

Pathogenicity of Coagulase Negative Staphylococcus Chicken Isolates to 10 Days Old Broiler Chickens

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ONE HUNDRED and sixty, 1-day old broiler chicks were grouped into 4 equal groups, at the 10th day birds of groups 1- 3 were s.c inoculated with 0.5 ml containing 1.5×10^8 of *S.xylois*, *S. sciuri* and *S.lentus*; respectively and group 4 was noninfected control.

Clinical signs in infected groups started at 2-3dpi as general signs. Signs disappear in Ciprofloxacin treated subgroups 24 hr post treatment and lasted to the 7th day in non treated.

Average body weight gain in *S.xylois* infected non treated was the highest (813.90 gm), followed by *S. sciuri* (778.50gm) and 773.75 in *S. lentus* infected treated . FCR was the highest in control (1.69 treated and 1.74 non treated) followed by *S. sciuri* infected (1.81 non treated and 1.82 treated) and the lowest 1.94 was in *S.lentus* infected non treated. *S. sciuri* was reisolated from intestine and spleen (5th dpi) and from intestine (7 and 10 dpi). While *S.lentus* was reisolated from intestine , liver and spleen (3rd dpi) ; from intestine and spleen (5th dpi) and intestine (10th dpi).

Histopathological lesion was recorded in infected group as hemorrhages with sinusoidal dilation, focal areas of vacuolar degeneration , fatty degeneration and shrinkage of hepatocytes in liver, necrotic changes of lymphocytes and vacuolion of corpuscle in spleen. Leucocytic infiltration , degeneration and necrosis of epithelium surface and intraepithelial as well as submucosal leucocytic infiltration were seen in intestine.

In conclusion the injected organisms induce mild subclinical disease with recording of histopathological lesions in liver, spleen and intestine. This area needs more investigation to explore pathogenicity of CoNS in chickens.

Keywords: Broilers, CoNS infection, FCR, Ciprofloxacin, Histopathology

Introduction

Staphylococcosis is a systemic disease of birds caused by avian strains of Staphylococcus spp. [1,2,3]. The infection is common in poultry and game birds; especially in turkeys and broilers. Birds 4 to 6 weeks of age are extremely vulnerable; the most frequent sites of infections are bones, tendon sheaths, and leg joints [4]. Coagulase-negative staphylococci (CoNS) are mostly normal skin commensals and are much less pathogenic than *S. aureus* [5]. CoNS infections in chickens are considered to be opportunistic [6] and under

the appropriate conditions became pathogenic. Research interest in CoNS has increased over the past decade due to their implication in infections in both humans and animals [7,8].

CoNS was reported to be isolated from clinically infected chickens with cellulitis in broiler chickens [9] granulomas in the liver and lungs [10,11], gangrenous dermatitis , sub-dermal abscesses with the wing tips and the dorsal pelvic region [12,13] *S. xylois* and *S. simulans* were recovered from infected bone [14]. Infections with staphylococcus are usually characterized

by increased heterophil counts and marked heterophilic infiltration of tendons, synovial membranes, and other affected organs [15].

Pathogenicity of *S. aureus* is due to toxic β -hemolysin and plasma coagulase [16]. Cunha Mde et al. [17] analyzed the CoNS virulence factors including hemolysins, lipase, lecithinase, DNase, thermonuclease, and enterotoxin A, B or C in 37.6% of tested isolates. Zell et al. [18] demonstrate hemolytic activity and the exfoliative toxin A (ETA). Enterotoxins producing *S. aureus* is the most common cause of food-borne human illness throughout the world [19], the other CoNS species such as *S. hyicus*, *S. sciuri*, *S. xyloso* or *S. cohnii* are also important, particularly because of carriage the genes encoding antimicrobial resistance and enterotoxins genes [13,20,21].

The present study is an attempt to detect pathogenicity of CoNS chicken isolates to 10 days old broiler chickens.

Material and Methods

Chickens

One hundred and sixty, 1-day old broiler chicks were obtained as hatch from commercial hatchery. The used chicks were floor reared and fed on a balanced commercial ration.

