An Outbreak of *Corynebacterium Diphtheriae* Infection in Broiler Chickens in Lagos, Nigeria

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Abstract- The outbreak involved 1,200 15 weeks old white leghorn growers in a poultry farm in Lagos out of which 163 died without any premonitory signs. Postmortem examination revealed congested lungs, haemorrhagic inflammation of the upper respiratory tract, crop, proventriculus and petechial haemorrhage of the cardiac muscles. *Corynebacterium diphtheria* was isolated from the intestine, heart blood, lung, upper respiratory tract and liver of all that died. The pathogenicity of the isolates was conducted on 8 15-weeks old white leghorn with two as controls using 0.5ml overnight broth culture administered orally. They were observed for up to 13 days with 100% mortality. The organism was re-isolated from the heart blood, liver, lung, trachea, proventriculus and crop of infected chickens. The control showed no sign of illness during the period of observation. This study revealed the zoonotic nature of *Corynebacterium diphtheriae* which association with poultry in Nigeria has not been reported as far as the authors knew.

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1. INTRODUCTION

Corynebacteria are gram-positive, catalase-positive, aerobic or facultative anaerobic, generally non motile rods. The genus contains the species Corynebacterium diphtheriae and the non-diphtherial corynebacteria, collectively referred to as diphtheroids (Burkovski, 2013). Nondiphtherial corynebacteria, originally thought to be mainly contaminants, have increasingly over the past two decades been recognized as pathogenic, especially in immune-compromised hosts (Ott and Burkovski 2013). Today, the more common scenario is nondiphtherial corynebacterial bacteremia associated with diverse infections as well as meningitis, septic arthritis, and urinary tract infections (Bonmarin et al., 2009). Nondiphtherial corynebacteria also cause chronic and subclinical diseases in domestic animals and can lead to significant economic losses for farmers (Bonmarin et al., 2009). Examples of widespread and difficult-to-control infections include Corynebacterium pseudotuberculosis caseous lymphadenitis in sheep, and goats; C. pseudotuberculosis ulcerative dermatitis in cattle; and urinary tract infections and mastitis in cattle due to infection with Corynebacterium renale, C. cytsidis, C. pilosum and C. bovis. (Yassin et al., 2003). C. diphtheria infection is typically characterized by a local inflammation, usually in the upper respiratory tract, associated with toxin-mediated cardiac and neural disease. Three strains of C. diphtheria are recognized in decreasing order of virulence: gravis, intermedius and mitis. These strains produce an identical toxin, but gravis strain is potentially more virulent because it grows faster and depletes the local iron supply, allowing for earlier and greater toxin production. Toxin production is encoded on the tox gene, which in turn, is carried on a lysogenic beta phage. When DNA of the phage integrates into the host bacteria’s genetic material, the bacteria develop the capacity to produce this polypeptide toxin. The tox gene is regulated by a corynebacterial iron-binding repressor (DtxR). Binding of ferrous iron to the DtxR molecule forms a complex that binds to the tox gene operator and inhibits transcription. Depletion of iron from the system removes the repression and allows the toxin to be produced. The toxin is a single polypeptide with an active (A) domain, a binding (B) domain and a hydrophobic segment known as the T domain, which helps release the active part of the polypeptide into the cytoplasm. In the cytosol, the A domain catalyzes the transfer of an adenosine diphosphate-ribose molecule to one of the elongation factors (eg elongation factor 2 EF2) responsible for protein synthesis. This transfer inactivates the factor, thereby inhibiting cellular protein synthesis. Inhibiting all the protein synthesis in the cell causes cell death. In this manner, the toxin is responsible for many of the clinical manifestations of the disease. As little as 0.1µg can cause death in guinea pigs. In 1890, von Behring and Kitasato demonstrated that sublethal doses of the toxin induced neutralizing antibodies against the toxin in horses. In turn, this antiserum passively protected the animals against death following infection. By the early 1900s, treating the toxin with heat and formalin was discovered to render it nontoxic. When injected into recipients, the treated toxin induced neutralizing antibodies. By the 1930s, many Western countries began immunization programs using this toxoid. The disease occurs mainly in temperate zones and is endemic in certain regions of the world. Humans are the known reservoir for the disease. The primary modes of dissemination are by airborne respiratory droplets, direct contact with droplets or infected skin lesions. Asymptomatic respiratory carrier states are believed to be important in perpetuating both endemic and epidemic disease (Collins and Cummings, 1986).
The toxin induced manifestations involve mainly the heart, kidneys and peripheral nerves. Cardiac enlargement due to myocarditis is common. The kidneys become edematous and develop interstitial changes. Both the motor and sensory fibers of the peripheral nerves demonstrate fatty degenerative changes and disintegration of the medullary sheaths. The anterior horn cells and posterior columns of the spinal canal can be involved and the CNS may develop signs of haemorrhage, meningitis and encephalitis. Death is mainly due to respiratory obstruction by the membrane or toxic effects in the heart or nervous system. The epidemiology of C. diphtheria infection has been changing. Increasing number of skin, pharyngeal infection in C. diphtheria has so far been made. This study describes a peculiar case of an outbreak of Corynebacterium diphtheriae infection in chicken in Nigeria have so far been made. Among 828 cultures of nontoxigenic C. diphtheriae isolated from different regions of Russia from 1994-2002, 14% carried the gene for the toxin (Burkovski, 2013). Molecular characterizations based on polymerase chain reaction (PCR) of some of these nontoxigenic strains have demonstrated that the bacteria often contain functional DtxR proteins, which could potentially produce toxin (Pitcher, 1983). No documented reports of an outbreak of Corynebacterium diphtheriae infection in chicken in Nigeria have so far been made. This study describes a peculiar case of an outbreak of C. diphtheria infection in a private poultry farm in Lagos, Nigeria.

