CASE REPORT OF MISDIAGNOSIS OF AVIAN COLIBACILLOSIS IN LAYING BIRDS

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ABSTRACT

Two freshly dead 27 weeks old Issa brown laying birds from a population of about 3000 birds with history of blindness, greenish-whitish diarrhoea, symptomatic diagnosis of coccidiosis, treatment failure, reduced egg lay and increased mortality was presented for postmortem examination and diagnosis. Postmortem examination revealed air sacculitis, egg peritonitis, misshapen ova with injected blood vessels and petechial haemorrhages in the epicardium, coronary, abdominal and intestinal fats and cecal tonsils. Diagnosis of colibacillosis was based on isolation and identification of pure cultures of Escherichia coli from cultured samples following standard procedure. Antibiotic sensitivity result showed that the isolate was resistant to ampicillin, nalidixic acid and gentamicin, but susceptible to ciprofloxacin, enrofloxacin and sulphamethoxazole-trimethoprim. Treatment was achieved by oral administration of enrofloxacin at the dose of 0.1mg/ml for 7 days with daily water renewal. Egg lay increased from 24% to 70% five days into treatment. This report has shown that symptomatic diagnosis of colibacillosis in layers results to misdiagnosis, mistreatment, treatment failure and great economic loss.

Keywords: *Escherichia coli*, Avian colibacillosis, Misdiagnosis, Multidrug resistance, Laying poultry birds, Issa brown

INTRODUCTION

Escherichia coli, a Gram-negative bacterium causes colibacillosis an economically important disease of poultry worldwide (Quinn and Markey, 2003). In adult birds, the clinical course of colibacillosis depends on the virulence of the associated *E. coli* strain (Barnes and Gross, 1997). The disease varies from a preacute and rapidly fatal septicemia (colisepticaemia) with little or no clinical signs, to a subacute or mild disease, or an acute form with signs including sudden onset of dyspnoea, depression and prostration ending in death. If the bird survives and not treated, it may progress to chronic infection characterized by a gradual onset of anorexia, unthriftiness, diarrhoea and progressive emaciation that is described by farmers as the sick bird syndrome (Charlton, 2006; Olarinmonye *et al*., 2013). In layers, drop in egg production is not uncommon (Quinn and Markey, 2003; Charlton, 2006). Postmortem lesions of avian colibacillosis include facial cellulitis (swollen head syndrome), air sacculitis, peritonitis, pericarditis, synovitis, coligranuloma or Hjarre's disease, and in layers salpingitis, omphalitis, misshapen and pedunculated eggs and egg peritonitis (Barnes and Gross, 1997; Charlton, 2006).

However, these clinical signs and postmortem lesions are not pathognomonic (specific) for colibacillosis and are manifested by an infected bird in several other diseases that affect layers such as Newcastle disease, pasteurellosis (fowl cholera) and salmonellosis (Quinn and Markey, 2003; Charlton, 2006; Hassan *et al*., 2010). In coligranuloma, the nodular lesions observed on visceral organs (such as the liver and heart) also occur in cases of aspergillosis, tuberculosis and salmonellosis (Charlton, 2006; Hassan *et al*., 2010). Because of this non-specificity, symptomatic diagnosis may result to misdiagnosis and mistreatment. Therefore, diagnosis of avian colibacillosis is based on isolation of the incriminated organism from faecal sample or postmortem tissues (such as the heart blood, liver and intestine) (Charlton, 2006).

In Nigeria, treatment of food-producing animals without conducting sensitivity test is a common practice by non-veterinarians. And because there are no strict regulations guiding the use of antibiotics in food-producing animals in Nigeria, farmers often use different antibiotics as growth-promoters/prophylaxis in brooding their birds (Mamza *et al*., 2010; Olarinmonye *et al*., 2013). This scenario has led to selection pressure and development of multidrug resistance among *E. coli* isolates. This in turn have resulted to the increased virulence of *E. coli* and consequently to high rate of treatment failures and heavy economic losses often experienced by farmers (Olarinmonye *et al*., 2013). Therefore, reports on clinical colibacillosis in which antimicrobial resistance profile of the isolates are reported would be helpful in empirical treatment of the disease.

