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Management of Concurrent Coccidiosis and Staphylococcosis In 14-Week-Old Isa Brown Pullets: A Case Report.

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ABSTRACT

This report investigated the outbreak of Coccidiosis in 14-week-old Isa Brown pullets. The farmer complained of high mortality in the farm and on the day of presentation picked thirty (30) dead birds. The mortality pattern for 3 days was (30, 20, and 30) which amounted to eighty (80) mortalities for the 3 days. Cumulative mortality from weeks 9 to 14 was 1200 (40%) in a flock of 3000 pullets. Clinical signs with gross pathological lesions include: pale comb, emaciated carcasses, pale shank, pale trachea, congested liver, congested and frothy lungs, pale spleen, and sloughing of the intestinal mucosa. Microscopic examination on a wet mount revealed *Eimeria* oocysts. Histological examination revealed different developmental stages of oocysts in the jejunum and the lung with congestion and widespread haemorrhages. The diagnosis of intestinal coccidiosi was made based on the mortality rate, necropsy and histopathological findings. The secondary bacterial infection was caused by Staphylococcus spp which was resistant to four antibiotics but susceptible to florum® (20% florfenicol) and Penstrep® (Penicillin +streptomycin). The apparent resistance of the *Eimeria* oocysts to Nutri-Amp 300® (300mg/g Amprolium) necessitated a change to sulfamore® (sulphadimidine 33.3%) injection for 2 days, followed by sulphamix® powder used for 5 days via drinking water which provided a better result.

Keywords: Coccidiosis, Mortality, Necropsy, Pullets, Staphylococosis, Sulphadimidine

INTRODUCTION

The poultry industry is one of the main suppliers of animal protein worldwide, contributing to both meat and eggs (Bogosavljevic-Boskovic *et al* 2010, Quiroz-Castañeda, and Dantán-González, 2015). However, the industry is faced with great pathogens challenges. Any pathogen that compromises the efficiency of a poultry

production system can pose a threat to food security worldwide (Godfray, 2010).

There are many pathogens of great importance in the poultry industry, and among these are several coccidiosis-causing species of *Eimeria* belonging to the Apicomplexa phylum. *Eimeria* spp *are* obligate intracellular parasites with special organelles within the apical complex. These organelles are necessary for the invasion of the host's intestinal cells (Cunha *et al.,* 2020). There are seven species of *Eimeria* recognized in poultry, each of them targeting a specific niche within the intestines and each with different pathogenicity characteristics (Vrba *et al.,* 2010).

The infection process begins with the ingestion of sporulated oocysts (infectious form). Depending on the species, infection can cause deficiencies in the absorption of nutrients, and poor growth rates, and in the case of the most pathogenic species, increased mortality (Chapman, 2014).

The life cycle of coccidia lasts from 7 to 9 days and includes 8 stages, made up of both sexual and asexual stages starting from oocysts to oocysts which is one generation. There are about one to three asexual (schizogony) stages and one sexual (gametogony or sporogony) stage before the completion of one life cycle. All development occurs within the epithelial cells of the intestinal mucosa. To be infective, oocysts must sporulate, which takes about 12 to 30 hours in the presence of oxygen and moisture at room temperature. Pathogenicity depends on the number of oocysts ingested by birds, the species of *Eimeria* or bird involved (Abdu, 2019). This clinical case report describes the management of an outbreak of coccidiosis.

CASE HISTORY

On the 5th of June 2023, ten (10) carcasses of 14-week-old Isa brown pullets were presented to the Poultry and Fish Clinic, the University of Jos Veterinary Teaching Hospital (UJVTH), Plateau State, Nigeria. The farmer complained of high mortality in the farm and on the day of presentation picked thirty (30) dead birds, The mortality pattern for 3 days was (30, 20, and 30) which amounted to eighty (80) dead birds within 3 days. The last vaccine administered was NDVK (Inactivated ND Komarov vaccine) on 23/4/2023 when birds were 12 weeks old. The birds were maintained on commercial poultry feed and borehole water which was sanitized with Sawke® (a 10% iodine solution) at 1ml/2/litres of water. The birds were sourced from a commercial hatchery in Jos.

On the day of presentation, the birds had been on Amprolium 300® and Alfaceryl®. The initial flock size was 3000 but on the day of presentation, there were 1,800 birds remaining in the flock.

CLINICAL SIGNS

Farm visit revealed the following clinical signs: decreased feed consumption, ruffled feathers, weakness, depression, loss of weight, paleness of comb and shanks, stunted growth and somnolence.