II. Materials and Methods

a) Collection of samples

The outbreak involved 1,200, 15-week-old white leghorn broiler chickens kept in battery cages. Out of this number 163 died without any premonitory signs. As a result they did not receive any veterinary attention. At post mortem samples of the heart blood, liver, lung, and intestine were aseptically collected for possible isolation of the causative agents.

b) Processing of samples

Samples of intestine, lung, liver and heart blood were aseptically placed in sterile universal bottles containing 9ml of nutrient broth and were subsequently incubated for 24h at 37°C. After 24h incubation, the broth was plated using sterile wire loop on Tinsdale selective medium (containing Tinsdale selective agar base and Tinsdale supplement) (Oxoid, UK) and incubated at 37°C for 24h. The resultant colonies were characterized by Gram stain and biochemical tests.

c) Pathogenicity test

Colonies from Tinsdale medium were inoculated into nutrient broth and incubated for 24h at 37°C to obtain pure culture. This was used to challenge eight 15 week old white leghorn at 0.5ml each orally while two served as control. They were kept in separate cages, fed and given clean water and were observed daily for 14days.

III. Result/Discussion

The original carcasses had lesions suggestive of acute gastroenteritis, pneumonia and septicemia. The postmortem picture was characterized by haemorrhagic inflammation and oedema of the gastro-intestinal tract, fibrinous pneumonia and petechial haemorrhages of the myocardium. Pure culture of Corynebacterium diphtheriae was isolated from the heart blood, intestine, liver and lung. Positive Corynebacterium diphtheriae identification was based on the presence of gram-positive pleomorphic rods with deeply metachromatic granules in smears. On Tinsdale medium grayish black colonies were obtained. The results were interpreted according to Barrow and Feltham (1995).

The isolate proved lethal for chicken killing all the inoculated eight birds: 6 in 11days and the rest in 13 days. Necropsy findings in the infected chickens were the same as the naturally infected chickens but in addition, the epithelial wall of the proventriculus was swollen with necrotic foci and heavily infiltrated with purulent exudate. The causal agent was re-isolated from all the infected chickens.

The isolation of Corynebacterium diphtheriae, a primary pathogen of human diphtheria infection from chicken is interesting as there appears to be no previous records of its incidence among chickens as far as the authors knew. The virtually wide host range makes Corynebacterium diphtheriae infection a zoonotic disease of both veterinary and public health importance. It is likely that many more cases might be occurring in chickens and other species than are reported. It is advisable to ensure individual sanitation of farm attendants as they could be the major source of infection, and a general sanitation of the farm. There should be culling of infected birds to limit the spread of infection to the healthy ones. The use of broad spectrum antibiotics in poultry feeds may be an effective prophylactic measures against Corynebacterium diphtheriae infection.

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REFERENCES Références Referencias