Moreover, in Nigeria most studies in which *E. coli* was isolated from layers were reported in southwestern (Raji *et al*., 2007; Ogunleye *et al*., 2008; Olarinmonye *et al*., 2013) and northern (Mamza *et al*., 2010) regions of the country. In the southeastern Nigeria, the only case of clinical colibacillosis in layers (Chah and Okwor, 2003) was reported in Nsukka (a University town) in Enugu State. None has been reported in Enugu town (the capital of Enugu State) in Southeast, Nigeria. Therefore, in this report we describe a case of colibacillosis in layers symptomatically misdiagnosed as coccidiosis at a farm located in Enugu Southeast, Nigeria.

MATERIALS AND METHODS

Two dead 27 weeks old Issa brown layers from a farm in Enugu Southeast, Nigeria were presented to the Department of Veterinary Pathology and Microbiology, University of Nigeria, Nsukka, for postmortem examination and diagnosis. Anamnesis revealed that a total of 3000 birds procured at day-old from a commercial hatchery in Southwestern Nigeria were randomly divided into 5 separate groups of 600 birds each, and kept in pens demarcated with wire gauze in a deep litter house in which they were brooded. Keproceryl (oxytetracycline) and Admacine (ampicillin and aluminum magnesium silicate) were administered during brooding. The birds were given Newcastle disease, infectious bursal disease (Gumboro) and fowl typhoid vaccinations following the recommended regimen.

At week 22, some of the birds in all the pens were either blinded in one or both eyes, while some others were passing watery faeces that were greenish-whitish. Mortalities occurred though initially low. The affected birds were isolated from the apparently healthy ones. Our client revealed that a poultry consultant in the farm suspected avian coccidiosis since the litter had never been replaced and subsequently administered Interfrimazole (toltrazuril), a coccidiostat, and Doxytylovet (doxycycline hydrochloride and tylosin tatarate) an antibiotic to the birds in drinking water. Few days thereafter, the feed intake of the birds in all the pens reduced and the total percentage egg lay in the farm reduced from 80% to as low as 24%. The apparently healthy layers were also placed on Doxytylovet. Our client reported that in spite of the drugs given, egg production did not improve and mortalities persisted (Table 1). Postmortem examination was done following standard procedure (Charlton, 2006). Samples for microbiological examination which included the liver, spleen and heart were collected from both birds using sterile Petri dishes.

Table 1: Number of layers that died in the farm after treatment

Week	Mortality
23	7
24	21
25	31
26	47
Total	106

They were processed in the Microbiology Laboratory, Department of Veterinary Pathology and Microbiology, University of Nigeria, Nsukka within 20 minutes of collection. The liver, spleen and heart blood were cultured on nutrient agar (NA) (Oxoid) and Mac Conkey agar (MCA) (Oxoid), incubated at 37°C for 24 hours aerobically. Then, colonies from both culture media were Gram stained following standard procedure. They were also sub-cultured on eosin methylene blue (EMB) agar (Oxoid), and incubated at 37°C for 24 hours. Preliminary identification of the isolate was done using morphological (colonial and microscopic) characteristics, while the final identification was done by conducting biochemical tests such as triple sugar iron agar, indole and Simmon's citrate following standard methods (Quinn and Markey, 2003).

Antibiotic susceptibility test of the isolate was done following disc diffusion procedure (Bauer *et al*., 1966). Nine antibiotic discs (Oxoid) consisting of 4 antibacterial classes were used. They include: ampicillin (10µg), amoxicillin/clavulanic acid (20/10µg), streptomycin (10µg), enrofloxacin (5ug), ciprofloxacin (5µg), orfloxacin (5µg), nalidixic acid (30µg), suphamethoxazole-trimethoprim (23.75/1.25µg) and gentamicin (10µg). For each antibiotic disc and for each isolate, the test was performed in duplicate and the mean inhibitory zone diameter (IZD) calculated. The isolates were classified as resistant, intermediate-resistant or susceptible to each of the tested antibiotic following the Clinical and Laboratory Standards Institute (CLSI) (2012) criteria for aerobic isolates. The antibiotic used for treatment was selected based on the result of the sensitivity test.

