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STUDIES ON AVIAN COLIBACILLOSIS IN BAHRI LOCALITY OF KHATROUM STATE, SUDAN

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ABSTRACT

Forty five poultry farms located in Bahri locality of Khartoum were investigated for the presence of avian colibacillosis and the study was extended for 6 months of the years 2016 and 2017. The prevalence rate of colibacillosis in Bahry locality poultry farms was 11.1%, the prevalence rate increased with broilers poultry farms 3 (6.67%) than layers farms 2 (4.45%) and it was inversely proportionate to the age of birds. The prevalence of avian colibacillosis was higher (8.9%) in closed system farms than open system (3.2%). This is the first report of incidence rate of avian colibacillosisin Bahri locality. In this investigation a total of 92 bacterial isolates were obtained from 81 diarrhoeic samples, according to the cultural characteristics, bacterial morphology and biochemical reactions results, the identified bacteria were: *Escherichia coli* 59.8%, *Proteus mirabilis* 19.6%, *Enterococcus faecalis* 6.5%, *Klebsiella pneumonae sub spp. ozaenae* 5.4%, *Salmonella spp.* 3.3%, and *Staphylococcus aureus* 5.4%. Gram negative

bacteria represented the predominant isolated bacteria (94.6%), compared to gram positive bacteria (5.4%). A total of 55 bacterial isolates were obtained from 45broilers diarrhoeic samples, According to the cultural characteristics, bacterial morphology and biochemical reactions results, the identified bacteria were: *Escherichia coli* 65.5%, *Proteus mirabilis* 16.3%, *Staphylococcus aureus* 9.1%, *Salmonella spp.* 5.5% and *Klebsiella pneumonae sub spp. Ozaenae* 3.6%. Gram negative bacteria represented the predominant isolated bacteria (90.9%), compared to gram positive bacteria (9.1%). A total of 37 bacterial isolates were obtained from 36layersdiarrhoeic samples, According to the cultural characteristics, bacterial morphology and biochemical reactions results, the identified bacteria were: *Escherichia coli* 51.4%, *Proteus mirabilis* 24.3%, *Enterococcus faecali*16.2% and *Klebsiella pneumonae sub spp. Ozaenae* 8.1%. Ten *E. coli* isolates were selected randomly and examined for their production of heat-stable enterotoxin using Suckling mouse test (SMT). Seven isolates (70%) out of 10 gave positive results with SMT test. This is the first report in Sudan using suckling mouse test (SMT) for detection of heat-stable enterotoxin (STa) in *E.coli* isolated from cases of avian colibacillosis.

I. INTRODUCTION

Poultry is an important source of egg and meat world widely and the consumption of poultry products is still increasing and this is due to increasing human population, religion (most world religions do not prohibit the consumption of poultry meat), the western beauty image (low fat percentage in poultry meat) and women working outdoors in the western (poultry meat is fast and easy to cook).[1]

In Sudan poultry products on commercial basis was commenced in 1979, by Sudanese Kwaiti Poultry Production Company. Poultry product as commodity is highly demanded by the domestic people and the public of the neighbouring countries especially the Arabs. The progressive market demand for poultry meat and table eggs encouraged investors to establish additional large scale poultry production projects. In 1980, the preceding commercial poultry projects achieved great stride in making poultry products available to consumers at favourable prices. The rapid expansion of poultry production in the Sudan in recent years has stimulated many workers to study many of major diseases of poultry which cause severe economic losses.[2]

Colibacillosis has an important economic impact on poultry production worldwide. The majority of economic losses resulted from mortality and decrease in productivity of the

affected birds. It is a common disease in poultry flock especially in the intensive farming system. The causative agent of colibacillosis is *E. coli* which is a gram negative, non-acid-fast, uniform staining, non-spore-forming bacillus that grows both in aerobically and anaerobically. Signs in birds affected with colibacillosis vary from sudden death to birds being off-color with their necks pulled into their bodies.[3]

