

Incidence of Some Bacterial Agent Causing Arthritis of Broiler Breeder Flocks at North Sinai Governorate

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ABSTRACT

One hundred broiler breeders from 16 to 72 weeks old were clinically examined they were suffering from symptoms of arthritis, necrosis of comb and congestion of liver in different breeders farms at North Sinai Governorate. Specimens from (liver, joint and comb) were collected from diseased or freshly dead birds for the detection & possible bacterial isolation that proved the presence of *Staphylococcus aureus*.

The experimental infection of 4 weeks old chicken was carried out, with the isolated *S. aureus* by I/V inoculation which revealed septicemia, morbidity and mortality rate reach to 33% and 20% respectively within 3 days post inoculation with congestion and enlargement in all internal organs. On the 6th day, chickens suffered from movement where the knee joint was inflamed with depression and ruffling feathers. On the 9th day post inoculation, 60% of the birds showed lameness.

On the 3rd week post inoculation (end of experiment), there were congestion and necrosis of the liver, and purulent exudates in the joint, accompanied by congestion of the blood vessels, and congestion of the kidney with precipitation of urates in ureters and also decrease of body weight of the bird.

The biochemical analysis showed a picture of hepatocellular and kidney changes, which was confirmed by an increased level of serum AST, ALT and associated with significant decrease of cholesterol and albumin with normal level of total protein and increase uric acid and creatinine. *Staphylococcus aureus* was found to be highly sensitive to Enrofloxacin, Amoxicillin, and Spectinomycin.

INTRODUCTION

The most frequent sites *Staphylococcus aureus* infections in poultry are bones, tendons sheaths and joints, especially tibiotarsal and stifle joints. *Staphylococcal* infections occur less frequently in skin, sternal bursa and granulomas in the liver and lungs (1). Infection is usually characterized by increased heterophils count and marked heterophilic infiltration of tendons, synovial membranes and other affected organs (2). *Staphylococcal* septicemia causes acute deaths in laying hens (3), it seems to be the most prevalent in hot weather and resembles fowls cholera. The routes of entry, pathogenesis and host response are not completely defined.

The disease is usually chronic and poultry responds to antimicrobial therapy (4). *Staphylococcal* microorganisms, which have

been isolated frequently from poultry, include *S. aureus*, *S. epidermidis* and *S. gallinarum* (5). In addition to being a major disease-producing organism, approximately 50% of typical and atypical *S. aureus* strains produce exotoxins that can cause food poisoning in human beings (6). Inoculation of 4- 6 weeks old broilers with *S. aureus* resulting in subacute fibrinopurulent arthritis (7).

S. aureus affected the biochemical constituents of serum such as increase of alanine aminotransferase (ALT), Aspartate aminotransferase (AST), Uric acid and creatinine (8). The aim of this study was to investigate the incidence of *staphylococcal aureus* in broiler breeder's farms suffering from arthritis in North Sinai and to chock the *in vitro* sensitivity test of the isolated *S. aureus* against different antimicrobial discs.

MATERIAL AND METHODS

A-Material

- Total numbers of 100 diseased and freshly dead birds broiler breeders suffering from arthritis were collected from different localities at North Sinai Governorate, were subjected to examination (16 - 72 weeks of age).
- Experimental birds, 50 Balady chicks 4 weeks old obtained from commercial broiler flocks were used to study the Pathogenicity of *S. aureus*.
- Media: - Baird Parker agar - nutrient agar - nutrient broth - blood agar - MacConkey agar
- Antibacterial sensitivity Disk produced by Oxoid
- Diagnostic Kits for liver and kidney function test from (Biomerieux / France)
- Blood samples were collected from sacrificed birds about of 0.5ml on EDTA used for hematological studies and about 3ml was collected in centrifuge tube, left to clot, centrifuged at 1500 RPM. For 10 minutes and the serum was separated in clean dry vial for clinico- biochemical studies.
- Experimental design, 5 birds were bacteriologically examined to prove that they are free from *S. aureus* spp. The other birds were divided into three equal groups each of 15 birds. Group 1 was infected non-treated group route of inoculation I/V and dose of inoculation (1×10^9) CFU / ml (9). Group 2 was infected I/V and dose of inoculation (1×10^9) CFU/ ml and treated with Enrofloxacin 10 mg / kg Bw. In drinking water, group 3 control group non infected non treated all birds were observed for 3 weeks and clinical findings were recorded, dead birds were necropsied and the trials of the re- isolation of *S. aureus* from organs and joint were carried out.

