Asian Journal of Poultry Science

ISSN 1819-3609

Academic Journals Inc.

www.academicjournals.com

Asian Journal of Poultry Science 4 (4): 173-181, 2010
ISSN 1819-3609

Outbreak of Colibacillosis among Broiler and Layer Flocks in Intensive and Semi intensive Poultry Farms in Kassala State, Eastern Sudan

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Abstract: The study aimed to provide documented information on the epidemiological and economical effects of the disease to assist in disease control policies and planning research priorities in the region. The outbreak of colibacillosis was reported among broiler and layer chicks reared in closed and semi closed system In Kassala State, Eastern Sudan. Mortality rate of the disease was 6.8% in the broiler flocks and 1.9% in the layers ones. Diagnosis of the disease was made on the basis of the case history, clinical signs, postmortem findings and laboratory examinations. *Escherichia coli* (*E. coli*) isolates were obtained from infected organs of broiler and layer flocks. Isolation and identification of *E. coli* were achieved by using biochemical diagnostic test kits. The isolates were highly resistant to most tested antibiotics. The cost of losses in broilers and layer chicks due to the outbreak was recorded. Factors which associated with the disease were discussed and some recommendations were outlined to avoid such outbreak.

Key words: *Escherichia coli*, strains, chickens, closed, industry

INTRODUCTION

Colibacillosis in chickens refers to any local or systemic infection caused entirely or partly by *E. coli* strains (Barnes *et al*., 2003). *Escherichia coli*, strains causing systemic disease in poultry (avian colibacillosis) are termed avian pathogenic *E. coli* (APEC) Ewers *et al*. (2003).

Colibacillosis is one of the main causes of economic losses in the poultry industry worldwide (Yogaratnam, 1995; Ewers *et al*., 2003). Traditionally, it was mostly associated with losses in broilers but, recently the incidence of the disease in layer hens has been substantially increased in many European countries (Zanella *et al*., 2000; Vanderkerchove *et al*., 2004c; Jordan *et al*., 2005).

Escherichia coli, has been associated with a variety of diseases in poultry such as pericarditis, perihepatitis, airsacculitis, peritonitis, salpingitis, panophthalmitis, omphalitis, cellulites, colispticemia, coligranuloma and swollen-head syndrome (Saif *et al*., 2003).

Strains of *E. coli* predominate among the aerobic commensal flora in the gut of humans and animals. These bacteria are widespread and present wherever there is faecal

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contamination, causing pollution of water sources, drinking water and food. The species encompasses a variety of strains, which may be purely commensal or possess combinations of pathogenic mechanisms that enable them to cause disease in man and other animals (Greenwood *et al*., 2002).

Escherichia coli may be sensitive to many antibiotics however; isolates from poultry are frequently resistant to one or more antibiotics, especially if they have been widely used in poultry industry over along period such as Tetracyclines (Allan *et al*., 1993; Watts *et al*., 1993; Blanco *et al*., 1997). Resistance to two or more classes of antibiotics is now commonplace in both veterinary (Gonzalez and Blanco, 1989; Harnett and Gyles, 1984; Irwin *et al*., 1989) and human medicine (Dennesen *et al*., 1998).

The aim of this study was carried out to investigate the outbreak of colibacillosis in poultry farms in Kassala State. The isolates were analyzed to determine their susceptibility to antimicrobial agents in order to aid in control of the disease in future. The present study appears to be the first documentation of outbreak of colibacillosis in closed system in the Eastern Sudan.

MATERIALS AND METHODS

Case History

During the dry summer season, ten affected chickens, 4 broilers Ross 4 weeks old and 6 layers Hisex 32 weeks old were brought to Kassala Veterinary Research Laboratory (KVRL) for investigation. The breeds were imported from poultry companies in Khartoum as day-old chicks. The flocks were vaccinated against Gumboro, infectious bronchitis, Mareks and Newcastle diseases. The outbreak reported in two poultry farms containing 26000 layers and 17000 broilers. The broilers were reared in semi-intensive poultry farm, while the layers were in intensive ones. The distance between these farms is about 0.5 km. Temperature in the poultry houses was 32°C and the flocks were suffering from heat stress. A sudden increase in daily mortality was recorded in these farms. Dead birds were removed daily; however, personal hygiene measures of the workers were not applied.