RESULTS

Postmortem examination of the presented carcasses showed vents pasted with whitish faeces, cloudy thoracic and abdominal air sacs, petechial heamorrhages in the abdominal fats, epicardium, coronary fats, proventricular mucosa and cecal tonsils of both carcasses. The peritoneum was thickened and coated with yolk material in both carcasses (Figure 1).

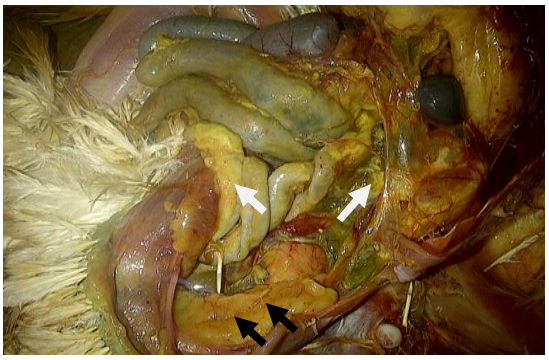

Figure 1: Flakes of yolk on the peritoneum and serosal surface of the intestines (white arrow) and petechial haemorrhages on the abdominal fats (black arrow)

The ova were misshapen and contained caseous materials in one, while the ova of the other bird were pedunculated with injected blood vessels (Figure 2). Based on these postmortem findings, the postmortem diagnoses were avian colibacillosis, pasteurellosis (fowl cholera) and salmonellosis.

From all the cultured samples, growth on NA were pure cultures of discrete, medium-sized, creamy-whitish, smooth, flat and circular colonies. On MCA, they were pure cultures of discrete, circular, dry, smooth and pinkish colonies indicating a lactose fermenting organism. On EMB, colonies from both MCA and NA showed greenish metallic sheen appearance incriminating *E. coli*. Microscopically, the isolates from both MCA and NA were Gram-negative medium-sized rods arranged singly or in pairs indicating they were the same isolate. Biochemical test showed that all the isolates fermented lactose and glucose, and produced gas and indole. Based on these characteristics, the isolates were identified as *E. coli* (Quinn and Markey, 2003).

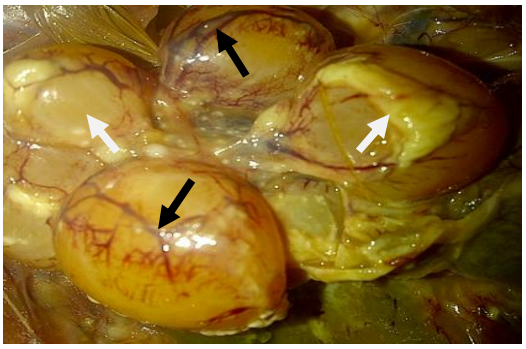

Figure 2: Pedunculated ovaries with flakes of yolk on the surface (white arrow) and severely injected blood vessels (black arrow)

The sensitivity result showed that isolates from both culture media were resistant to ampicillin, nalidixic acid and gentamicin, intermediately-resistant to streptomycin, amoxicillin/clavulanic acid and orfloxacin, but susceptible to ciprofloxacin, enrofloxacin and sulphamethoxazole-trimethoprim. Because of this similarity in biochemical characteristics and sensitivity results, the isolates were regarded as one strain. Based on the observed sensitivity results, enrofloxacin was recommended, to be administered at the dose of 0.1mg/ml in drinking water with daily water renewal for 5 – 7 days. Five days into treatment, egg production reportedly increased from 24.8% to 70% and the mortalities ceased.