Diagnostic Tests

Necropsy

Postmortem examination was conducted on the birds brought to the Poultry and Fish Clinic of the Veterinary Teaching Hospital on the 5th of June 2023 and gross lesions observed were: Pale combs,nasal discharge, soiled vent, emaciated carcasses, pale shanks,

loss of breast muscle with prominent keel bone , pale tracheas , congested livers , congested and frothy lungs , petechial haemorrhages on the proventricular mucosa , pale spleen , enlarged and congested kidneys with slightly distended ureters and sloughing of the intestinal mucosae . Microscopic examination (x40 objective lens) of intestinal mucosal scrappings on a wet mount revealed *Eimeria* oocysts. Affected organs were harvested and divided into two parts, one for microbial analysis and the other preserved in 10% formalin for histopathologic investigation.

Microbial analysis

Organs harvested were lungs, liver, spleen, kidney and intestine, and under sterile condition, inocula from the liver, spleen, kidneys, and lungs were streaked on blood agar, MacConkey agar and Mannitol salt agar. Inoculated agars were incubated aerobically at 37°C for 24 hours. Pure colonies of bacteria on blood agar were identified using routine standard bacteriological methods as described by Olutiola *et al*., (1991). With the aid of a sterile inoculating loop, a few colonies of the isolated bacterium were suspended in sterile normal saline and the suspension was allowed to be turbid before immersing a sterile swab soaked with the broth suspension of bacterium. This was used in seeding the surface of Mueller hinton agar. The antibiotic discs were placed on this before incubating for antibiotic susceptibility test.

Histopathologic Investigation

Harvested organs such as intestine and lungs were preserved in 10% formalin and subjected to dehydration under various concentrations of ethanol (70%, 80%, 90%, and 100%), cleared in xylene, and embedded in paraffin blocks before sectioning on the microtome and staining with hematoxylin and eosin were done as described by Akpavie, (2014).

RESULTS

Microbial analysis of tissue inocula streaked on Mannitol salts agar yielded yellow colonies of bacterial growth. On MacConkey agar, there were a pale pink colony of bacterial growth. Gram staining and other biochemical tests conducted on the isolate as shown in (table 1), confirmed the organism to be *Staphylococcus spp.* The antibiotic susceptibility test conducted showed that the isolate was susceptible to Floricol® (Florfenicol) and Penstrep® (Penicillin + Streptomycin), intermediate to enrofloxacin, colistin, gentamicin, and resistant to oxytetracycline, streptomycin, furaltadone, tylosin and alfaceryl® (Erythromycin, Furaltadone, Oxytetracycline, Streptomycin, and Neomycin).

TABLE I. Antimicrobial susceptibility pattern of Staphylococcus spp isolated from clinical case of coccidiosis in 14-week-old chickens.

Susceptible	Intermediate	Resistant
Floricol® (Florfenicol) Penstrept®	Enrofloxacin Colistin Gentamicin	Oxytetracycline Streptomycin Furaltadone Tylosin Alfaceryl®

TABLE II. Biochemical tests for bacterial identification of Staphylococcus spp

TESTS	**RESULTS**
Gram reaction	Gram positive
Shape	Cocci in clusters
Catalase	+
Oxidase	-
Indole	-
Lactose	+
Sucrose	+
Growth on MacConkey agar	Pale pink colonies
Hemolysis (on blood agar)	-
Citrate utilization (on SCA)	-
Growth on Mannitol salt Agar	Yellow bacterial colonies
Coagulase	……

TABLE III: Medication and mortality pattern

DATE	MEDICATION	MORTALITY PATTERN
4TH JUNE 2023	Nutri-Amp 300® + Alfaceryl®	30
5TH JUNE 2023	Nutri-Amp 300® + Alfaceryl®	30
6TH JUNE 2023	Nutri-Amp 300® + Alfaceryl®	15
7TH JUNE 2023	Nutri-Amp 300® + Alfaceryl®	20
8TH JUNE 2023	Only water	45*
9TH JUNE 2023	Only water	43
10TH JUNE 2023	Sulphadimidine injection	40

11TH JUNE 2023	Sulphadimidine injection	30
12TH JUNE 2023	Florum® + Sulphamix®	33
13TH JUNE 2023	Florum® + Sulphamix®	12
14TH JUNE 2023	Florum® + Sulphamix®	7
15TH JUNE 2023	Florum® + Sulphamix®	6
16TH JUNE 2023	Florum® + Sulphamix®	3