The slaughter house of broilers is about 25 m from the poultry houses. Moreover, rodents and flies were seen in these farms.

Treatment with Oxytetracycline, Getamicin, Tylosine and vitamins failed to reduce losses. The system of water sterilization (ultraviolet lamps) was completely stopped, but chlorine added to the drinking water was reduced mortality rate of the disease. Groundnuts oil has been added by 1-5% to the ration of the broiler and layer flocks as growth promoters.

Outbreak

The disease outbreaks occurred in broiler and layer flocks in intensive and semi-intensive poultry farms in Kassala State, Eastern Sudan. The farms are about 4 km from Kassala town centre. Outbreak occurred during the dry summer season in May, 2008.

Diagnosis of the Disease

The disease was diagnosed on the basis of the history, clinical signs, postmortem examination and isolation and identification of the causal agents. Information on management, biosecurity measures and housing conditions was recorded.

Sample Collection

Ten affected birds of both broiler and layer flocks at varying stages of the disease were submitted to the KVRL for examination. The affected organs of the necropsied birds were processed for bacteriological and Virological examination. Specimens for bacteriological

examination were collected aseptically from lungs, livers and ovaries. Small fragments from kidneys and lungs of the same birds carcasses were taken, processed and then sent to CVRL for Virological examination of infectious bronchitis and Newcastle Disease Virus (NDV).

Virological examination: Isolation of the causative agent was performed as described by OIE (2004).Tissue samples were processed by grinding each sample in PBS to make a 20% tissue suspension, this was clarified by centrifugation at 3000 rpm for 10 min. The supernatant was then treated with Penicillin , Streptomycin , Getamicin and Mycostatin.

0.2 mL from each sample was inoculated via the allantoic sac into 10 day old embryonated chicken eggs . Eggs were incubated at 37°C for 7 days and candled daily , all death occurring within 24 h post inoculation were regarded as nonspecific and discarded. The allantoic fluid from each egg were harvested and tested for Haemagglutination Activity (HA), fluid with negative reaction were passed two times more.

Bacterial Isolation

Specimens were directly inoculated on Blood and MacConkey Agar media and incubated aerobically at 37°C for 24 h, the colonies were examined with the naked eye for their cultural characteristics, morphological properties and any changes in the media. Then the organism was stained with Gram's method (Cheesebrough, 2000). Identification of the isolates was performed according to methods described by Barrow and Feltham (1993).

Identification of the Isolates

The isolates were identified by using standard biochemical test kits (KB001 HiIMViC, India) including, Indole, Methyl Red, VogesProskauers, Citrate Utilization and carbohydrates fermentations (Glucose, Adonitol, Arabinose, Lactose, Sorbitol, Manitol, Rhamnose and Sucrose).

Antimicrobial Susceptibility Test

The disk diffusion method was used to test susceptibility of the isolates to: Ampicillin/Sulbactam (AS_{20}), Co-Trimoxazole (BA_{25}), Cefotaxime (CF_{30}), Piperacillin/Tazobactam ($TZP_{100/10}$), Chloramphenicol (CH_{30}), Ciprofloxacin (CP_5), Ceftizoxime (CI_{30}), Tetracycline (TE_{30}) Ofloxacin (OF_5), Gentamicin (GM_{10}), Amikacin (AK_{30}) and Pefloxacin (PF_5). Concentration of antibiotics was in micrograms. Interpretation followed criteria recommended by National Committee for Clinical Laboratory Standards (2001).