B-Methods

- Clinical and postmortem examination

-Bacteriological examination: Under aseptic condition a loopful from liver, joint (synovial fluid) and comb were inoculated into nutrient broth and incubated at 37°C for 18- 24 h followed by subculturing on selective media for *S. aureus*, as bird - parker agar media at 37°C for 48h.

Also a loopful from inoculated broth subculture on blood agar ; macConkeys agar media and aerobically incubated at 37°C for 24h the suspected growing colonies of *staphylococcus aureus* were kept on to slope tubes for further identification (5&9).

- **Bacterial titration:** infecting dose (10).
- Biochemical analysis, liver function tests, kidney function tests and hematological techniques (11).
- Pathogenicity test

Fifty Balady chickens 4 weeks old were used in this study , 5 birds were slaughtered to be sure free from *S. aureus* and others chicken were divided into 3 groups each group contained 15 birds, group1 were inoculated I/V with 1ml (1×10^9 CFU/ml) of live cell of *S. aureus* (isolates). Group 2 birds were inoculated I/V with 1ml (1×10^9 CFU/ml) of isolate *S. aureus* and treated with Enrofloxacin, 10 mg/kg body weight in drinking water for 5 successive days after infection, group 3 birds kept as control. All birds kept under observation for 21days clinical symptoms; postmortem examination and reisolation were done.

- Sensitivity test: The determination of sensitivity of isolated organism against different antibiotic discs was done (12).
- The obtained data were statistically analyzed (13).

RESULTS

Clinical Signs

The affected breeders (male and female) had swollen joints, on their hocks, keel bone, and were unable to stand, reluctance to move, gradual emaciation and finally died.

Gross Lesion

Postmortem examination of naturally infected chickens showed swelling and necrosis of the comb, necrosis and congestion of liver, spleen, kidneys and lungs other birds had affected swollen joints filled with inflammatory exudates.

Isolation and identification of causative agent

Bacteriological examination revealed that the suspected colonies were 1-2 mm in diameter on blood agar within 24 hours, and give β -hemolytic, most colonies were pigmented white to orange on nutrient agar. On Baird - Parker agar media colony was black surrounded by faint yellow zone, Gram positive cocci arrange in clusters prove that the isolates was *staphylococcus aureus*. Other microorganisms were isolated and not identified and that's due to lack of qualities.

In vitro sensitivity of *staphylococcus aureus* isolates to antibacterial agents are showed in Table 1.

Pathogenicity of *staphylococcus aureus* to chickens after short incubation period (48h). The inoculated chicks showed, Ruffled feathers, lameness, followed by depression, congestion and enlargement of internal organs. On the 6th day (PI) birds surviving the acute disease had swollen joint on their hocks and were unable to stand. Beside this, there were weakness and decrease body weight. The mortality rate reached 20% and morbidity (66%). On the 9 days (PI) 66% of infected birds had lameness and at the end of experiment (3weeks PI) when we opened joint, we noticed white to yellow purulent exudates and congestion of blood vessels. In addition, there was congestion, focal area of necrosis in the liver, congestion of kidney with precipitation of urates in the ureters. There were absence of signs, lesions or death in any bird in 2nd group and control group. Re-isolation of *staphylococcus aureus* was performed as shown in Table 2. Results of biochemical and hematological studies of the blood picture and serum samples at 7, 15 and 21 days post infection were recorded in Table 3,4.

Table 1. Results of sensitivity test of *S. aurous* isolates strain.

Antimicrobial agents	Disk potency	Stander inhibition zone	No. Of isolates	No. Of sensitive isolates	%
Kanamycin	30 ug	> 18 > 23	10	10	100%
Penicillin (P)	10 ug	> 13 > 18	10	3	30%
Spectinomycin (SM)	100 ug	> 13 > 18	10	10	100%
Amoxicillin (AMX)	10 ug	> 18 > 23	10	9	90%
Lincomycin	10 ug	> 11 > 13	10	7	70%
Tetracycline (T)	10 ug	> 11 > 13	10	5	50%
Gentamycin (GM)	10 ug	> 15 > 19	10	0	0%
Neomycin (N)	30 ug	> 13 > 18	10	2	20%
Enrofloxacin	5 ug	> 13 > 18	10	10	100%
Novobiocin (NOB)	10 ug	>10>15	10	1	10%

Table 2. Results of 1/V injection with *staphylococcus aureus* isolates to 4weeks old chickens, comparison with those groups treated with Enrofloxacin.

