gentamycin, neomycin, tylosin and chloramphenicol between the year 2002 and 2004 (7). Undocumented field surveys in other parts of the country had presented with comparable statistics.

This work therefore aims to investigate and isolate the causative agent (s) of the recent increasing cause of death in day-old-chicks; and report on its management and controls.

Materials and Methods

Reports of increasing mortality in day-old-chicks were made by poultry farms around Vom (8°45'E, 9°43'N). Similar confirmed and unconfirmed reports were received from other parts of Plateau, Bauchi, and Kaduna states. Data from Ekiti state indicated similar findings (7). Though the chicks were sourced from different hatcheries and distributors; histories of huddling, somnolence, weakness, droopy wings, sudden death without premonitory signs and off-feed were reported by most farmers. Increasing mortality usually starts at about day three

to five of chicks' lives. One of the case farms confirmed the usage of combined Gentamycin and Diaziprim based on manufacturers' instruction. Based on the relative ineffectiveness of the recommended dosages, the dosage of the Gentamycin in the combination was doubled to achieve the desired results (initial reduction in mortality followed by complete cessation of mortality).

Visits were made to farms and carcasses were collected. All carcass samples collected were transported in sealed containers to the laboratory on wet ice (+4°C).

Carcasses were opened aseptically inside a microbiological safety cabinet class II (SterilGARD® III Advance, The Baker Company, Sanford ME, www.bakerco.com). At post mortem, carcasses were investigated for post mortem lesions indicative of aspergillosis, management errors (dehydration, asphyxiation, etc) and other diagnostic pathological lesions of sudden death in day-old chicks. The congested tissue samples (lung, liver, spleen, heart and intestine); and the brain were harvested for further laboratory analyses.

Virologic analyses

Pooled tissue samples were homogenized to make a 20% suspension using PBS enriched with penicillin, streptomycin, gentamycin and amphotericin B (PSGA), inoculated into 9-day-old specific antibodies negative chicken embryonating

eggs (SAN-CEE) and monitored for pathogenicity for seven days using standard protocols (OIE, 2004a; OIE, 2005). The non-haemagglutinating allantoic fluid was passaged into another set of 9-day-old SAN-CEE and monitored as previously described (8, 9). Agar-gel-immunodiffusion test was performed on the 20% pooled tissue homogenate using standard protocol to test for antigen to infectious bursal disease virus (IBDV) (10).

Bacteriologic analyses

Whole samples of lung, liver, spleen and heart were sectioned aseptically; the parenchyma portion of each organ was plated directly on 5% sheep blood agar (Oxoid, Basingstoke, England) using sterile wire loop and incubated for 24 hours in a humidified incubator (temperature: 37°C approx., Humidity: 44.50 approx.). The resultant colonies were sub-cultured onto blood, MacConkey (Oxoid) and Salmonella-Shigella (Fluka, UK) agars and incubated at 37°C for 24 hours (11, 12).

These colonies were further sub-cultured and Gram-stained. Catalase and oxidase tests were performed according to previously described procedures (13). The isolates from the plates were pre-enriched in peptone water and incubated for 4-6 hours. Motility test was performed using the hanging drop method (14). Indole test was conducted by using Kovac's reagents according to previously described methods (15). Triple sugar iron agar test

was performed by streaking three colonies of the pure culture on the sloped surface and the stabbing of the butt centrally, followed by incubation for 24-48 hours at 37°C.

Sugar fermentation test was carried out on Mannitol, Dulcitol, Lactose, Sucrose, Inositol, Glucose, Arabinose and Xylose. Citrate and urease tests were also performed.

Antibiotic Sensitivity

Antibiotic sensitivity test was conducted using antibiotic disc (Oxoid, UK) according to Kirby-Bauer antibiotic disc diffusion techniques. Briefly described, Mueller-Hinton agar was prepared in petri-dishes (Bibby Sterilin, UK). Pure colonies of the isolated organisms were emulsified in normal saline and the turbidity matched against McFarland No. 0.5 turbidity standard. The bacteria were plated on the Mueller-Hinton agar and antibiotic disc was placed centrally using the antibiotic disc dispenser (Oxoid, UK). The Petri-dish and its content were incubated for 24 hours in a humidified incubator at 37°C. The organisms were observed for antibiotic sensitivity by measuring the zone of inhibition on the plate.

