Research Article

Experimental Induction and Control of Cellulitis in Broiler Chickens

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Abstract | In the commercial broiler, cellulitis is considered one of the most economically prevalent problems due to the presence of the lesion leading to increased condemnations and carcass downgrading. Clinically cellulitis is a deep infection of broiler chicken skin caused by many bacterial species, mainly *Escherichia coli* (*E. coli*) and/ or *Staphylococcus aureus* (*S. aureus*), causing severe economic losses in poultry. This study was done on 14-day old broiler Ross 308 chickens subcutaneous (s.c) injected with *E. coli* and/ or *S. aureus* to induce cellulitis. Clinical signs, mortality, pathological lesion, and growth performance were determined. Hematological parameters, liver and kidney functions were also recorded. Colistin+ Doxycycline combination (Doxyforte®) was used to control the infection. Clinically, site of infection was appeared red, swollen accompanied with increased skin thickness, postmortem lesions in the 3rd day post infection with s.c. yellowish suppurative exudates, pericarditis and perihepatitis were prominent *E. coli* infected with hepatic subcapsular hemorrhage mostly in *S. aureus* groups. Hematological parameters were mostly affected in all infected non-treated groups compared to negative control without significant difference. Histopathological changes of infected non-treated groups showed inflammation of s.c tissue with massive heterophils and mononuclear cell infiltration, hydropic degeneration of the hepatocytes and congested splenic sinusoids. While treated groups showed limited skin inflammatory condition at the site of injection and return of skin to normal color and thickness. Doxycycline+ colistin combination helps in reduction of lesions in treat infected birds, with marked improvement in measured parameters. We recommended active actions to prevent causes and factors helping in including cellulitis, regular lowering bird density, enhancing restrict biosecurity, modulating the vaccination timing, improving management practices, as well as application of probiotics to improve and restore good gut health.

Keywords | Cellulitis, Experimental infection, Broiler chickens, Clinical signs, Pathological lesion, Control, Colistin, Doxycycline, Hematology, Bacterial diseases

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INTRODUCTION

Avian cellulitis (AC) is a relatively recently recognized acute and diffuse suppurative inflammation, affecting all s.c tissues and sometimes extends to muscular tissue. It is frequently associated with abscess formation, discoloration and thickening of the broilers skin leading to an increase in condemnation rate at slaughterhouse (Amer et al., 2019). AC was detected on different parts of chicken body including the head, dorsum, thighs, breast, legs, and

abdomen (Randall et al., 1984; Norton, 1997; Fallavena et al., 2000; Gomis et al., 2000). Clinically, cellulitis may be observed in diseased chickens in case of occurrence the infection in the head area. Involvement of other parts of the body, on the other hand, is only discovered incidentally on post-mortem (PM) or slaughterhouse inspections (Morley and Thomson, 1984; Bianco et al., 2016). Skin traumas and scratches are considered the main predisposing factors for occurrence of cellulitis in broilers, facilitating invasion of pathogenic bacteria (Sanches et al., 2020). Also, other factors like cannibalism, insect bites, poor litter conditions, immunodeficiency, foot problems in addition to systemic infections are thought to predispose AC (Wang et al., 2005; Bianco et al., 2016).

Economic losses occur chiefly as a result of increased condemnation rate and/or downgrading of affected carcasses (Bianco et al., 2016). Losses due to cellulitis in broilers were up to 30% in USA, 0.8% in Canada, at least 18 thousand tons in Brazil and, 0.9-1.7% of total carcass condemnation in Egypt (Norton, 1997; Paniago, 2009; Barbieri et al., 2013; Amer et al., 2019).

The lesions showed varying-colored secretions from yellow to green, which were either serous, fibrous yellow, green, or suppurative. Many bacterial agents were involved to be the cause of AC as *Aeromonas* spp., Clostridia, *Enterobacter* spp., *E. coli*, *Proteus mirabilis*, *P. aeruginosa, Staphylococci* and *Streptococci*, where *E. coli* was the predominant (Derakhshanfar and Ghanbarpour, 2002; Barros et al., 2013). Recently, *E. coli* was recorded as the most prevalent in AC (45.2%), followed by *Staphylococci* (33.2%) (Amer et al., 2019). Immunosuppressed chickens were confirmed to possess more possibility for acquiring cellulitis. Experimental induction of AC was done via s.c injection of clostridia, *E. coli*, and *S. aureus* in 25-day-old broilers (Gomis et al., 1997b). In two separate experiments s.c injection of *E. coli* in 25 and 39-day-old broilers, 98% and 100% of birds developed characteristic cellulitis lesions as early as 28 and 18 hs post infection (PI) (Gomis et al., 1997b; Norton et al., 1997). Also, *E. coli* was recovered from > 75% of lesions (Gomis et al., 1997b; Johnson et al., 1997; Olkowski et al., 2005). Both *E. coli* and *Staphylococcus* spp isolates from cellulitis are multidrug resistant (Amer et al., 2019).

Leukocyte profiles that deviate from normal are useful in conservation physiology because its changes are linked to the causes of stress and directly related to stress hormone levels (Dhabhar et al., 1996) as well as diseases and infections. Total white blood cell counts (TWBC) distributions and hematological parameters indicated leukocytosis, leukemoid reactions, and a high frequency of atypia (Cotter, 2015). Polymicrobial bacteremia and fungemia are the reasons for the hematological observations, both of which could account for high TWBC and atypical cells (Weinstein et al., 1983).

The normal range of red blood cell (RBC) count is $3.5x10^6$ /mm 3, packed cell volume (PCV) is 22-35%, hemoglobin is 7-13 g/dL in broilers (Aksu et al., 2010). In addition to causing relative neutrophilia and lymphopenia, infections commonly cause an increase in monocytes (Jain, 1986; Campbell, 1996; Davis et al., 2004), and general increases in total WBC count (Jain, 1986, 1993; Latimer et al., 1988; Thrall, 2004). Heterophils and lymphocytes are the most abundant white WBC in birds, they play an important role in innate and acquired immunity, respectively (Minias, 2019).