Pathogenicity of *E. coli* strains is due to the presence of one or more virulence factors including invasiveness factors invasins, heat labile and heat stable enterotoxins, verotoxins and colonization factors or adhesins.[4] Pathogenic *E. coli* in poultry are divided into three types namely as avian pathogenic *E.coli* (APEC), enterotoxigenic *E. coli* (ETEC) and enteropathogenic *E. coli* (EPEC), *E. coli* causes a variety of diseases in poultry such as pericarditis, airsacculitis, peritonitis, salpingitis, cellulites, colicepticemia, panophthalmitis, omphalitis, coligranuloma, and swolen-head syndrome.[5] Pathogenic *E. coli* is usually identified by detection of a specific virulence factor associated with avirulence. Over 700 antigenic types (serotypes) are recognized based on 0, H and K antigens. Different serological test such as fluorescent antibody test (FAT), enzyme-linked immunosorbent assay (ELISA) test, serum agglutination test (SAT), growth inhibition test (GIT) are used for the detection of enterotoxigenic and enteropathogenic *E. coli*.[6]

This study was aiming to determine the incidence of colibacillosis in poultry farms in Bahri locality of Khartoum State, to isolate and identify important bacteria associated with diarrhoea in poultry including and to characterize *E. coli* as the most common organisms associated with diarrheoa in poultry using biochemical and biological methods.

II. MATERIALS AND METHODS

Area of Study

This study was conducted out in Bahri-North during the years 2016 and 2017 covering layers and broilers poultry farms with different rearing systems (open and close).

Source of samples

Eighty one cloacal swabs were collected from diarrhoeic poultry in Bahri locality of Khartoum State.

Sampling procedure

Faecal swabs were put in ice box containing ice and transported to the laboratory. In the laboratory samples were kept in a deep-freezer at -20^{o}C. On the next day samples were removed from the deep freezer and left on the bench to thaw. Samples were then subjected to bacteriological analysis (isolation, identification and characterization of the isolated *E. coli*).

Preparation of culture

Samples were enriched in nutrient broth at 37°C for 24 hours.

Isolation, identification and characterization of bacterial isolates

All media (Oxoid media) were prepared and sterilized according to the manufacturer instructions. For the primary isolation of bacteria, a loop full of the enriched broth streaked onto blood agar, McConkey's agar, and nutrient agar using sterile wire loop. The cultures were incubated aerobically at 37oC for 18-24 hours. Cultures on semi-solid media were examined grossly for colonial morphology and haemolysis on blood agar. Whereas, broth media were checked for turbidity, change in colour, accumulation of gases in CHO media and for sediment formation. One half colony from each plate was used for performing gram staining. Purification was based on the characteristics of colonial morphology and smear. This was obtained by sub culturing of a typical discrete colony on blood agar plate. Pure cultures were preserved on slants of blood agar and egg media at 4C^{o}.

Biological and biochemical identification

The purified isolates were identified as previously described[7] and.[8] The identification include: Gram's reaction, presence or absence of spores, shape of organism, motility, colonial characteristics on different media, aerobic and anaerobic growth, sugars fermentation ability and biochemical tests (staining of smear, catalase test, oxidase test, coaggulase test, oxidation fermentation test, motility test, glucose breakdown test, fermentation of carbohydrates, urease activity, citrate utilization, gelatin hydrolysis test, nitrate reduction test).

Suckling mouse test (SMT)

According to[9] volumes of 100 ml of Brain Heart Infusion Broth in 250ml conical flasks were inoculated with three to four colonies of *E. coli* grown on blood agar plates. The flasks were incubated in a water path shaker (100 rpm) at 37C^{o} for 24 hours. The broth cultures were coldly (4^{o}C) centrifuged at 4000 rpm for 15 minutes and the supernatant was collected. This supernatant constituted the STa which was aseptically stored at 4^{o}C before use. Two

days after birth infant mice were inoculated orally with 0.1ml STa into the stomach using a special canula. After inoculation the mice were kept at room temperature for 4 hours and then decapitated or killed. The abdomen was opened (after killing of mice); the small intestines were examined for distension and then removed by forceps. The intestines were then weighted using a sensitive balance and the ratio of gut weight to the body weight was calculated. Ratios of less than 0.070 were considered negative. Those in range of 0.070-0.090. were considered doubtful positive and those over 0.090 were positive.