Ration

The chicks were fed on ration according to the Ross broiler management manual and *NRC* [22] requirement for broiler. All housed chickens were given ration ad libitum.

Bacterial strains

CoNS strains *S. xyloso*, *S. sciuri* and *S. lentus* isolated and identified from broiler chicken flocks [23]. The isolates were separately inoculated on 5% blood agar base and Mannitol salt agar (Difco) then incubated for 24-48 hours at 37°C. The resulted colonies were examined for identical morphological character of *Staphylococcus* species [24].

Preparation of Bacterial Inoculums

Used isolates were inoculated into Tryptic Soy Broth (TSB) and incubated at 37°C for 24 hours. Infective dose of *S. xyloso*, *S. sciuri* and *S. lentus* was adjusted using colony forming units (cfu) was determined by plating tenfold dilutions on Tryptone soya agar as 0.5 ml contains approximately 1.5×10^8 colony forming units (CFU/ml) [25].

Reisolation of inoculated CoNS

Cultures from liver, spleen and intestine of broiler chicks in tryptone soya broth then subcultures on mannitol salt agar finally Gram stained smears from these colonies were examined [24].

Histopathological examination

Tissue samples were collected from liver, spleen and intestine of all birds. These samples were fixed in neutral formalin 10% for 48hrs then underwent processing and staining according to Bancroft and Gamble [26]. After fixation, the sample were washed in running water, dehydrated in graduated ethyl alcohol, cleared in xylene and embedded and blocked in paraffin wax. Five microns tissue sections were mounted on clean glass slides and stained with hematoxyline and Eosin (H&E stain).

Experimental design

At the 10th day of age the used 160 chicks were divided into 4 equal groups (1-4); 40 chicks each. Chickens of groups 1, 2 and 3 were S/C inoculated with 0.5 ml 1.5×10^8 of *S. xyloso*, *S. sciuri* and *S. lentus*; respectively. Birds of group 4 were kept as noninfected control group. At the 15th day with appearance of signs each group was divided into two equal sub groups. Subgroup a was treated with Ciprofloxacin and subgroup b was kept as infected not treated. The treatment was done using Ciprofloxacin (sensitive *in vitro*) in a dose of 1cm/litre for 5 days. All groups were subjected to daily observation for clinical signs and mortalities with recording of average weekly BWG and FI for calculation of FCR [27]. Two birds/group were randomly weighted and sacrificed at 3, 5, 7 and 10 days post infection (dpi) as well as 2 birds from control group for P.M with recordings of lesions and collection of intestine, spleen and liver samples in formal saline for histopathological examination.

Results and Discussion

To study pathogenicity of CoNS in broiler chicks at the 10th day birds of groups 1, 2 and 3; 45 chicks each; were S/C inoculated with 0.5 ml 1.5×10^8 of *S. xyloso*, *S. sciuri* and *S. lentus*; respectively. Clinical signs in infected groups at 2-3dpi in the form of general signs of illness including depression, ruffled feather and off food. Group 3 infected with *S. lentus* showed additionally slight brownish diarrhea in 2 chicks.

After 24 hours post treatment signs started to disappear in treated subgroups while nontreated ones restored their activity at the 10th day (the 5th post treatment). This result can prove the possible efficacy of used drug as signed in disc sensitivity.

Performance of infected groups those consumed higher feed intake than noninfected control group (Table 1) while the control noninfected showing the lowest feed intake. Average body weight gain of infected non treated subgroups with *S.xylois* (Gr 1b) was the highest

(813.90 gm), followed by (778.50gm) in *S. sciuiri* (Gr 2b) and 773.75 in the infected treated *S. lentus* Gr 3a). In the other hand treatment an infection not markedly affect body weight gain. FCR of control noninfected group was the highest 1.69 in treated and 1.74 in non treated while the lowest rate was in group 3b (1.94) followed by 1.92 in groups 1a and 3a. The result proved no marked effect of CoNS on performance [6]. Becker et al. [28] reported that CoNS have become a major nosocomial pathogen despite the normally sub acute and low inflammatory course of these infections in human.