The utility of the avian Heterophils/lymphocytes (H/L) ratio was firstly realized by Gross and Siegel (1983) and used now to assess the welfare of chickens under rearing conditions (Altan et al., 2000; Davis et al., 2000; Elston et al., 2000; Onbasilar and Aksoy, 2005; Nicol et al., 2006), and infections or diseases causing increases in stress (Lindström et al., 2005). The H/L ratio is an indicator of stress and welfare of hens caged in modern systems (Cotter, 2015). The H/L ratio may reflect a readiness to cope with infection through injury (via heterophils) rather than with a communicable disease (via lymphocytes) (Minias, 2019). Blood H/L ratio reflects the status of immune system (Lentfer et al., 2015).

The usage of antibiotic *or Bifidobacterium bifidum* with avoiding immunosuppression can reduce cellulitis lesion and condemnation rate (Randall et al., 1984; Fallavena et al., 2000; Gomis et al., 2000; Amer et al, 2019).

This study aimed to induce cellulitis experimentally by s.c infection of broiler chickens, recording clinical signs, pathological lesion, performance parameters and trial to use Colistin and Doxycycline in control of this infection.

MATERIALS AND METHODS

Bacterial strains

In the present study, *E. coli* (O78) and *S. aureus* cultures were purified and used. These strains were originally derived from cellulitis lesions in broiler chicken. *E. coli* strain O78 was molecular identified to be positive for 5 genes by PCR (Amer et al., 2019, 2020a). These isolates were sensitive to both Doxycycline and Colistin according to antibiotic susceptibility test using disc diffusion method according to Watts (2008) and CLSI (2016).

Experimental infection

E. coli O78 and *S. aureus* isolates were cultured and propagated as stated formerly by Matthijs et al. (2003). Both isolates were prepared for usage at a concentration

of 4.5X10^8 colony forming units (CFU)/ml. Each bird was infected by s.c injection of 0.5 ml of *E. coli* O78 and/or *S. aureus* over the left breast muscle (Cookson et al., 2007).

Chickens

One hundred and fifty (150) 1-days old Ross 308 broiler chickens were purchased from a private commercial hatchery. The chickens were reared on straw deep litter.

Ration

The chickens were nourished on ready-made commercial pelleted rations free from feed additives (NRC, 1984) involving starter ration (CP not less than 23%) as well as growing ration (CP not less than 21%). Chickens were supplied ad libitum with drinking water and feed.

Vaccination

All chickens were immunized with ND+IBV vaccine at age of 5 days, IBD intermediate 228E vaccine at age of 10 days, in addition to La Sota vaccine at age of 16 day. All vaccines were given by ocular instillation route.

Drug

Doxyforte® is produced by Jordan Veterinary and Agriculture Medical Industry Company. P.O. Box: 2760 Amman 11953 JORDAN. Formula characteristics: Each gram contains: Doxycycline Hcl 240 mg and Colistin sulphate 500,000 IU. Formula: Water soluble powder. Dosage: 100 gm/ 200 liters drinking water for 5 days, Batch Number: 190326. Mfg. Date: 03/2019. Exp. Date: 03/2022.

Experimental design

At 14th days of life, chicks were randomly divided into 4 groups. Group 1 was kept as non-infected and non-treated (30 birds were kept as control negative group). Groups 2, 3 and 4 were (challenge groups, 40 chickens in each group) infected with *S. aurous*, *S. aurous*+ *E. coli and E. coli;* respectively. Chickens were s.c injected in the thigh fold with 1 ml of *S. aureus* (group 2) or *E. coli* (group 4), while chicks of group 3 were injected with 0.5 ml of both strains. Infected birds were observed daily for clinical signs, mortalities and examination of the inoculation site. At the 3rd day post infection (DPI) groups 2, 3 and 4 were subdivided into two subgroups (A and B), 20 chicken each. Subgroups A were kept as infected non-treated groups while subgroups B were infected treated groups with doxycycline + colistin in 1 gm/ liter drinking water for 5 days.

Broiler performance parameters

1. Feed consumption and conversion ratio were determined using the following Formula: Feed consumption (FC) g/bird = Feed intake in a replication/ No. of live birds in a replication. Feed conversion ratio (FCR) = Feed intake (g)/ Live weight (g). Parameters were recorded for each chicken in 4 groups at third and fourth week of age according to NCR (1984).
2. Daily examination of injection site with record of clinical signs, mortalities and post-mortem examination.
3. Measuring of skinfold thickness with skinfold meter of inoculation side (left) as compared with non-inoculated side (Right) at 3rd (DPI), 3rd (DPT) and the 7th DPT. Brest skin close to thigh web was drawn out far enough to apply the skinfold meter and measure thickness (Harpenden Skinfold Calipers).
4. Clinical signs: The infected and treated groups were observed daily for clinical signs just after infection till the end of the observation period.
5. Mortality rate: The number of positive dead birds/ group was recorded daily till 35 days of age (end of the experiment).
6. Evaluation of deterioration in hematological parameters: Clotted blood for serum analysis and non-clotted blood for blood picture were collected from all groups at 3 DPI as well as 5 and 7 DPT. Blood samples from each group were collected for determination of hematological parameters such as RBC, WBC, PCV, Hemoglobin (Hb) concentration and platelets using Natt and Herrick blood diluent and hemocytometer. Blood smears were done for differential leucocyte count and H/L ratio (Fidan et al., 2017).
7. Samples from each group for the assay of liver function parameters (GOT, GPT, ALP), kidneys function parameters (Urea, creatinine) using BioSystems S.A. Kits.
8. Histopathological Examination: Skin specimens were collected at 3 and 5 DPI; fixed in 10% neutral buffered formalin, paraffin embedded (FFPE) block of tissue (Sadeghipour and Babaheidarian, 2019). Paraffin tissue were sectioned at 4-6 µm thickness and stained with hematoxylin and eosin (H and E) (Bancfort and Stevens, 1996).

Statistical analysis

The obtained results were statistically compared by ANOVA without detection of marked statistical significance.