III- RESULTS

Cases of avian colibacillosis were reported in 5 out of 45 (25 broilers and 20 layers) poultry farms in Bahri locality, with incidence rate of 11.1%. The incidence rate increased with broilers poultry farms 3 (6.6%) than layers farms 2 (4.5%) and it was inversely proportionate to the age of birds. The incidence of avian colibacillosis was higher (8.9%) in closed system farms than open system (3.2%).

Characteristics of samples collected

A total of 81 cloacal swabs were collected from birds showing clinical signs of avian colibacillosis and did not receive any treatment. Samples were collected from broilers and layers poultry farms, following closed, semi closed and open housing systems and located in Bahri locality of Khartoum state. 76.5% of the samples were collected from closed housing systems and 23.5% were collected from open system. Diarrhoeic samples were classified as: watery yellowish diarrhoea 49.3%, watery-mucoid diarrhoea 33.3% and white-watery diarrhoea 17.2% (Table 1).

Bacteria isolated from diarrhoeic samples

A total of 92 bacterial isolates were obtained from 81 diarrhoeic samples (Table 2). According to the cultural characteristics, bacterial morphology and biochemical reactions results (Table 3) the identified bacteria were: *Escherichia coli* 59.8%, *Proteus mirabilis* 19.6%, *Enterobacter cloacae* 6.5%, *Klebsiella pneumonae sub spp. Ozaenae* 5.4%, *Salmonella spp.* 3.3%, and *Staphylococcus aureus* 5.4%. Gram negative bacteria represented the predominant isolated bacteria (94.6%), compared to gram positive bacteria (5.4%) (Figure 2).

Bacteria isolated from diarrhoeic samples collected from broilers

A total of 55 bacterial isolates were obtained from 45broilers diarrhoeic samples (Table 4). According to the cultural characteristics, bacterial morphology and biochemical reactions results (Table 3) the identified bacteria were: *Escherichia coli* 65.5%, *Proteus mirabilis* 16.3%, *Staphylococcus aureus* 9.1%, *Salmonella spp.* 5.5% and *Klebsiella pneumonae sub spp. Ozaenae* 3.6%.

Bacteria isolated from diarrhoeic samples collected from layers

A total of 37 bacterial isolates were obtained from 36broilers diarrhoeic samples (Table 5). According to the cultural characteristics, bacterial morphology and biochemical reactions results (Table 3) the identified bacteria were: *Escherichia coli* 51.4%, *Proteus mirabilis* 24.3%, *Enterobacter cloacae* 16.2% and *Klebsiella pneumonae sub spp. Ozaenae* 8.1%.

Suckling mouse test (SMT)

Seven isolates 7(70%) out of ten isolates gave positive results with SMT test (Table 6).

Table (1): Characteristics of the faecal samples collected from diarrhoeic birds.

Watery yellowish Diarrhoea	Watery-mucoid Diarrhoea	White-watery Diarrhea	Total (%)
40 (49.4%)	27 (33.3%)	14 (17.3%)	81 (100%)

Table (2): Bacteria isolated from diarrhoeic samples.

Isolated bacteria	Number	Percentage%
Escherichia coli	55	59.8%
Proteus mirabilis	18	19.6%
Staphylococcus aureus	5	5.4%
Salmonella spp.	3	3.3%
Enterococcus faecalis	6	6.5%
Klebsiella pneumonae sub spp. Ozaenae	5	5.4%
Total	92	100%

Table (3): Cultural characteristics, bacterial morphology and biochemical tests of the isolated bacteria.