TABLE 1. Average Feed intake/gm, Body weight gain and FCR of CoNS infected Ciprofloxacin treated and nontreated control chickens at 10 days post infection.

Group	Sub-group	Infection	Treatment	Feed intake/gm	Body weight gain	FCR
1	a	<i>S. xylois</i>	+	1434	748.40	1.92
	b		-	1506	813.90	1.85
2	a	<i>S. sciuiri</i>	+	1330	732.05	1.82
	b		-	1405	778.50	1.81
3	a	<i>S. lentus</i>	+	1487	773.75	1.92
	b		-	1453	747.40	1.94
4	a	non infected control	+	1213	717.05	1.69
	b		-	1190	685.25	1.74

S. sciuiri was reisolated from intestine and spleen of infected chickens at the 5th dpi and from intestine at 7 and 10 dpi. While *S.lentus* was reisolated from intestine, liver and spleen at the 3rd dpi and from intestine and spleen at the 5th dpi as well as from intestine at the 10th dpi (Table 2). Cheville et al. [29] detected *S. hyicus* was isolated from birds in all stages of multiple outbreaks of acute severe fibrinopurulent lesions

of the eyelids occurred in chickens and turkeys. Awan [30] studied pathogenicity of field isolates in 5-day-old embryonated eggs. *S. intermedius* or *S. lentus* demonstrated some pathogenicity in embryos while, these bacterial species caused neither clinical signs of acute or chronic nor mortality in 3-week-old broilers. Osman [31] stated that CNS was one of the main causes of avian cellulitis in chickens.

TABLE 2 . Reisolation of CoNS from infected chicken groups.

Group	Infection	Subgroup	Treated	DPI	Organs		
					Intestine	Liver	Spleen
1	<i>S.xylois</i>	a	+	3-10	0	0	0
			-	3-10	0	0	0
		b	+	3	0	1	0
			-	5	1	0	0
			-	7	1	0	0
2	<i>S. sciuiri</i>	a	+	10	1	0	0
			-	3	1	1	0
		b	+	5	1	0	1
			-	7-10	0	0	0
			-	3	2	1	1
3	<i>S.lentus</i>	a	+	5	0	2	1
			-	7	0	0	0
		b	+	10	0	0	1
			-	3	2	2	1
			-	5	1	0	2
4	-	a	+	7	0	0	0
			-	10	0	0	0
		b	+	3-10	0	0	0
			-	3-10	0	0	0
			-	3-10	0	0	0

The recorded histopathological lesion *S.xylois* infected group 1b: Liver at 3rd day dpi showing hemorrhages with sinusoidal dilation (Fig. 1), while focal areas of vacuolar degeneration in the form of fatty change and hydropic degeneration with moderate widening of hepatic sinusoids (Fig. 2) were recorded at 5th and 7th dpi. Spleen having mild to moderate necrotic changes of lymphocytes especially pyknosis and karyorrhexis (Fig. 3) at 3rd,

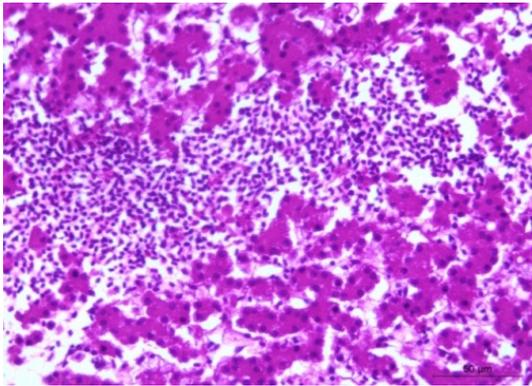


Fig. 1. Liver showing hemorrhages with sinusoidal dilation. (H & E- stain). (X400).