RESULTS AND DISCUSSION

Avian cellulitis is a diseased condition affecting broiler chickens and characterized by inflammation of the s.c tissue, particularly in the thigh and abdomen, with the presence of suppurative exudates and fibrino-necrotic plaques (Gomis et al., 1997a; Norton, 1997; Amer et al., 2020a, b). In the poultry industry, cellulitis represents one of the most essential reasons of partial or total condemnation

of carcasses. The major causative agents for cellulitis are *E. coli* and *S. aureus* (Messier et al., 1993; Gomis et al., 1997a; Amer et al., 2020a, b).

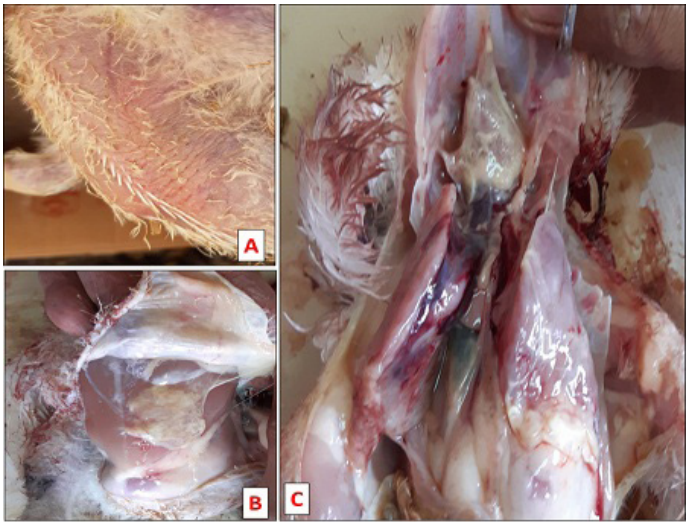

Figure 1: Lesion in broiler chicken s.c infected with *S. aureus* and /or *E. coli* showing. A: Thick rough dark colored skin. B: S.c lesion. C: S.c lesion, pericarditis and prehepatitis with subscapular hemorrhage in liver.

The obtained results revealed that, at the 1st DPI infected chickens showed low feed intake with ruffled feather followed by red swollen skin at site of infection with increase in thickness at the 3rd DPI (Figure 1A), and birds with severe lesions were reluctant to move. In postmortem lesions revealed s.c yellowish exudates, pericarditis and perihepatitis with subscapular hemorrhage in liver (Figure 1B, C). Mortality started at the 3rd DPI as 1 bird with ratio of 2.5% in 2nd and 3rd groups injected with *S. aurous* and *S. aurous* + *E. coli* respectively, and 2 birds in 4th group, *E. coli* infected group with ratio of 5%. After subgrouping at the 4th DPI, mortalities were 1 bird in *S. aureus* infected group (2a) with ratio (5%) and 3 birds in *S. aurous* + *E. coli* subgroup (3a) (15%). While, at 5th DPI, 2 birds in *E. coli* subgroup (4a). The highest mortality was 17.5% in subgroups (3a) followed by 15% in subgroup (4a) and the lowest mortality (7.5%) was in subgroup (2a). While control group and all treated subgroups showed no mortality. Our results were in accordance with that obtained by Norton et al. (1997) who administered an *E. coli* strain in broilers for experimental induction of cellulitis with suppurative plaques at 18 h pi. Macroscopic lesions induced by *E. coli* were distinguished by the existence of suppurative exudates, skin thickness and hemorrhages similar to those obtained in our study (Messier et al., 1993; Peighambari et al., 1995). Sanches *et al.* (2020) experimentally reproduce cellulitis in chickens by *P. mirabilis* and recorded cellulitis lesions (suppurative exudates and hemorrhages) in the chest region. Some birds showed additional congestion and infusions in the musculature with petechiae, bruises, and edema within the 24 h pi. Amer et al. (2020a) experimentally induced cellulitis in broiler chickens by s.c injection of *E. coli* O78 where yellowish to suppurative s.c exudate with thick yellowish red skin. The mortality in low and sporadic cases, without septicemia were recorded (Elfadil et al., 1996a; Derakhshanfar and Ghanbarpour, 2002). The injected *E. coli* and /or *S. aureus* were successfully reisolated from the developed lesions (Asmaa, 2013; Mellata, 2013; Amer et al., 2020a; Szafraniec et al., 2022; Wilczyński et al., 2022).

In poultry farms, antibiotics are administered commonly for therapeutic applications and growth promotion (Kariuki et al., 1999; Apata, 2009; Suleiman et al., 2013). In the current study treatment with doxycycline + colistin (0.5 gm/ liter in drinking water for 5 days), at the 2nd DPT birds of subgroups B, showed increased feed intake and activity than infected non-treated subgroups (A).

Table 1: Average body weight gain, feed intake and feed conversion rate of infected non-treated and treated broiler chickens.

Group	Infection	Treatment	Age/ weeks	Av. FI	ABWG	FCR
1	Control	Negative	1	145.69	139.42	1.04
			2	386.75	283.20	1.37
			3	840.60	511.80	1.64
			4	1038.48	728.80	1.42
2a	*S. aurous*	-	3	697.25	489.70	1.42
			4	956.9	510.40	1.87
2b		+	3	721.65	588.10	1.23
			4	981.40	736.67	1.33
3a	*S. aurous* + *E. coli*	_	3	688.90	486.50	1.42
			4	910.10	327.733	2.78
3b		+	3	722.40	632.30	1.14
			4	726.75	577.90	1.26
4a	*E. coli*	_	3	686.35	394.90	1.74
			4	975.80	416.80	2.34
4b		+	3	726.75	577.90	1.26
			4	1014.60	590.47	1.72

The recorded ABW as well as FCR of control gr 1 (Table 1) at 1 and 2 weeks of age were 139.42 and 1.04 as well as 283.20 and 1.37, respectively. ABW and FCR of at the 3rd and 4thweek of age (2 and 3wpi) of group 1 (511.80, 1.64, 728.80 and 1.42) were the highest followed by those of *S. aurous* infected group 2a (489.70, 1.42, 510.40 and 1.87) followed by *E. coli* infected group 3a (394.90, 1.74, 416.80 and 2.34), while group 2a showed the lowest values (486.50, 1.42, 327.733 and 2.78). This result indicates that both *E. coli* or *S. aurous* solo infections resulted in lower ABW and FCR (Barnes et al., 2003; Kamel, 2011; Asmaa, 2013; El-Sawah et al., 2018; Abd Elatiff et al., 2019), and the dual infection were resulted in more severe losses. Infected

treated an improvement in ABW and FCR was seen but still lower than control (Table 1). This result indicated that treatment can improve of ABW and FCR (Asmaa, 2013; El-Sawah et al., 2018) but not completely eliminate the pathological lesions (Barnes et al., 2003; Asmaa, 2013; El-Sawah et al., 2018; Abd Elatiff et al., 2019).