Test	*E. coli*	*Salmonella sp.*	*S. aureus*	*Proteus mirabilis*	*Enterococcus faecalis*	*Klebsiella pneumoniae*
Aerobic growth	+	+	+	+	+	+
Colonies on MacConkey	Bright pink	Pink	Pink	Pale	Pale	Pink
Haemolysis on blood agar	+	-	+	+	+	+
Gram reaction	-	-	+	-	+	-
Shape	Rods	Rods	Cocci	Rods	Cocci	Rods
Motility	+	+	-	-	-	-
Catalase	+	+	+	+	-	+
Oxidase	-	-	-	-	-	-
Indole	+	+	-	-	-	+
Methyl red	+	+	+	+	-	-
VP	-	-	-	-	+	-
Citrate	-	-	-	+	-	+
Nitrate	+	+	+	+	+	+
Arginine	-	-	-	+	-	-
KCN	-	-	-	-	-	-
Urease	-	-	-	+	-	-
H_2S	-	-	-	+	-	-
Gelatinase	-	-	-	+	-	-
Eikjman	+	-	-	-	-	-
O/F	+	+	+	+	+	+
Glucose	+	-	+	+	+	+
Lactose	+	-	+	+	+	+
Maltose	+	+	+	+	-	+
Inositol	-	-	-	-	-	-
Sucrose	+	-	+	+	+	-
Mannitol	+	+	+	+	+	+
Xylose	+	+	+	-	-	+
Raffinose	-	-	+	-	-	+

Sorbitol	+	-	-	-	+	+
Trehalose	+	+	+	+	+	+
Dulcitol	-	-	-	-	-	+
Cellobiose	-	+	+	-	-	+

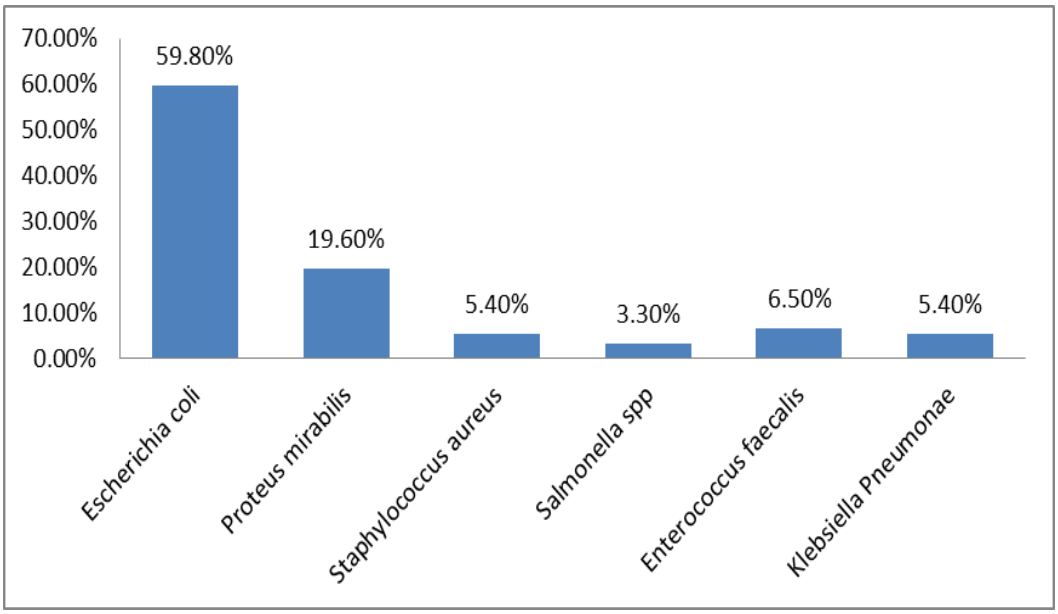

Fig. (1): Bacteria isolated from diarrhoeic samples.