Groups	Clinical signs	Mortality rate	Swollen hock	Re-isolation
1-1/V injection group with 1ml of 1×10^9 CFU of <i>S. aureus</i> /chicken	5/15	3/15	2/10	3/4
2-1/V injection with 1ml of 1×10^9 CFU of <i>S. aureus</i> / chicken - treated with Enrofloxacin 10 mg/kg b.w for 5 successive days for chicken	1/15	0	0	0
3-Control group (non infected and non treated)	0	0	0	0

Table 3. Hematological studies in broiler experimentally infected with *S. aureus* at 7, 15 and 21 days post infection

Time of sampling (day)	Group	RBCs M / ul	PCV %	WBCs M / ul	Absolute count $\times 10^3$ cell / ul					HB%
					L	M	H	E	B	
7	G1	1.6±0.1	41.9±0.9	18.22±0.33	51.95±0.52	7.12±0.1	29.55±0.42	1.77±0.15	1.47±0.1	6.5±0.12
	G2	1.62±0.07	41.3±0.35	17.72±0.24	49.51±0.5	7.22±0.07	28.7±0.5	1.72±0.02	1.8±0.15	6.75±0.16
	G3	3.3±0.04**	46.5±0.5**	16.89±0.04***	48.6±0.44	7.33±0.2	25.9±0.34	1.63±0.14	1.43±0.3	11.3±0.5**
15	G1	1.26±0.05	24.02±1.25	16.13±0.4	57.85±0.57	7.63±0.1	27.65±0.34	1.67±0.15	1.83±0.1	6.55±0.12
	G2	1.95±0.04	23.03±0.02	16.32±0.5	58.73±0.45	7.67±0.09	28.19±0.8	1.59±0.12	1.74±0.24	7.45±0.42
	G3	3.16±0.16**	32.4±0.36*	15.53±0.36	62.83±0.54*	7.41±0.2	27.46±0.49	1.54±0.14	1.9±0.2	11.6±0.42
21	G1	3.26±0.07	31.02±1.25	16.14±0.5	59.75±0.55	7.68±0.52	28.85±0.5	1.58±0.15	1.68±0.1	7.55±0.12
	G2	2.3±0.07	24.5±1.01	16.64±0.32	58.49±1.13	7.53±0.49	28.57±0.45	1.57±0.34	1.75±0.14	7.74±0.39
	G3	3.45±0.6*	43.8±0.5	15.2±0.3	62.31±0.44	6.54±0.3	26.76±0.33	1.45±0.4	1.76±0.03	10.98±0.4

L = lymphocytes M = Monocytes H = Heterophils E = Eosinophils B = Basophils
 WBCs = total leucocytic count RBCs = total erythrocytic count PCV% = Paked cell volume

Table 4. Biochemical studies for liver and kidney function tests in broiler experimentally infected with *S. aureus* at 7, 15 and 21days post infection

Time of sampling (day)	Group	AST (U/L)	ALT (U/L)	Creatinine (Mg / dl)	Cholesterol (Mg / dl)	Uric acid (Mg / dl)	Total protein (Gm / dl)
7	G1	118.20±4.54	24.08±0.37	1.1±0.01	94.5±5.84	9.56±0.1	2.81±0.1
	G2	115.1±1.37	23.89±0.5	1.4±0.14	88.2±4.57	8.64±0.52	3.15±0.3
	G3	47.6±3.4**	12.89±0.3***	0.49±0.05	80.12±5.3	4.98±0.9*	4.2±0.03
15	G1	142.20±0.89	22.08±0.3	1.15±0.012	130.5±3.84	10.8±0.5	1.42±0.14
	G2	91.88±0.83	21.1±0.41	0.95±0.03	118.6±4.97	7.03±1.02	2.53±0.02
	G3	87.3±3.5*	13.2±0.18*	0.45±0.05	79.5±0.9	5.8±0.5	3.92±0.01
21	G1	127.20±1.89	23.08±0.27	0.97±0.01	97.5±.84	9.7±1.4	1.85±0.04
	G2	69.74±2.15	22.29±0.19	0.69±0.07	61.4±2.23	7.8±0.06	2.42±0.05
	G3	65.5±0.54	14.5±0.3	0.47±0.03	64.1±0.9	5.9±0.8	2.99±0.02

Week significant P < 0.1

Highly significant P < 0.5

DISCUSSION

The economic significance of *S. aureus* infection in poultry industry is difficult to determine because of prolonged mortality seen over time with poultry flocks, this mortality results not only in the breeder but also in the potential loss of broilers. *Staphylococcal* infections are a worldwide problem in chicken and turkeys and cause economic losses due to and weight gain, decrease of egg production (14).

S. aureus was isolated from swelling and necrotic comb, a finding that has been recorded previously in layer breeder chickens (4). In addition, *S. aureus* was isolated from birds suffering from joint affection and lameness, congestion, necrosis of liver, kidney and lung, of chickens and turkey (1).