Table 2: Thickness of skinfold in the site of infection (left) and the non-infected side (Right) and their difference.

Gr.	Infection	Treatment	Time	L	R	Difference
				M ± SD	M ± SD	Mean
1	Control	Negative	3 DPI	13.2±1.1	14.6±0.55	1.4
			3 DPT	20.6±0.89	18.2±2.05	2.4
			7 DPT	21.4±2.07	20.6±2.61	0.8
2a	*S. aurous*	-	3 DPI	24.8±17.54	14±1	10.8
			3DPT	39±17.36	18.8±2.68	20.2
			7 DPT	42.2±23.66	20.4±0.89	21.8
2b		+	3 DPT	29.4±20.84	20.4±1.52	9.0
			7 DPT	27.8±7.53	19±2.24	8.8
3a	*S. aurous* + *E. coli*	-	3 DPI	65.8±12.85	15.6±1.34	50.2
			3 DPT	36.4±13.48	23.6±4.93	12.8
			7 DPT	60.8±49.24	21±2.35	39.8
3b		+	3 DPT	48±41.92	14.6±1.67	33.4
			7 DPT	34.6±19.71	20.2±0.45	14.4
4a	*E. coli*	-	3 DPI	55±15.05	15±0	40.0
			3 DPT	61±40.45	23.4±6.84	37.6
			7 DPT	57.2±52.03	25.6±6.66	31.6
4b		+	3 DPT	77.8±6.69	30±0	47.8
			7 DPT	32.4±6.23	19.8±0.84	12.6

DPI: day post infection; DPT: day post treatment.

Skin fold thickness describes the s.c inflammation and deposits where the skin fold thickness is measured by specialized (Table 2). Results of skinfold thickness revealed that all infected groups show an increase in skin thickness at site of injection (left) as compared to control negative group. The highest thickness was in *S. aurous*+ *E. coli* non-treated group which was 60.8±49.24 at 7 DPI, then infected non-treated *E. coli* which was 57.2±52.03 at 7 DPI (Table 2). Then infected non treated *S. aurous* group which was 42.2±23.66 at 7 DPI, then followed by infected with *S. aurous*+ *E. coli* treated group which was 34.6±19.71 at 7 DPT, followed by infected treated *E. coli* which was 32.4±6.23 at 7 DPT, followed by infected treated *S. aurous* group which was 27.8±7.53 at 7 DPT, and the lowest was control negative group at 7 DPI as it was 21.4±2.07. The non-infected side (Right) shows no lesions; it was 20.6±2.61 while in infected non-treated *E. coli* group is much thicker (22.6±6.66) (Table 2). Increase of skin thickness in injected chicken's groups indicate inflammation of s.c tissue and consequently increase skin thickness due to increase amount of s.c deposits, this was firstly recorded by Randall et al. (1984), later cellulitis was worldwide diagnosed in poultry (Gomis et al., 2003; Nain and Smits, 2011; Chen et al., 2016). *E. coli* cause cellulitis with subsequently increase skin thickness (Santana et al., 2008; Andreasen, 2020). Amer et al. (2019) reported that infection with pathogenic *E. coli* causing cellulitis is due to excessive production of s.c exude and increase deposits in s.c region. The highest skin thickness was found in group injected with both *E. coli* and *S. aureus,* that can be attributed to co-infection which increase severity of the s.c inflammation. This result was parallel with that of Radwan et al. (2018) who found that *E. coli* and *S. aureus* are the most prevalent bacteria in poultry cellulitis.

The RBC count, Hb and (PCV% are helpful in diagnosing nutritional deficiencies, acute illnesses, and chronic medical conditions (Sarma, 1990; Samour, 2011). The normal chicken blood parameters are arranged (RBC: 2.5-3.5 x10^6 µl, PCV: 22-35 %, Hb: 7-13 g/dl and WBC: 12-30 x 10^3 µl) (Bounous and Stedman, 2000; Odunitan-Wayas et al., 2018). At the 7th day, the values of RBC count, Hb and PCV% of control group were 2.91±0.37, 6.45±0.49 and 28±1.41 higher than 2.53±0.14, 6.55±0.35 and 30±4.24 in S. aurous infected group, 2.56±0.34,7.05±0.49 and 26.5±0.71 in *S. aurous*+ *E. coli* group, as well as in 2.14±0.37, 7.1±0.28 .and 29. 5±2.12 in *E. coli* group (Table 3) this result indicates that *E. coli* and/or *S. aureus* infections reduce RBC parameters.

Values of RBC, Hb and PCV% in doxycycline + colistin treated groups were 2.25±0.02, 6.2±0.14, and 28.5±2.12 in *S. aureus,* 2.28±0.11, 6.85±0.07, and 27.5±2.12 in *S. aurous*+ *E. coli* group, as well as 2.35±0.07, 6.3±0.71, and 27.5±0.71 in *E. coli* group. These values are nearly close to each other but still lower than control non infected non treated.

The TLC of control group (20.5±8.71) was nearly similar to *E. coli* (20.0 ±7.07) and both are higher than that of *S. aureus* (19.5±13.54), while that of *S. aureus and E. coli* showed the highest values (22.2±11.0) at the 7th day (Table 4). Values in treated groups were 17.5±3.54, 15.0±0.0, and 22.5±3.54 in *S. aurous, S. aurous*+ *E. coli* and *E. coli* infected group, respectively, but all were still lower than nontreated and control group. So, the result reflects the bad effect of treatment on measured TLC. Generally, blood platelets were nearly similar not in all treated or infected groups. Regarding differential leucocytes count the control negative group 1 showed Heterophils (H), Lymphocytes (L) and Monocytes (M) values of 16.5±0.50, 76.0±0.00, and 3.50±0.50; respectively. While the infected groups showed H, L, and M values of 16.0±1.00,77.5±0.50, and 3.00±1.00 in *S. aurous*, 18.0±0.00, 75.5±0.50, and 3.00±0.00 in *S. aureus* +*E. coli* group, while *E. coli* infected values were 17.5±0.50,76.0±1.00 and 3.50±0.50.