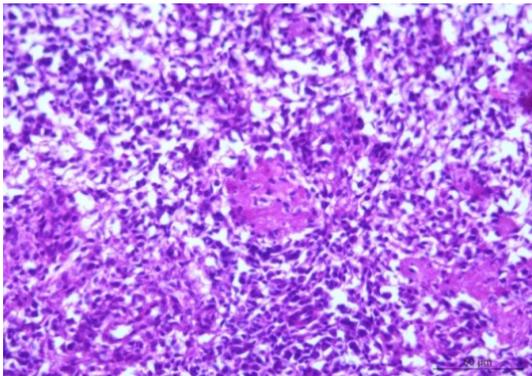


Fig. 3. Spleen having mild to moderate necrotic changes of lymphocytes. (H & E-stain) (X400).

5th and 7th day dpi. Intestine at 3rd day dpi showing moderate enteritis having diffuse leucocytic infiltration (mostly macrophages and lymphocytes) with degeneration and necrosis of intestinal mucosa and muscularis mucosa appeared wavy (Fig. 4) as well as focal submucosal mononuclear cell infiltration with mild degenerative changes intestinal epithelium and cystic dilation of a crypt (Fig. 5) at 5th and 7th dpi.

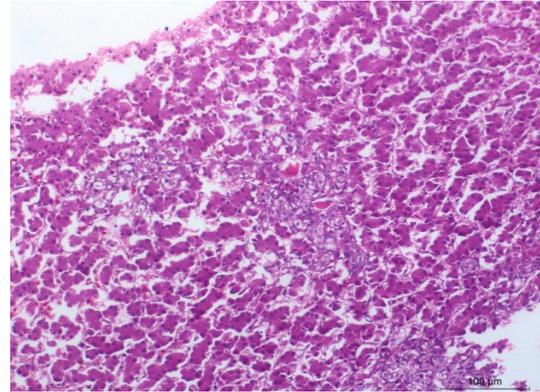


Fig. 2. Liver showing focal areas of vacuolar degeneration in the form of fatty change and hydropic degeneration with moderate widening of hepatic sinusoids. (H & E-stain) (x200).

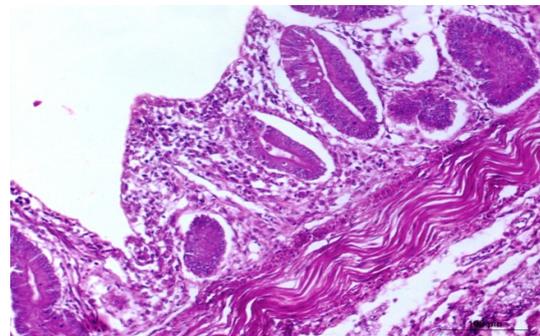


Fig. 4. intestine showing moderate enteritis with degeneration and necrosis of mucosa and muscularis mucosa appeared wavy. (H&E-stain) (X200).

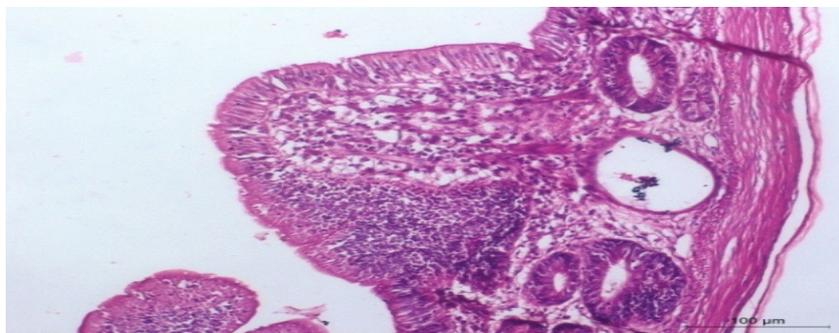


Fig . 5. intestine showing focal submucosal mononuclear cell infiltration with mild degenerative changes intestinal epithelium and cystic dilation of a crypt. (H & E- stain) (X200)

S. sciuri infected group 2b: Liver at 3rd day dpi showing marked widening of hepatic sinusoids with moderate congestion (Fig. 6), at 5th day dpi liver shows mild degeneration with perivascular leucocytic infiltration around central veins (Fig.7). Moreover, diffuse degenerative changes and congestion of blood vessels and hepatic