RESULTS

Mortality Rate

Mortality rate of the disease was 6.8% in the broilers flocks and 1.9% in the layer ones. Table 1 shows that a total of 1655 broilers and layers died during the period of outbreak (28 days) with mortality rate of 3.8%. The total loss in U$ dollars was 5281.5. Figure 1 shows that during 28 days of the disease, the maximum mortality rate in broilers in the period between 13-19 days while, in layers it was in 11-14 days. However, the number of broiler chickens died in day 18 was > 160. For layers in day 13 more than 40 chickens died.

Clinical Manifestations

The clinical signs of the affected birds were depression, respiratory distress, reduced food consumption, loss of weigh, weakness, ruffling of feathers, decrease in egg production and yellowish diarrhea or sometimes vents pasted with faeces. Conjunctivitis was also observed as sporadic cases of layer chicks.

Table 1: The cost of losses in broiler and layer chicks due to the outbreak of colibacillosis in Kassala State

Type of flocks	No. of chicks	No. of chicks died	Mortality rate (%)	No. of chicks eradicated	Cost of one day old chick (U$)	The total cost in U$
Broiler	17000	1164	6.8	1657	1.58	4457.2
Layer	26000	491	1.9	98	1.40	824.6
Total	43000	1655	3.8	1755	2.98	5281.8

This did not include the cost of transportation, ration, medication, expenses of labor, rent of poultry houses (electricity and water) and other miscellaneous

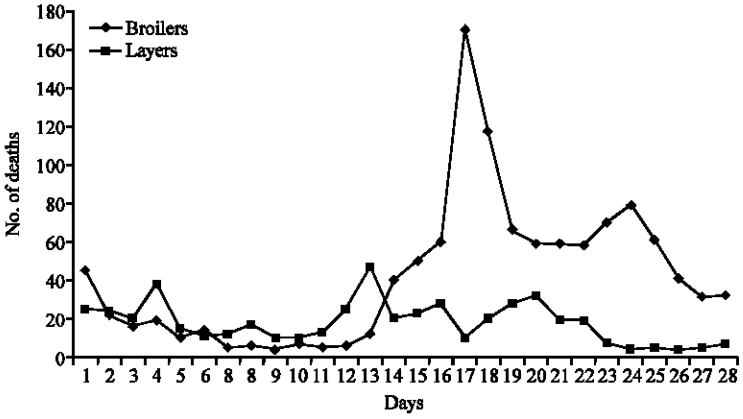

Fig. 1: The pattern of colibacillosis disease among broiler and layer flocks during the period of outbreaks in Kassala State

Postmortem Findings

At necropsy, the birds showed characteristics lesions in the livers, intestines and gallbladders, these lesions almost in every case. The livers were enlarged, pale, friable and sometimes necrotic. The gallbladders were distended with bile and petechial hemorrhages were seen in the intestines. Also, pericarditis was the most prominent lesion recorded in some cases of broilers and layer chicks. Pneumonia and airsaculitis were the most striking lesions observed in the broiler birds while, salpingitis was the more advanced pathological changes noted in the layer ones.

Virus Isolation

All samples of broiler and layer hens were negative for infectious bronchitis and Newcastle disease virus.

Isolation and Identification of the Bacteria

Cultures from lungs of a broiler chick and ovaries of a layer hen onto Blood Agar plates yielded pure growth of one type of colonies, Gram stained smears from these colonies showed uniform Gram negative bacilli. Then subcultures of the same colonies on MacConkey Agar media revealed lactose fermenting microorganisms. All isolates were identified biochemically as *E. coli* using standard biochemical test kits.

Antibiotic Sensitivity Test

The isolates were sensitive to Amikacin, Gentamicin, Co-Trimoxazole and Ofloxacin, but they were resistant to Ciprofloxacin, Chloramphenicol, Piperacillin/Tazobactam, Ceftizoxime Cefotaxime, Pefloxacin, Tetracycline and Ampicillin/Sulbactam. The isolates were completely resistant to Tetracycline and Ampicillin/Sulbactam, where no inhibitory zones were observed.