Table 3: Blood picture at 3 DPI (0 DPT), 3 and 7 DPT of infected non-treated and doxycycline + colistin treated groups of broiler chickens.

Gr.	Infection	Treat-ment	Time /DPT	RBC X10^6	Hb mg/ml	PCV %	Platlets X10^3	TLC X 10^3
				Mean± SD	Mean± SD	Mean± SD	Mean± SD	Mean± SD
1	Control	Negative	0	2.68±0.04	6.95±0.21	31±2.83	10.0±0.0	20.5±8.71
			3	2.61±0.79	5.8±0.42	27.5±0.71	9.5±0.71	27.5±10.61
			7	2.91±0.37	6.45±0.49	28±1.41	10.0±0.0	27.5±10.61
2a	*S. aurous*	_	0	2.26±0.08	6.65±0.07	28±0	10.0±0.0	21.0 ±14.0
			3	2.31±0.34	6.85±0.92	26±2.83	10.0±0.0	17.5±13.54
			7	2.53±0.14	6.55±0.35	30±4.24	10.0±0.0	19.5±13.54
2b		+	3	2.39±0.23	6.45±0.21	28±0	9.5±0.71	21±1.41
			7	2.25±0.02	6.2±0.14	28.5±2.12	10.0±0.0	17.5±3.54
3a	*S. aurous* + *E. coli*	-	0	2.35±0.07	5.15±0.35	25±1.41	10.5±0.71	20.0±8.0
			3	2.60±0.28	7.15±0.78	31±2.83	10.0±0.0	22.5±3.54
			7	2.56±0.34	7.05±0.49	26.5±0.71	10.0±0.0	22.2±11.0
3b		+	3	2.81±0.37	7±0.57	28.5±0.71	12.5±3.54	27.5±10.61
			7	2.28±0.11	6.85±0.07	27.5±2.12	10.0±0.0	15.0±0.0
4a	*E. coli*	-	0	2.43±0.04	5.25±0.35	26.5±0.71	10.0±0.0	22.2±10.0
			3	2.23±0.49	5.85±0.64	25±2.83	8.0±1.41	20.0±7.07
			7	2.14±0.37	7.1±0.28	29.5±2.12	10.0±0.0	20.0 ±7.07
4b		+	3	2.85±0.32	6.3±1.56	28.5±0.71	12±4.24	27.5±10.61
			7	2.35±0.07	6.3±0.71	27.5±0.71	10.0±0.0	22.5±3.54

RBC: red blood cell; Hb: Hemoglobin. PCV: Packed cell volume. TLC: Total leucocytes count.

Table 4: Leukocyte profiles of infected non-treated and doxycycline + colistin treated groups of broiler chickens.

Gr.	Infection	Treatment	DPT	Heterophils Mean± SD	Lymphocytes Mean± SD	H /L ratio*	Monocytes Mean± SD	Eosinophils Mean± SD	Basophils Mean± SD
1	Control	-	0	15.7±2.52	73.0±4.00	21.51	4.00±1.00	1.00±0.00	1.00±0.00
			3	17.5±0.50	76.0±0.00	23.03	3.50±0.50	1.00±0.00	1.00±0.00
			7	16.5±0.50	76.0±0.00	21.71	3.50±0.50	1.50±0.50	1.00±0.00
2a	Staph	-	0	15.7±1.53	79.0±1.00	19.87	3.00±0.00	0.67±0.58	1.00±0.00
			3	17.0±0.00	77.5±0.5	21.94	3.00±0.00	1.00±0.00	0.50±0.50
			7	16.0±1.00	77.5±0.50	20.65	3.00±1.00	1.50±0.50	1.00±0.00
2b		+	3	16.5±0.50	76.5±0.50	21.57	4.00±0.00	1.00±0.00	1.00±0.00
			7	16.5±0.50	74.5±0.50	22.15	4.00±0.00	2.50±0.50	1.50±0.50
3a	Staph + *E. coli*	-	0	15.7±0.58	78.7±1.53	19.95	3.67±0.58	1.00±0.00	0.67±0.58
			3	17.5±0.50	76.0±0.00	23.03	3.00±0.00	2.00±0.00	1.00±0.00
			7	18.0±0.00	75.5±0.50	23.84	3.00±0.00	1.50±0.50	1.00±0.00
3b		+	3	16.5±1.50	72.5±2.50	22.76	5.50±0.50	3.50±3.50	1.00±1.00
			7	15.5±0.50	75.0±3.00	20.67	5.00±2.00	2.50±1.50	1.00±0.00
4a	*E. coli*	-	3	17.0±1.00	76.7±0.58	22.16	3.33±0.58	1.67±0.58	1.00±0.00
			3	16.5±1.50	72.5±1.50	22.76	6.00±2.00	2.50±1.50	1.50±0.50
			7	17.5±0.50	76.0±1.00	23.03	3.50±0.50	1.50±0.50	0.50±0.50
4b		+	3	15.0±0.00	76.0±3.00	19.74	5.00±2.00	2.00±1.00	1.00±0.00
			7	16.5±0.50	76.0±1.00	21.72	3.50±0.50	2.00±0.00	1.00±0.00

* H/L Ratio: Heterophils/ lymphocytes ratio

Heterophils were higher in infection than control, while L was lower in infected. It was found that at infection site the *S. aureus* stimulates the immune cells to secrete chemokines, released WBC in the bone marrow into the blood, where the WBC and neutrophils are significantly increased, with a shift to the left in the nuclear index, toxic granules appeared in the cells, and decreased number of eosinophils (Peralta et al., 2020). The detected inflammatory response at the inoculation sites showing a significant increase in absolute count and of pseudo-eosinophils, basophils%,

and monocytes in infected group than in control group (Moiseeva et al., 2020). Intracellular accumulation of antibacterial medicines in active form facilitates transformation of phagocytic activity of macrophages and neutrophils and affects viability of phagocytized bacteria (Cirz et al., 2006; Liu et al., 2012; Moiseeva et al., 2020; Ulfig and Leichert, 2021).