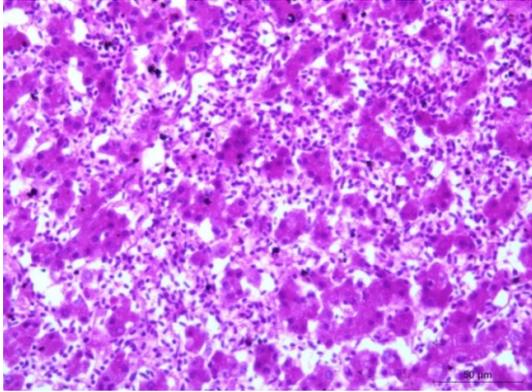


Fig. 6. liver showing marked widening of hepatic sinusoids with moderate congestion. (H & E- stain) (X400).

sinusoids (Fig. 8) were seen at 7th day dpi. Spleen at 5th day dpi showing mild degree of vacuolion of splenic corpuscle (Fig. 9). Intestine at 7th day dpi showing marked enteritis which characterized by severe degeneration and necrosis of intestinal mucosa and leucocytic infiltration (Fig. 10).

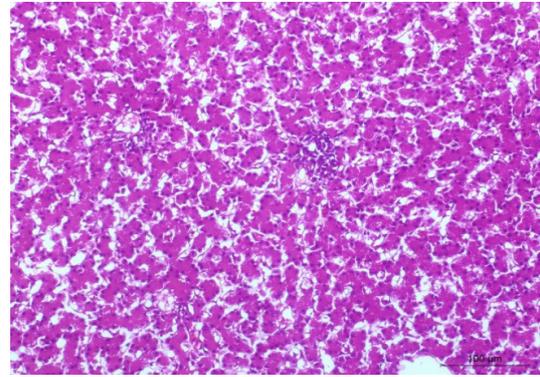


Fig.7. Liver show mild degeneration with perivascular leucocytic infiltration around central veins. (H & E- stain) (X200).

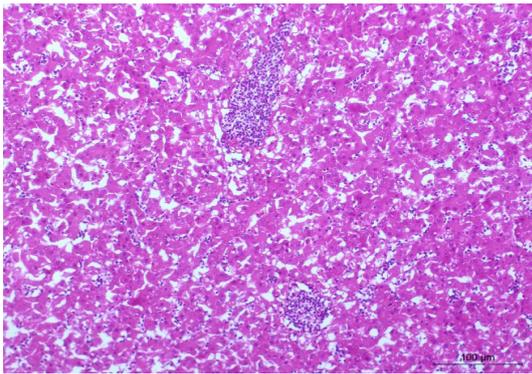


Fig. 8. Liver with diffuse degenerative changes and congestion of blood vessels and hepatic sinusoids (H & E-stain) (X200).

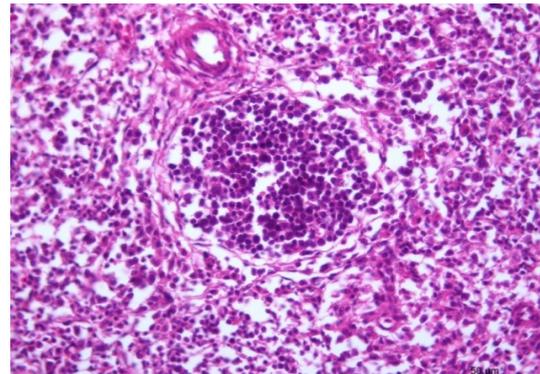


Fig. 9. Spleen showing mild degree of vacuolion of splenic corpuscle. (H & E- stain). (X400).