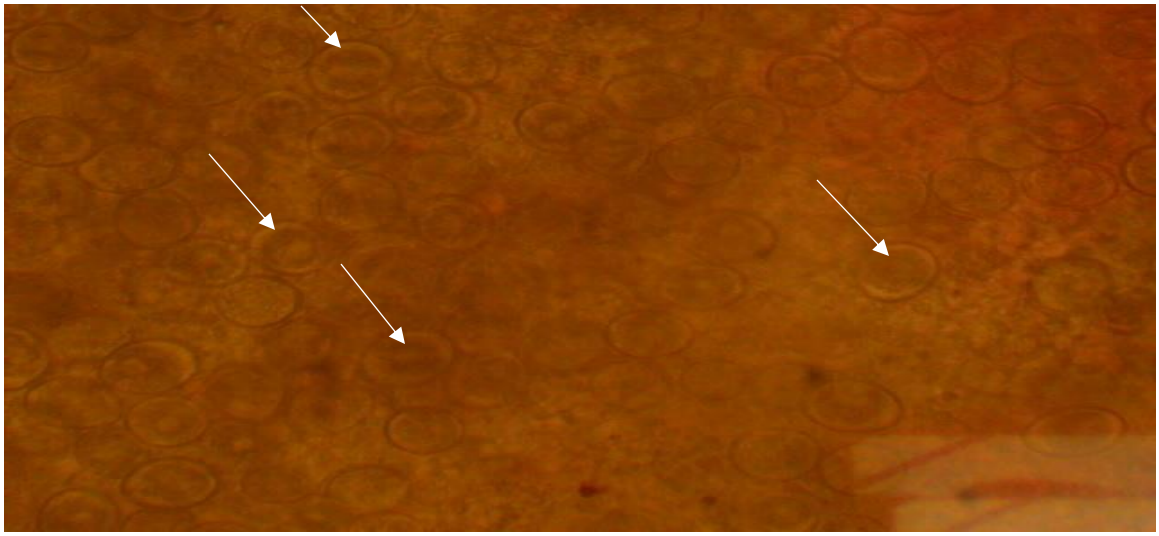

Plate 1 shows massive presence of *Eimeria* oocysts under the microscope using x400 mag. This indicates coccidiosis.

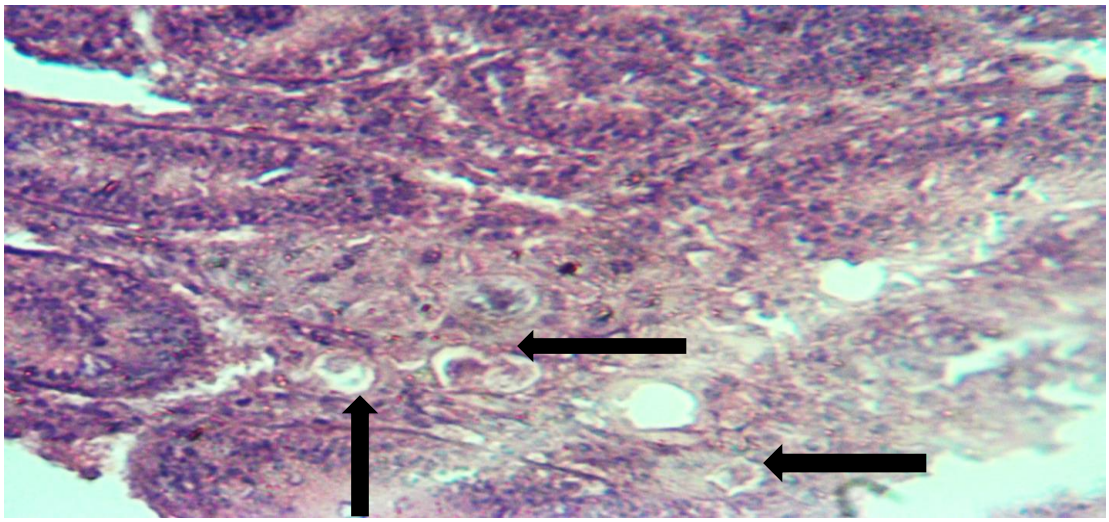

Plate 2: Photomicrograph of jejunum of Isa Brown pullets showing oocyst at different stages of development. H&E ×400 (black arrow).