Counts of H, L, and M in treated were 16.5±0.50, 74.5±0.50, and 4.00±0.00 in *S. aureus*, 15.5±0.50, 75.0±3.00, and 5.00±2.00 in *S. aureus* + *E. coli*, 15.5±0.50, 75.0±3.00, and 5.00±2.00 in *E. coli* (Table 5). It was observed that M count was H and L decreased, while M was increased in treated than non-treated and control groups. H/L ratios were 21.71 in control, infected with *S. aureus* (20.65), *S. aureus* + *E. coli* (23.84), and *E. coli* (23.03), while in infected treated was generally lower 22.15 in *S. aureus*, 20.67 in *S. aureus* + *E. coli*, and 21.72 *E. coli* (Table 4). Gross (1989) indicated that injected *E. coli* affects the H/L ratio. There is no detectable difference in the count of Eosinophils and Basophils in between groups.

Liver function test GPT, GOT, and ALP results in control group 1 were 8.5±0.71, 15± 4.24, and 887.5±17.68, respectively. GPT, GOT, and ALP for *S. aureus* infected group were 11±1.41, 22±5.66, and 632.5±519.72; in *S. aurous* + *E. coli* were 14.5±3.54, 22.5±4.95, and 1120±28.28; while in *E. coli* were12.75±5.3, 32.25±6.01, and 920±197.99 (Table 5), so results of infected groups are generally higher than control. GPT, GOT, and ALP results of doxycycline + colistin treated groups were 8.75±1.77, 16.25±2.47, and 975±49.5 in *S. aurous;* 8.75±1.77, 16.25±2.47, and 975±49.5 in *S. aurous* + *E. coli;* 9.5±2.12, 13.25±1.77, and 952.5±74.25 in *E. coli*. Results of treated groups showed lower values than non-treated and close to the control negative group. The raise in serum AST is signal for cellular damage to heart muscles and liver cells. Where the increase in serum ALT is mostly resulting from hepatic injuries (Sharma et al., 2015; Zoppini et al., 2016; Lala et al., 2023). An increase in serum ALT, AST, LDH activities, globulin concentration and a decrease ALP activity in *E. coli* O78 at 107 CFU/0.5 ml intraperitoneally infected groups were recorded (Eleiwa et al., 2011; Petrov et al., 2011; Zaki et al., 2012; Kumari et al., 2014; Sharma et al., 2015). The volume of AST and ALT rising alters according to the cause of liver cells injury (Leoni et al., 2018). It was reported that there was no significant effect on level of ALT and creatinine in *E. coli* infected broilers after administration of colistin in feed (Fitri et al., 2021). Rising in ALT can be occured through direct effect of bacterial toxins, drugs, chemicals on hepatocytes, particularlly those are near to the central vein (Schulze et al., 2019; Sharma and Nagalli, 2023). Colistin does not affect hematobiochemical serum on broilers (Saleemi et al., 2014).

Kidney function test represented by urea and creatinine values were 10.5±0.71 and 0.54±0.02 in control. While infected groups showed values of 9.5±0.71 and 0.51±0.08 in *S. aurous*; 9±1.41 and 0.6±0.06 in *S. aurous* + *E. coli;* as well as 9.75±2.47 and 0.48±0.04 in *E. coli*. The urea of infected groups was lower than control, while creatinine showed no marked difference. The urine and creatinine of

Table 5: Serum biochemical parameters at 3 DPI (0 DPT), 3 and 7 DPT of infected non-treated and doxycycline + colistin treated groups of broiler chickens.

Group	Infection	Treatment	Time/days/ DPT	GPT (U/L)	GOT (U/L)	ALP (IU/L)	Urea (mg/dl)	Creatinine (mg/dl)
				Mean±SD	Mean±SD	Mean±SD	Mean±SD	Mean±SD
1	Control	Negative	0	8.5±0.71	11.5±0.71	770±183.85	8.5±0.71	0.55±0.03
			3	7.5±0.71	39.5±14.85	260±98.99	9±1.41	0.52±0.06
			7	8.5±0.71	15±4.24	887.5±17.68	10.5±0.71	0.54±0.02
2a	*S. aurous*	-	0	7±1.41	14±1.41	875±304.06	9±1.41	0.51±0.08
			3	9±1.41	17±1.41	360±134.35	9.5±0.71	0.51±0.08
			7	11±1.41	22±5.66	632.5±519.72	9.5±0.71	0.51±0.08
2b		+	3	7.5±0.71	17±1.41	1020±169.71	9.5±2.12	0.53±0.05
			7	7.5±0.71	17±4.24	722.5±215.67	9.5±0.71	0.54±0.05
3 a	*S. aurous* + *E. coli*	-	0	7.5±0.71	14.5±4.95	1010±56.57	9.5±0.71	0.54±0.04
			3	16.5±6.36	36.5±14.85	780±311.13	11.5±2.12	0.51±0.08
			7	14.5±3.54	22.5±4.95	1120±28.28	9±1.41	0.6±0.06
3b		-	3	9±1.41	20.5±0.71	845±388.91	10±0	0.52±0.08
			7	8.75±1.77	16.25±2.47	975±49.5	9.75±0.35	0.54±0
4 a	*E. coli*	-	0	8.5±0.71	11.5±0.71	770±183.85	8.5±0.71	0.55±0.03
			3	6.5±0.71	26.5±12.02	1230±42.43	8±0	0.5±0.07
			7	12.75±5.3	32.25±6.01	920±197.99	9.75±2.47	0.48±0.04
4b		+	3	9±1.41	12±0	875±318.2	10±2.83	0.53±0.01
			7	9.5±2.12	13.25±1.77	952.5±74.25	10.75±2.47	0.58±0.02

GPT: Glutamate pyruvate transaminase. GOT: Serum glutamic oxaloacetic transaminase. ALP: Alkaline phosphatase.

treated *S. aurous* groups were 9.5±0.71 and 0.54±0.05; 9.75±0.35 and 0.54±0 for *S. aurous* + *E. coli, as* well as10.75±2.47 and 0.58±0.02 for *E. coli*. The values in treated groups were close to those of control. Marked reduction in serum albumin concentration and TP was seen in *E. coli* infected groups (Saini, 2004; Raheja and Jakhar, 2005; Zaki et al., 2012; Kumari et al., 2014; Sharma et al., 2015).