DISCUSSION

The fact that pure cultures of *E. coli* were isolated from all the cultured samples established the incidence of colibacillosis in the farm (Quinn and Markey, 2003; Nolan, 2013). The blindness reported by the client is likely due to "swollen head syndrome" (cellulitis) caused by the *E. coli* isolate (Nolan, 2013; Olarinmonye *et al*., 2013). It may as well be due to sinusitis caused by respiratory pathogens such as Infectious bronchitis virus, *Haemophilus paragallinarum* or *Mycoplasma gallisepticum* if these organisms acted as primary agents destroying the epithelial lining (deciliation) of the upper respiratory tract of the birds (Ewers *et al*., 2003), thereby enabling secondary invasion by the *E. coli* isolate from the contaminated environment (Ewers *et al*., 2003; Nolan, 2013). But concurrent infection by these respiratory pathogens was ruled out because respiratory symptoms were not reported in the history. Also no postmortem lesion indicated upper respiratory tract infection (Charlton, 2006).

Clinical signs observed in this present case have also been reported in Newcastle disease, salmonellosis and pasteurellosis (Charlton, 2006; Hassan *et al*., 2010; Miller, 2014). Gross lesions observed including isolation of *E. coli* from visceral organs suggest there was colisepticaemia (Charlton, 2006; Nolan, 2013). But the gross lesions are not pathognomonic (specific) for colibacillosis; they have also been reported in salmonellosis and pasteurellosis (fowl cholera) (Quinn and Markey, 2003; Charlton, 2006). Petechial haemorrhages on the proventricular mucosa enabled us to eliminate ND in which haemorrhages would have occurred on the tip of the proventricular glands (Charlton, 2006). Mortality rate of ND is usually as high as 70 – 100% in few days (Jibril and Umoh, 2014), but in the present case, mortality was 6.6% in 4 weeks. Hence, the observed petechial haemorrhages on the cecal tonsils may likely be due to a ND vaccine recently given to the birds (Olarinmonye *et al*., 2013). Although the aetiology of fowl cholera (pasteurellosis) – *Pasteurella multocida* type A grows on MCA, it produces a subtle characteristic odour and microscopically it exhibits a bipolar staining characteristic (Quinn and Markey, 2003) which were not observed in this case. Salmonellosis was eliminated because *Salmonella* species are non-lactose-fermenting (i.e. they yield tan coloured colonies on MCA) and microscopically they appear as Gram-negative short rods (Quinn and Markey, 2003). It was a misdiagnosis for coccidiosis to be suspected in the first instance by the poultry consultant. This is because coccidiosis is characterized by passage of frank blood in the faeces, and cecal cores (hemorrhagic necrotic debris) in the small intestines and ceca (McDougald, 2012). But none of these were observed from the history and postmortem result. No wonder the condition exacerbated in spite of administration of Interfrimazole.

Resistance of the isolate to three classes of antibiotics tested (ampicillin – β-lactam, nalidixic acid - quinolone and gentamicin - aminoglycoside), indicated that it was a multidrug resistant *E. coli* strain. This multidrug resistance may be due to exposure to different antibiotics since they were brooded with Keproceryl and Admacine. And this multiple resistance may have led to exacerbation of the disease despite administration of Doxytylovet by the poultry consultant. The use of Keproceryl at the brooding stage may have resulted to selection pressure against tetracycline which doxycycline (one of the component of Doxytylovet) belongs to and hence the treatment failure.

The layers could have contracted the organism by inhaling contaminated dust from the litter, since the litter has never been replaced (from the history) and there could have been ammonia buildup in the pens (Barnes and Gross, 1997). Ammonia induces immunosuppression in birds allowing for opportunistic *E. coli* infection (Charlton, 2006). All the groups were affected since they were only demarcated with wire gauze.

However, the increased egg lay from 24% to 70% and ceasation of mortalities following therapy showed that enrofloxacin (chosen based on sensitivity test) was effective. This report has shown that symptomatic diagnosis of colibacillosis is difficult and would likely result in misdiagnosis, mistreatment and treatment failure. There is therefore the need for poultry farmers in Nigeria to consult qualified veterinarians for laboratory-based diagnosis and treatment. This report is also a call for stricter policies guiding the use of drugs in food producing animals in Nigeria.

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