Results

Virology

The allantoic and chorio-allantoic fluids were declared negative for Newcastle disease (ND) and avian influenza (AI)

viral infection respectively following negative haemagglutination tests, and the performance of haemagglutination-inhibition on allantoic fluid (for ND) and agar-gel-immuno-diffusion on chorioallantoic membrane (for AI) tests using monospecific antisera of ND and AI. The 20% homogenate was negative for IBDV antigen since no line of identity was recognized between the test antigen and the IBDV monospecific antibodies used.

Bacteriology

On blood agar, uniform growth of smooth, circular and convex colonies without haemolysis was observed from the different organ plated. The cultures in MacConkey revealed both pale non lactose fermenting translucent colonies suggestive of *Salmonella*, and reddish lactose fermenting colonies surrounded by a turbid zone suggestive of *E. coli*; while yellowish/pale colonies with black dots indicative of hydrogen sulphide (H₂S) production was seen on Salmonella-Shigella agar 24-48 hours post incubation. Gram Staining revealed numerous Gram negative short rods. All isolates were catalase positive, oxidase negative and motile confirming *E.coli* and *S. paratyphi*. Indole test revealed both indole negative and positive organisms. On Triple Iron Sugar, some organisms produce acid and gas, a characteristic of *E. coli* while others produce acid, gas and H₂S, an indication of *S. paratyphi*.

Sugar fermentation test revealed some lactose fermenting organisms and some non-lactose fermenters; the organisms ferment mannitol with gas production (*E. coli* and *S. paratyphi*), on dulcitol, the organism ferment it with gas production (*S. paratyphi*), there was no fermentation on sucrose (*Salmonella* organisms). The reaction in inositol test was delayed but there was fermentation (*S. paratyphi*), fermentation on glucose with acid and gas production was observed (*E. coli* and *S. paratyphi*). The fermentation reaction on arabinose was also delayed but acid and gas production was observed (*S. paratyphi* and *E. coli*) and fermentation reaction was also observed on xylose. Based on the bacteriological and biochemical tests performed above, a confirmation of *Salmonella paratyphi* and *Escherichia coli* was reached.

Antibiotic Sensitivity

The pure isolate of *S. paratyphi* was sensitive to Ciprofloxacin, Kanamycin, Chloramphenicol, Gentamycin and Norbafloxacin but resistant to Penicillin. However, the isolate of *E. coli* was sensitive to Chloramphenicol, Kanamycin, Gentamycin but resistant to Ciprofloxacin, Norbafloxacin and Penicillin. In view of the virologic and bacteriologic analyses of the samples, paratyphoid diseases complicated by colibacillosis was diagnosed.

Discussion

Our investigation revealed a pattern of infection that appears to be spreading in the country in the form of epizootics. The causative organisms-in this case *S. paratyphi* and *E. coli* are resistant to a range of antibiotics commonly used in the veterinary and human practices in Nigeria. This presents a serious cause for concern considering that the uninformed farmers/quacks may continue to use increasing level of ineffective antibiotics in the management of infection in farms with possible residues in poultry meat, eggs and other products meant for human consumption. Previous workers have confirmed similarity in antibiotic resistance pattern in poultry and humans within the same locality (3, 16, 17). The cause of the resistance to these antibiotics needs to be investigated more thoroughly, to prevent possible continued resistance to currently sensitive and newer range of antibiotics.

The transfer of potential residues of these antibiotics through the food chain to human presents an opportunity for the development of resistance by human pathogens to this range of antibiotic products.

In conclusion, this work has demonstrated that there appear to be resistant strains of *Salmonella* organism and *Escherichia coli* circulating in the field in Nigeria, and calls for concerted effort from the field veterinarians in informed drug administration; and for the policy makers

to make and implement standardized legal classification of veterinary drugs to prevent continued abuse of these various products. More work need to be done to comprehensively assess the national prevalence of these organisms and carry out full characterization of the various serotypes that exist.

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