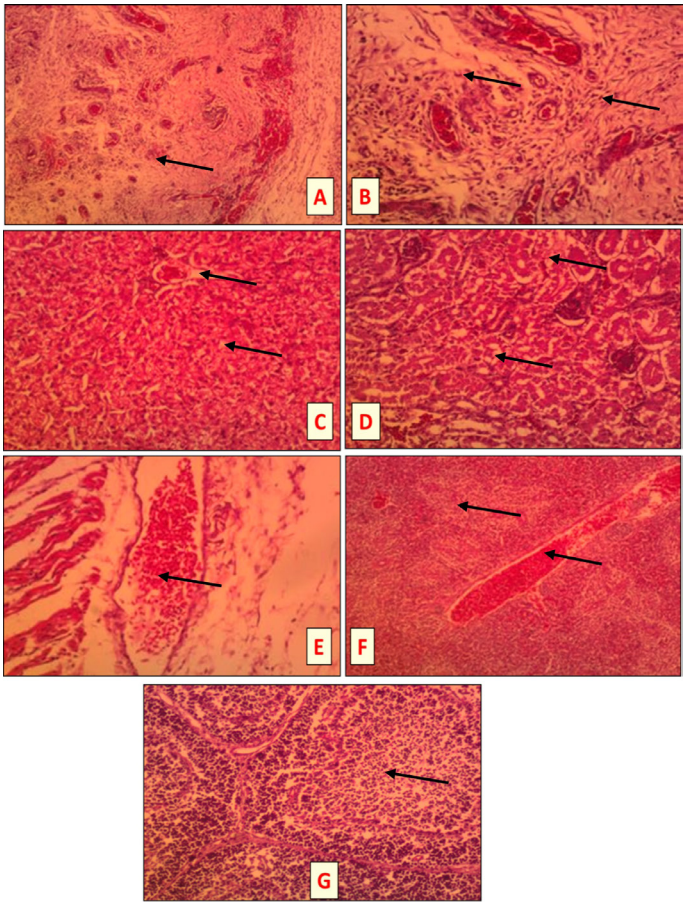

Figure 2: Tissue sections of *S. aureus* and /or *E. coli* infected birds at 3 DPI (HandEX, 200) showing the following lesions. A: Subcutis of *E. coli* infected bird: Severe suppurative inflammation characterized by heterophils and mononuclear cells infiltration in s.c tissues (head of arrow). B: Subcutis of *S. aureus* and *E. coli* infected bird: severe suppurative inflammation with marked accumulation of heterophils and mononuclear cells in s.c fatty tissue (head of arrow). C: Liver of *S. aureus* infected bird: hydropic degeneration of the hepatocytes (head of arrow). D: Kidney of *S. aureus* + *E. coli* infected bird: severe hydropic degeneration (head of arrow). E: Subcutis of *S. aureus* infected bird: subcutaneous hemorrhages (head of arrow). F: Spleen of *S. aureus* infected bird: congested red bulb and blood vessels with necrotic area (head of arrow). G: Bursa of *E. coli* and/or *S. aureus* infected bird: depletion of the lymphoid follicle (head of arrow).

Histopathological examination of tissue section of control non-infected non-treated (control negative) group showed normal tissue structure in bursa, kidney, liver, muscles,

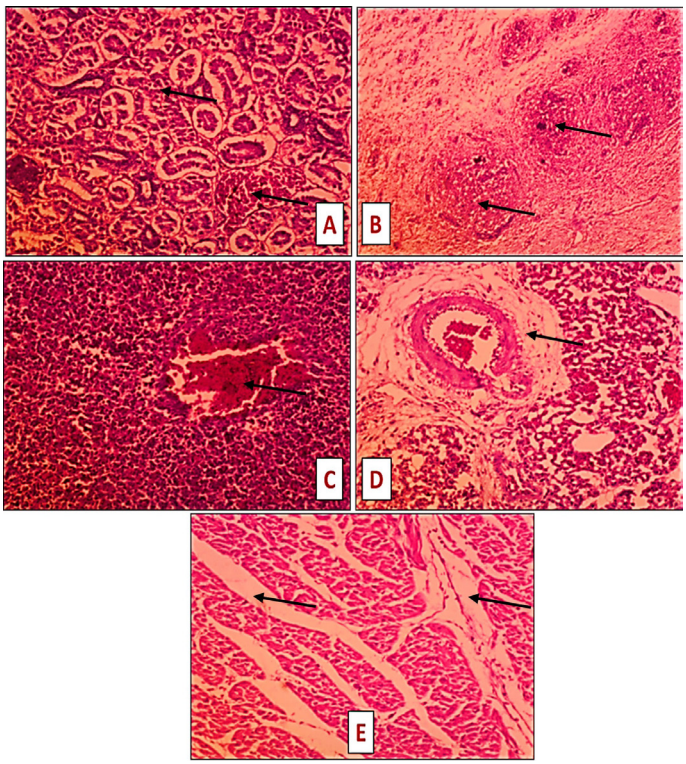

Figure 3: Tissue sections of *S. aureus* and /or *E. coli* infected non treated birds (7 DPT) (HandE, X 200) showing. A: *E. coli* infected bird: kidney hemorrhages accompanied with coagulative necrosis of some tubules (head of arrow). B: *E. coli* infected bird: s.c severe suppurative inflammation with marked accumulation of heterophils and mononuclear cells in s.c fatty tissue (head of arrow). C: *E. coli* infected bird: spleen hemorrhages (head of arrow). D: *S. aureus* infected bird: perivascular edema of the pulmonary artery (head of arrow). E: *S. aureus* + *E. coli* infected bird: muscle edema (head of arrow).