DISCUSSION

Poultry production plays an important role in providing valuable proteins, poverty alleviation and economic development. Kassala State consists of 42 semi intensive poultry farms and 5 intensive ones, most of these farms are managed by the private sector. Despite great potential and opportunities, poultry production is threatened by many disease outbreaks, these diseases are the major constrains for developing the poultry industry (Ewers *et al*., 2003). To establish poultry farms, the incidence of the disease should be considered for prevention and control.

Colibacillosis was reported by many researchers in different countries (Omer *et al*., 2008; Vandekerchove *et al*., 2004c; Yang *et al*., 2004; Saenz *et al*., 2003; White *et al*., 2000). Therefore, the disease is considered one of the principal causes of mortality and morbidity in poultry, responsible for high economic losses to poultry industry world wide (Ewers *et al*., 2003; Gomis *et al*., 2001; Allan *et al*., 1993).

In the present study outbreak of colibacillosis was encountered in both closed and semi closed system. The occurrence of colibacillosis in closed system, has never been reported in Kassala State, therefore, this report is to record an outbreak of colibacillosis in intensive system in Kassala State, Eastern Sudan. The present study revealed 6.8% mortality rate of colibacillosis in broiler chicks and 1.9% in the layer ones, these findings were in agreement with Zanella *et al.* (2000), who reported 5-10% mortality due to *E. coli* infections. However, the findings disagree with Omer *et al*. (2008) who reported 1.8% mortality rate of the disease in layers and 1% in broilers.

In the present study the higher mortality rate was observed in the broiler chicks than in the layer ones, this could be attributed to the housing condition where, broilers were reared in semi intensive system while the layers in intensive system.

From the study the clinical manifestations were observed in the affected birds considered as typical signs of colibacillosis in addition to, inflammatory changes such as pericarditis, salpingitis and airsaculitis were seen in the autopsied birds considered as pathognomonic lesions of the disease, this in agreement with Omer *et al.* (2008) and Landman and Cornelissen (2006) who reported similar clinical signs associated with colibacillosis. However, contrary to the findings, was reported by Vandekerchove *et al.* (2004b), who reported acute mortality of colibacillosis in laying hens without prior clinical signs of the disease.

In this study *E. coli* was isolated from lesions of lungs and ovaries of the sick chickens. Since, the isolation of the organisms were obtained as pure form from these organs and absence of other bacteria, this emphasized that *E. coli* act as the primary causative agent of this outbreak. However, only two isolates were obtained from lungs and ovaries and failed to isolate the organisms from other organs, this might be due to pre-treatment with antibiotic of such cases, these findings were in agreement with Omer *et al.* (2008) who obtained only one isolate from chickens pre-treated with antibiotics. The results from this study indicate that outbreaks of colibacillosis are not necessarily associated with IBV or NDV infections, this similar previous study conducted by Vandekerchove *et al*. (2004b).

From this study it was observed that there were many factors might play an essential role in the occurrence of this outbreak, such as bad management system and hygiene measures as mentioned by Vandekerchove *et al.* (2004a). Factors like presence of rodents and flies in the poultry farms, can act as vectors of the disease. Furthermore, distances between

poultry farms may have a little importance in transmission of bacterial diseases. Since, the outbreak has been reported in the hot summer season (May) the environmental factors possibly have a role in this case (Aielo, 1998). In addition to, temperature in side the poultry houses of broilers was about 32°C and the cooling system was stopped, this might cause heat stress to the poultry. Failure of ultraviolet lamps as disinfectant system, this might be the exact cause of this outbreak.