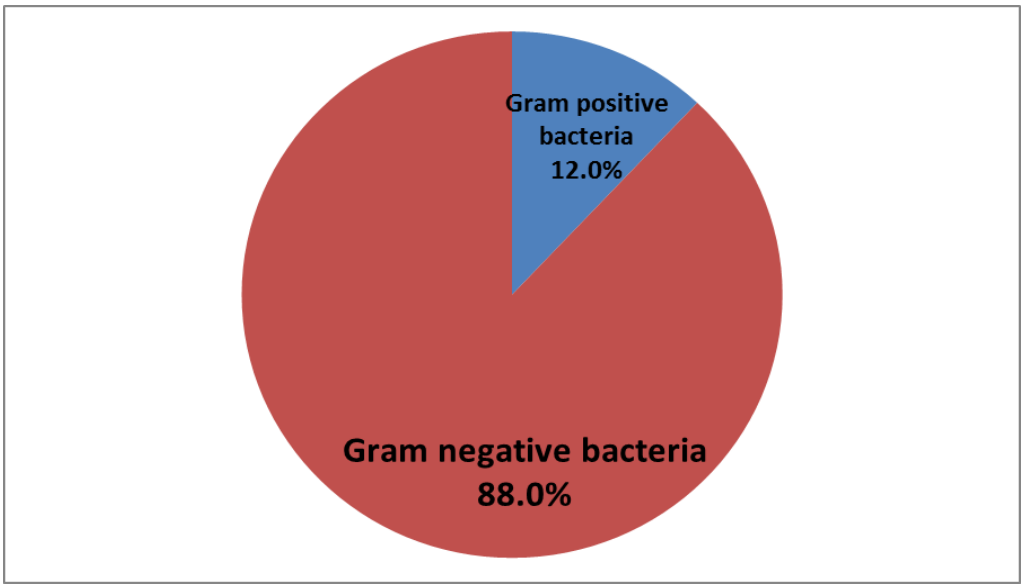

Fig. (2): Gram negative and gram positive bacteria isolated from diarrhoeic samples collected from bahri locality.

Table (4): Bacteria isolated from broilers' diarrhoeic samples.

Isolated bacteria	Number	Percentage%
Escherichia coli	36	65.5%
Proteus mirabilis	9	16.3%
Staphylococcus aureus	5	9.1%
Salmonella spp.	3	5.5%
Klebsiella pneumonae sub spp. Ozaenae	2	3.6%
Total	55	100.0%

Table (5): Bacteria isolated from layers' diarrhoeic samples.

Isolated bacteria	Number	Percentage%
Escherichia coli	19	51.4%
Proteus mirabilis	9	24.3%
Staphylococcus aureus	6	16.2%
Klebsiella pneumonae sub spp. Ozaenae	3	8.1%
Total	37	100.0%

Table (6): Detection of STa enterotoxin produced by *E. coli* isolates using Suckling Mouse Test (SMT).

E. coli isolate	Intestinal weight/g	Carcass weight/g	Ratio/g
1	0.35	3.74	0.093
2	0.41	4.56	0.090
3	0.29	4.47	0.064
4	0.35	5.66	0.061
5	0.38	3.88	0.099
6	0.41	4.65	0.088
7	0.34	3.22	0.105
8	0.40	3.85	0.103
9	0.39	3.57	0.109
10	0.42	4.57	0.091

IV. DISCUSSION

Colibacillosis was reported by many researchers in different countries[10], therefore, the disease is considered one of the principal causes of mortality and morbidity in poultry and responsible for high economic losses to poultry industry worldwide.[11] Avian colibacillosis