spleen and skin. Microscopical examination of tissues for infected non treated groups showed that, subcutis tissue of *E. coli* infected and mixed infected (*S. aureus* + *E. coli*) groups exhibited severe inflammatory reaction with accumulation of suppurative exudate with massive heterophils and mononuclear cells infiltration in s.c fatty tissue (Figures 2A, B and 3B) accompanied with muscle edema (Figure 3E) while showed milder s.c reaction in case of *S. aureus* infected group (Figure 2E). The liver of mixed infected group and *S. aureus* infected group showed hydropic degeneration (Figure 2C), while *E. coli* infected bird showed milder liver lesion. Kidney of mixed infected (*S. aureus* + *E. coli*) group exhibited severe hydropic degeneration (Figure 2D) while in case of *E. coli* or *S. aureus* infected group, kidney appeared hemorrhagic accompanied with coagulative necrosis of some tubules (Figure 3A). The bursa of all infected groups showed depletion of the lymphoid follicle (Figure 2G). The spleen of all groups appeared hemorrhagic (Figure 3C) but also accompanied with congested red bulb and blood vessels in addition to necrotic area in case of *S. aureus* infected

group (Figure 2F). Also, the lung of *S. aureus* infected group exhibited perivascular edema of the pulmonary artery (Figure 3D). Similar histopathological lesions were detected (Barnes et al., 2003; Kamel, 2011; Asmaa, 2013; El-Sawah et al., 2018; Abd Elatiff et al., 2019).

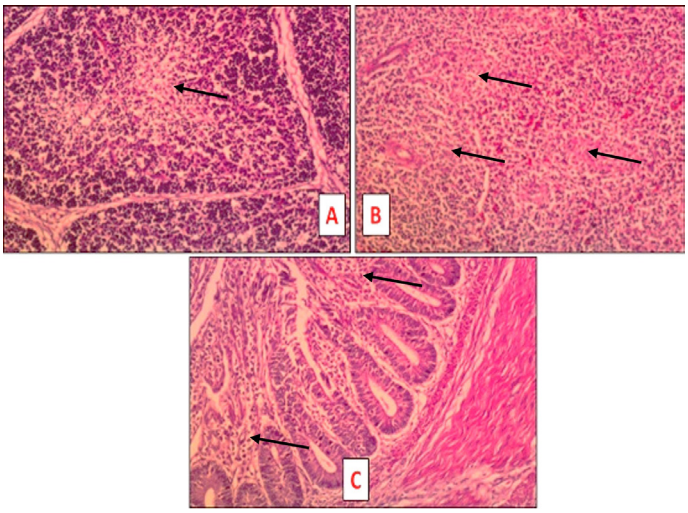

Figure 4: Tissue sections of *S. aureus* and /or *E. coli* infected treated birds at 7 DPT (HandE, X 200) showing the following lesions. A: *S. aureus* + *E. coli* infected treated bird: bursa slight depletion of the lymphoid follicle (head of arrow). B: *S. aureus* + *E. coli* infected treated bird: spleen slight depletion of the lymphoid follicle (head of arrow). C: *S. aureus* infected treated bird: intestine light inflammation characterized by lymphocytic infiltration of the mucosa (head of arrow).

Microscopical examination of tissues for *S. aureus* and/or *E. coli* infected treated groups revealed slight depletion in both bursal tissue (Figure 4A) and spleen (Figure 4B) was seen in mixed infected treated group. Also, intestine of *S. aureus* infected treated group showed light inflammation (Figure 4C).

CONCLUSION AND RECOMMENDATIONS

Cellulitis in broilers composes an essential reason for carcass condemnation at slaughterhouses. Our result pointed out that experimental infection of broiler chicken with *S. aurous* and/or *E. coli* resulted in induction of cellulitis with marked pathological and histological changes. Skin lesions were measured. Effect on broiler performance was detected. Blood analysis and serum biochemical parameters were studied but were of low value in diagnosis. Doxycycline + colistin combination was used to treat infected birds where it helps in reduction of lesions, with somewhat improvement in measured parameters.

We recommended several actions can be taken to oppose or minimize cellulitis as reinforcing feather coverage, observing birds density, continuous enhancing of biosecurity, modulating the vaccinations timing, improving management practices, control immunosuppressive factors as well as application of probiotics in order to improve and restore good gut health.

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NOVELTY STATEMENT

The primary purpose of this research is to study the influences of experimentally induced cellulitis on broiler performance and recognize the gross and histopathological changes. In addition to investigate the effect of antibiotic treatment in control of this infection.

AUTHOR'S CONTRIBUTION

AMM, KME-B designed this study and supervised laboratory work. HSF, HMM, AAE-S collected samples and performed all laboratory work. BMA carried out histopathological examination. All authors shared manuscript writing, drafted, revised the manuscript, and approved the final manuscript.

Data availability

The authors affirm that the data bolstering the results of this research are accessible within the article in addition to its supplementary materials.

Ethical approval

All work design and procedures were approved by Medical Research Ethics Committee (MREC), National Research Centre, Egypt with Approval number 7427082021.

Abbreviations

ABWG, Average body weight gain; ALP, alkaline phosphatase; ALT, alanine transaminase; AST, aspartate transaminase; CFU, colony forming units; DPI, day post infection; DPT, Day post treatment; FC, Feed consumption, FCR, Feed conversion ratio; FI, feed intake; GOT, glutamic oxaloacetic transaminase; GPT, Glutamate pyruvate transaminase; HandE, hematoxylin and eosin; H/L, Heterophils/lymphocytes; Hb, Hemoglobin; LDH, lactate dehydrogenase; PCV, Packed cell volume; PM, postmortem; RBC, Red Blood Cell; TLC, Total Leukocyte Count; TWBC, Total white blood cell; WBC, White Blood Cell.

Conflict of interest

The authors have no conflict of interests regarding the publication of this paper. Also, the authors declare that the work was self-funded.

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