In the present study chickens naturally infected with *S. aureus* showed clinical signs as swollen joints and keel bone and unable to stand, similar signs were recorded for experimental birds that inoculated with *staphylococcus aureus* this observation was previously noticed by several investigators (7 & 9).

The isolates were β hemolytic colonies with golden color and Gram-positive cocci arranged in clusters. Similar findings, were cited (7).

Experimental infection of the isolated *staphylococcus aureus* to 4 weeks old chicken showed the clinical signs and post mortum findings manifested by depression, swollen hocks joint, unable to stand, congestion and enlargement of all internal organs. The liver was congested and had focal area of necrosis with mortality rate reached 20% which may be due to enterotoxin of *S. aureus*. Similar results were previously reported (2 & 4).

Also intravenous inoculation of chickens with the isolated *S. aureus* revealed lameness and joint affection where bacterial arthritis in the knee joint, an important causes of lameness

in broilers with clinical evidence of joint problem. The pathogenesis of these lesions depend mainly on the ability of the organism to reach the circulatory system which may be through wound infection due to trauma or mosquito bites in naturally infected cases or through intravenous inoculation experimental study. *S. aureus* has a high affinity for collagen rich surfaces such as the articular surface of the joints and synovial sheaths located around joints and tendons, the organism tend to localize in the growth plate of actively growing bones (5).

S. aureus organism could be re-isolated from experimentally infected birds (4). The antibiogram test revealed that Enrofloxacin and Amoxicillin were the most effective drugs; which confirm previous study in birds and turkey (1).

The biochemical findings of liver function test in this study denoted very high significant increase in the level of AST, ALT and cholesterol, indicating hepatocellular damage after infection of *S. aureus*. In addition, the significant increase in the level of uric acid and creatinine indicating kidney function was affected with infection. Hematological findings showed macrocytic hypochromic anemia, leucocytosis associated with heterophillia as compared with healthy control group these results were similar to that carried out in rabbits infested with coccidiosis (15).

Conclusion: This study proved that *S. aureus* infection caused large economic losses in broiler breeding as high morbidity, swollen joints, and mortality in the laboratory and field cases, so the *Staphylococcal* infection in poultry farm must be controlled.

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المخلص العربي

مدى انتشار بعض البكتريا المسببه للعرج فى قطعان امهات التسمين بمحافظة شمال سيناء

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تم جمع ١٠٠ عينه (الكبد,المفصل والعرف) من دجاج عمر ١٦- ٧٢ اسبوع مريض وناقح حديثا , وكانت الاعراض الظاهرية عباره عن عرج بالارجل وتتركزبالعرف ويعمل الصفه التشريحيه وجد احتقان بالكبد والكلى وتتركزبالكبد مع ترسيب اليوريك فى الحالب وذلك من مزارع مختلفه بمحافظة شمال سيناء وتم زرع العينات على مستنبتات بكتيرييه مختلفه خاصه بميكروب المكور العنقودى الذهبى وبعد عمل الاختبارات البيوكيميائيه تم التعرف على ميكروب المكور العنقودى الذهبى وتم عزل ميكروبات اخرى ولم نتمكن من التعرف عليها لعدم توفر الامكانيات.

تم اجراء العدوى الصناعيه على دجاج عمر ٤ اسابيع بالعترات المعزوله عن طريق الحقن الوريدي والذى ادى الى اعراض ونفوق بنسبة ٢٠% خلال يومين من بداية العدوى مع وجود احتقان وتضخم فى الاحشاء الداخليه وفى اليوم السادس من العدوى ظهرت مشاكل فى حركة الطيور وظهرت التهابات فى المفصل مع ضعف ظاهر على الطيور المصابه وفى اليوم التاسع من بداية الحقن كان حوالى ٦٠% من الطيور المصابه يعانى من صعوبة الحركة مع التهاب فى مفاصل الارجل .

وفى نهاية التجريه (٣ اسابيع من بداية العدوى) وجد تضخم فى الكبد مع تتركز واحتقان فى الاوعيه الدمويه و احتقان بالكلى مع ترسيب حمض اليوريك فى الحالبين وكذلك وجد صديد فى المفصل المصاب مع احتقان بالاوعيه الدمويه بالاضافه الى ضعف الطيور ونقص الوزن.

اما بالنسبه للمجموعه التى اخذت العلاج بعد العدوى كانت الاعراض اقل وضوحا عن المجموعه الاولى وباجراء اختبار الحساسيه على ميكروب المكور العنقودى الذهبى المعزول وجد ان الانتروفلوكساسين والاسبكتنومايسين الاكثر تاثيرا على العترات المعزوله.