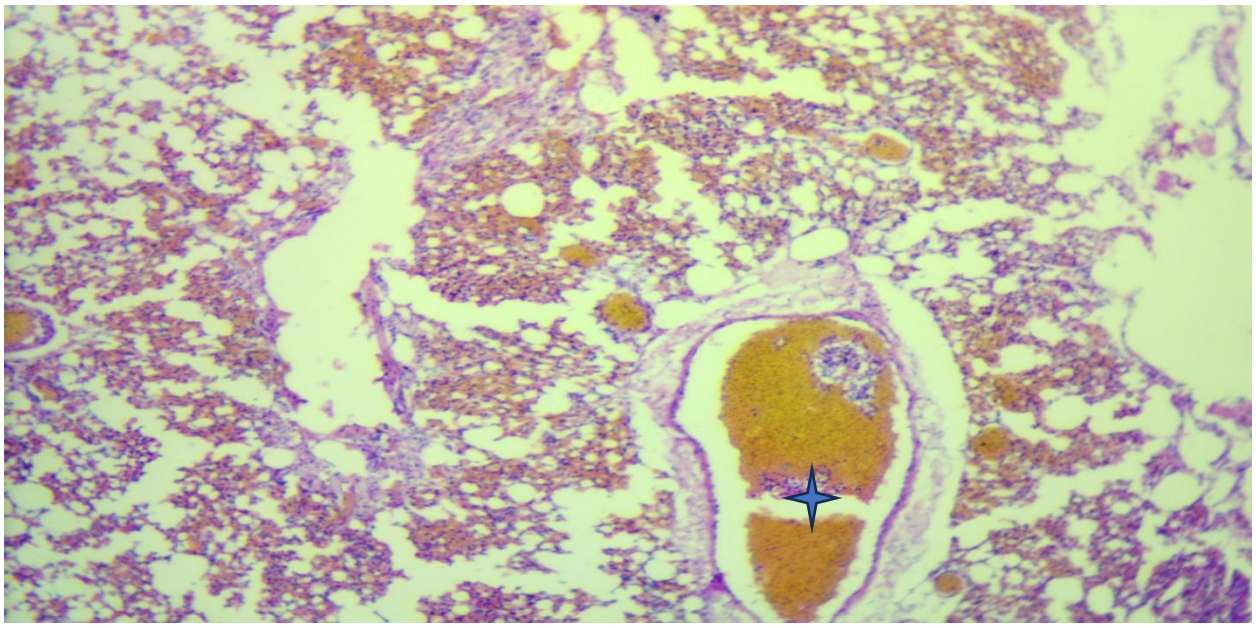

Plate 3: Photomicrograph of lung showing congestion (asterisk) and widespread haemorrhages x 40 H&E