caused by *E. coli* is a major health problem in poultry industry in Sudan.[10] The incidence of colibacillosis in Bahri locality was found to be 11.1%. The incidence rate in broilers poultry farms (6.67%) was greater than in layers farms (4.45%) and it was inversely proportionate to the age of birds. A study conducted in 1.9% Kassala in Eastern State of Sudan, revealed 6.8% mortality rate of colibacillosis in broiler chicks and in the layers.[12] found that the incidence of colibacillosis was 32.6% in broiler flock and 27.2% in layer in South korea. In Pakistan[13] found that the prevalence of colibacillosis was 8.9% in poultry farms around Faisalabad.[14] determined the incidence of 9.52- 36.73% of colibacillosis in all age group of bird in Bangladesh. 76.5% of the samples were collected from closed housing systems and 23.5% were collected from open system.[15] reported that several factors might be responsible for higher incidence of colibacillosis such as: inadequate ventilation condition of farm, unrestricted movements of individuals, vehicles and wild life. Diarrhoeic samples were classified as: watery yellowish diarrhoea 49.3%, watery-mucoid diarrhoea 33.3% and white-watery diarrhoea 17.2%. Forty (72.8%) out of 55 *E. coli* isolates were isolated from watery yellowish diarrhoea, and 10 (18.1%) were isolated from watery-mucoid diarrhoea and 5 (9.1%) from bloody-watery diarrhoea. This high percentage of watery diarrhoea confirmed the involvement of *E. coli* as studied by[16] and.[17] Out of 81 diarrhoeic samples collected from poultry farms in Bahri locality of Khartoum State a total of 92 bacterial isolates were obtained. These were *Escherichia coli* 59.8%, *Proteus mirabilis* 19.6%, *Enterococcus faecali* 6.5%, *Klebsiella pneumonae sub spp. Ozaenae* 5.4%, *Salmonella spp.* 3.3%, and *Staphylococcus aureus* 5.4%. A total of 55 bacterial isolates were obtained from 45 broilers diarrhoeic samples. These were *Escherichia coli* 65.5%, *Proteus mirabilis* 16.3%, *Staphylococcus aureus* 9.1%, *Salmonella spp.* 5.5% and *Klebsiella pneumonae sub spp. Ozaenae* 3.6%. A total of 37 bacterial isolates were obtained from 36 broilers diarrhoeic samples. These were *Escherichia coli* 51.4%, *Proteus mirabilis* 24.3%, *Enterococcus faecali* 16.2% and *Klebsiella pneumonae sub spp. Ozaenae* 8.1%. In some cases more than one isolate was recovered from the same sample. *E. coli* represented the highest percentages (59.8%, 65.5% in broilers and 51.4% in layers) of the bacteria isolated from cases of avian colibacillosis. The result is similar to that found by[18], Mohamed[19], [16] and.[20] Ariful Islam (2014) reported that *E. coli* represented 83.33% of the total bacteria isolated from cases of layers colibacillosis in Bangladesh and 72.22% of broilers. In this study Gram negative bacteria represented the predominant isolated bacteria (94.6%, 90.9% in broilers and 100.0% in layers), compared to gram positive bacteria (5.4% and 9.1% in broilers). This result is similar to that reported by[21] and.[20] Isolation of *E. coli* doesn't necessarily means the

presence of the disease unless, virulence factors are identified i.e. toxins and or fimbriae.[22] In this study 10 randomly selected *E.coli* isolates which tested by the suckling mouse test (STM) for production of heat-stable (STa) enterotoxin. seven isolates (70%) gave positive result with SMT test. This finding agrees with[16] who found that 90% of isolated *E. coli* produced STa enterotoxin. The reability of SMT test was also confirmed by[23] and this study disagrees with[24] who stated that the infant mouse test is un satisfactory as a method for detection of STa enterotoxin.

V. CONCLUSION

The incidence rate of avian colibacillosis in Bahri locality of Khartoum state is 11.1%. The incidence rate increased with broilers poultry farms 6.67% than layers farms 4.45% and it was inversely proportionate to the age of birds. *E. coli* represents the predominant Bacterial spp. (59.8%), isolated from diarrhoeic poultry. Other bacteria are *Proteus mirabilis* 19.6%, *Enterococcus fecalis* 6.5%, *Klebsiella pneumonia sub spp. Ozaenae* 5.4%, *Salmonella spp.* 3.3%, and *Staphylococcus aureus* 5.4%. *E. coli* isolates were tested by the suckling mouse test (SMT) for production of heat-stable (STa) enterotoxin. Seven isolates (70%) gave positive results with (SMT) test.

VI. ACKNOWLEDGEMENT

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