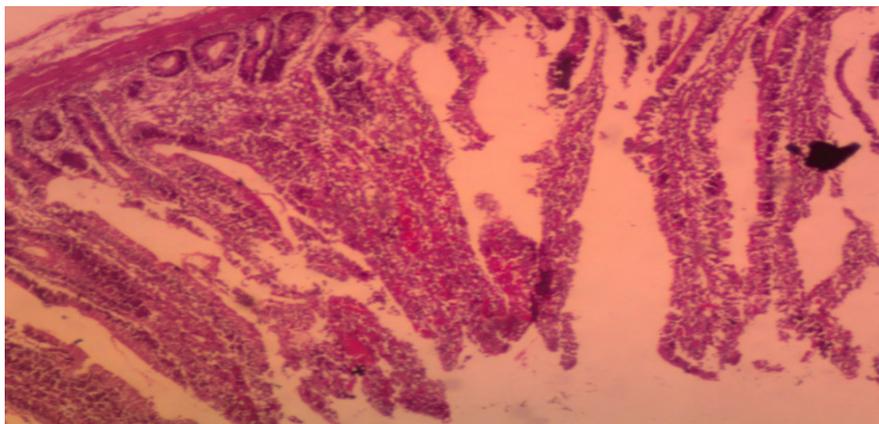


Fig. 10. Intestine showing marked enteritis which characterized by severe degeneration and necrosis of intestinal mucosa and leucocytic infiltration. (H & E- stain) (X200).

S.lentus infected group 3b: Liver at 3th dpi showing mild degree of vacuolar degeneration in the form of early fatty infiltration and hydropic degeneration (Fig. 11), at 5 and 7 days shrinkage of hepatocytes and marked widening of hepatic sinusoids with congestion (Fig. 12) and at 10th dpi of gr 3b showing small fat droplets within

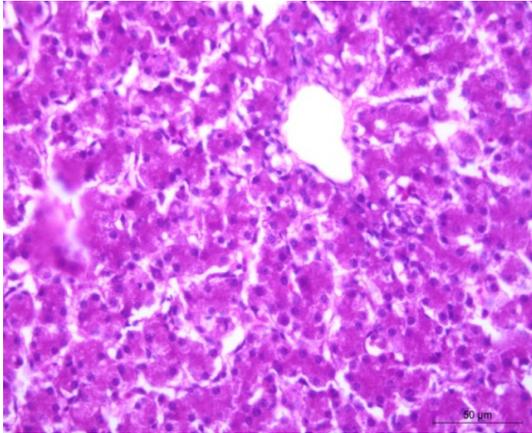


Fig. 11. Liver showing mild degree of vacuolar degeneration in the form of early fatty infiltration and hydropic degeneration. (H & E- stain) (X400).

hepatocytes with widening and mild congestion of hepatic sinusoids (Fig. 13). Spleen 3th dpi showing hyperplasia of lymphoid follicle (Fig. 14), vacuolation and necrosis of splenic corpuscle (Fig 9) at 5th day dpi. Intestine shows mild degeneration of surface epithelium with intraepithelial and submucosal leucocytic infiltration (Fig. 15).

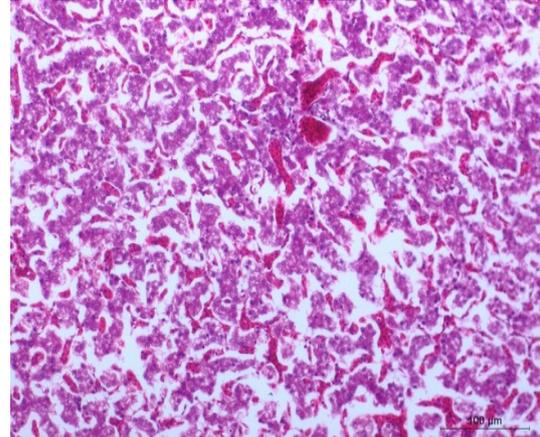


Fig. 12. liver Showing shrinkage of hepatocytes and marked widening of hepatic sinusoids with congestion (H & E-stain) (X200).

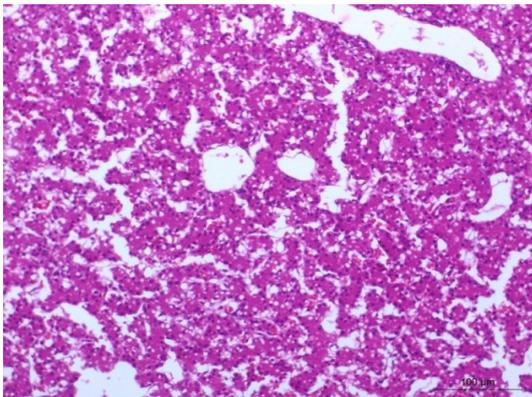


Fig. 13. Liver Showing small fat droplets within hepatocytes with very mild congestion of hepatic sinusoids (H & E-stain) (X200).