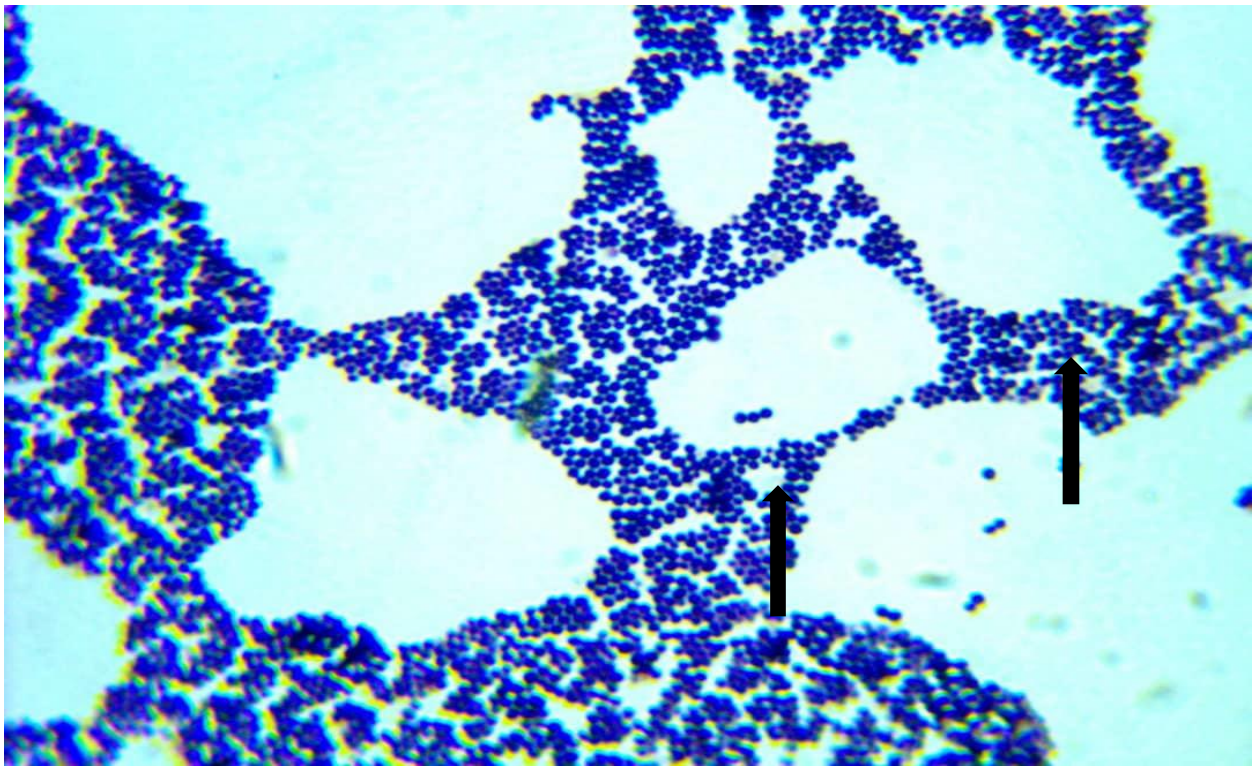

Plate 4: Photomicrograph of Gram-positive cocci in clusters (arrows) x 1000 Mag

Management of condition and discussion

From the laboratory findings, coccidiosis and Staphylococcosis were diagnosed. The *Eimeria* pathogen was, however, resistant to Nutri-Amp 300® + alfaceryl®, which were earlier administered through drinking water by the farmer before visiting the University of Jos Veterinary Teaching Hospital. Alfaceryl® which contains the following blend of antibiotics (Erythromycin, Furaltadone, Oxytetracycline, Streptomycin, and Neomycin) was administered to the birds by the farmer for five (5) days, with no positive response, therefore, susceptibility disc was prepared to further confirm why birds were not responding to this blend of antibiotics and antibiotic susceptibility testing also showed that the organism was resistant to Alfaceryl®. However, the mortality pattern started declining when Sulfamore ® (sulphadimidine 33.3%) injection was administered (0.3ml/bird) intramuscularly for 2 consecutive days and was followed up with florum® (20% florfenicol) at 1ml/2L of drinking water for 5 days plus sulphamix® at. 1 g/L of drinking water for 5 days. The mortality declined from 40 dead birds per day on day one of the sulfamore® injection to 3 dead birds on day 5 of Sulphamix® administration plus Florum® (20% Florfenicol). It is also paramount to say that while managing the coccidiosis, the complicating bacterial infection was also been controlled even as the bacterial isolate was found to be resistant to 5 antibiotics but susceptible to 2 (table I). Disc diffusion technique was used in determining the antibiotic to which the bacterial pathogen was most susceptible.

The poultry litter was changed after treatment to break the cycle of infection because faecal oocysts shedding was apparent and had been reported (Arabkhazaeli *et al*., 2013; Abdelrahman *et al*., 2014; Lan *et al*., 2017), however, oocysts shed per gram of faeces by birds taking anticoccidial drugs was significantly less than in birds not on anticoccidial drugs. This implies that drug administration may reduce transmission of oocysts.

Coccidiosis can be complicated with secondary bacterial infection as in this case that was complicated with *Staphylococcus spp*. This could also be responsible for the cause of the high mortality recorded on the farm. Ojimelukwe *et al* (2018), reported that, regardless of the prophylactic treatment of birds with anticoccidial drugs, coccidiosis can still reduce production efficiency in infected birds. Since the parasite damages the lining of the intestine, it is expected to impair the process of digestion and absorption of nutrients, hence poor growth and reduced egg production in the affected flocks. This highlights the economic importance of chicken coccidiosis.

Resistance to anticoccidial drugs may be a result of the high preponderance of oocysts (Chapman *et al*., 2013), invasiveness of the *Eimeria* spp involved (Luu *et al*., 2013; Prakashbabu *et al*., 2017), or co-occurrence of multiple *Eimeria* spp in a given site of infection (Ojimelukwe *et al*., 2018). *Eimeria tenella* and *Eimeria necatrix* are considered highly pathogenic species of *Eimeria* spp (Blake *et al*., 2015; Silva *et al.*, 2022). The circulating of such pathogenic species of *Eimeria* can contribute to high morbidity and mortality in young chickens particularly when conditions of poor nutrition, poor sanitation and hygiene, and co-infection with other pathogens persist (Williams *et al*., 2009). To avoid colossal losses, it is important for poultry farmers to abstain from abuse of anticoccidials and antibacterials especially when they depend only on clinical signs in diagnosis of disease conditions. This will

further prevent the issue of antimicrobial resistance which is already a global concern.

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