Control and prevention of poultry diseases especially colibacillosis, antimicrobial agents are administered to chickens via food and water. This practice also improves feed efficiency and accelerates weight gain (Bower and Daeschel, 1999). However, the treatment of whole flocks with antimicrobials for disease prevention and growth promotion has become controversial practice (Van den Bogaard and Stobberingh, 1999; Witte, 1998). Therefore, this practice is reported to have caused high resistance to antimicrobial agents in both normal flora and pathogenic organisms (Allan *et al*., 1993; Amara *et al*., 1995). There is also concern that antimicrobial use in food animals can lead to the selection of antimicrobial resistant zoonotic enteric pathogens which may then be transferred to people by the consumption of contaminated food or by direct animal contact (Caudry and Stanisch, 1979; Turtura *et al*., 1990).

In this study almost all *E. coli* isolates showed high resistance to Ciprofloxacin, Chloramphenicol, Piperacillin/Tazobactam, Ceftizoxime Cefotaxime, Pefloxacin, Tetracycline and Ampicillin/Sulbactam, but they were sensitive to Amikacin, Gentamicin, Co-Trimoxazole and Ofloxacin, these findings were similar to the previous studies done in Germany and other countries (Guerra *et al*., 2003; Saenz *et al*., 2003; Tricia *et al*., 2006), but the findings disagree with Raji *et al*. (2007) who found high numbers of *E. coli* isolates were sensitive to Ciprofloxacin. Furthermore, the isolates were highly sensitive to Amikacin, Gentamicin, Co-Trimoxazole and Ofloxacin this can explain that these agents commonly used in human.

In the present study the isolates clearly demonstrated high resistance to most examined antibiotics. This is most probably due to increased use of antibiotics as feed additives for example so far, Tetracyclines are extremely used in poultry industries for growth promotion or prevention of diseases. For this reason, these antibiotics are inactive against a vain pathogenic *E. coli* strains at the present time. Moreover, inappropriate use of antibiotics, resistance transfers among different bacteria and possible cross resistance between antibiotics used in poultry (Allen *et al*., 1993). The major factors responsible for antimicrobial resistance in bacteria is antibiotic use, crowding and poor sanitation therefore, these factors are explain the high degree of resistance in *E. coli* isolates, this in agreement with these studies (Van den Bogaard and Stobberingh, 1999). Furthermore, this might be explained by the fact that antimicrobial use and misuse have contributed to the emergence and spread of antimicrobial resistant microorganisms (Levy, 1994). Also, this may suggest that the extent of resistance of antibiotic is associated with the extent of it use.

The most striking findings from this study that the isolates were almost totally resistance to Tetracyclines; this might be explained by the fact that this drug is heavily used in the poultry industry in Kassala State and could be the result of misuse or antagonism effect between the drug and other bactericidal antibiotic(s), especially when used concurrently (Allen *et al*., 1993).

In conclusion, introduction of surveillance programs to monitor antimicrobial resistance in pathogenic bacteria is strongly needed. Good management system and biosecurity measures should be adopted in the poultry industries. To avoid multi-drug resistant organisms, it is recommended that potentially synergistic antimicrobial combinations be used. Treatment strategies include attempts to control predisposing infections or environmental factors and early use of antibacterials indicated by susceptibility tests should

be applied for control of colibacillosis (Aiello, 1998). Furthermore, other approaches to prevent and control outbreaks of colibacillosis in the poultry industries include improved preventive strategies such as hygienic methods, appropriate husbandry methods, routine health monitoring and immunization. However, each of these practices have had limited success (La Ragione *et al*., 2001, 2004; Gomis *et al*., 2003) and it has necessitated the use of antimicrobial chemotherapy to control outbreaks of colibacillosis.

Thus, poultry producers should approach the treatment of diseases with antibiotics very cautiously and the use of these agents should be given consideration and full attention. Therefore, the producers and veterinarians should work closely when antibiotic therapy is needed and both must continue to work toward ensuring a safe food supply for consumers.

ACKNOWLEDGMENTS

The authors gratefully acknowledge the technicians and laboratory workers in Kassala Veterinary Research Laboratory for their help and cooperation during processing of the specimens. Special thanks to Salih, D.A for reviewing the manuscript.

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