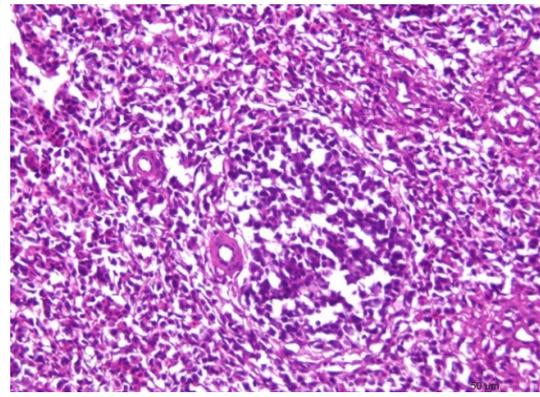


Fig. 14. Spleen showing hyperplasia of lymphoid follicle. (H & E- stain) (X400).

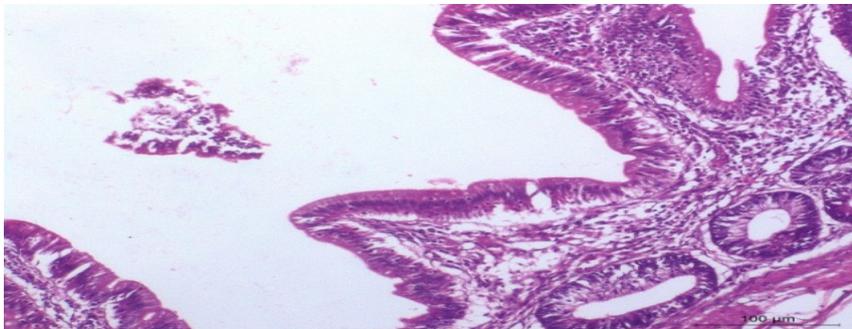


Fig. 15. Intestine of showing mild degeneration of surface epithelium with intraepithelial and submucosal leucocytic infiltration with. (H & E- stain) (X200).

S.lentus infected treated gr 3a: Liver at 5dpi showing small fat droplets within hepatocytes with very mild congestion of hepatic sinusoids (Fig. 13) Spleen *S.lentus* infected treated at 5th day dpi showing hyperplasia of lymphoid follicle (Fig. 14).

Stępień-Pyśniak et al. [32] reported that heart revealed multifocal conglomerates of bacterial colonies attached to the valvular endocardium, threads of fibrin, and inflammatory cells with the presence of heterophils. The detection of histological change in infected nontreated groups can support the statement CoNS are shown to have role as opportunistic pathogens [33]. Tsai et al. [34] and Stępień-Pyśniak et al. [32] suggested a strong association between CoNS and endocarditis in broiler chickens. This lesions can be due to action of virulence factors including slime-producing [35,36,37,38], toxic shock syndrome toxin 1 (TSST-1) [18,39], enterotoxin [18,39] and biofilms formation [40].

As Pathogenicity of *S.aureus* is due to toxic b-hemolysin and plasma coagulase [4]. Cunha Mde et al. [17] analyzed the CoNS virulence factors including hemolysins, lipase, lecithinase, DNase, thermonuclease, and enterotoxin A, B or C in 37.6% of tested isolates. Zell et al. [18] demonstrate hemolytic activity and the exfoliative toxin A (ETA). Shimaa El-Nagar et al. [21] stated that enterotoxins genes in CoNS *sec* and *see* were found in 6 isolates with 10.3% for each. Valle et al. [41] found a toxigenic capacity in 45 (16.5%) CoNS isolates, including *S. xylosus*.

In conclusion, from results of this study used organisms can induce subclinical disease while, histopathological lesions in liver, spleen and intestine of infected chickens were recorded. This area needs more investigation to explore factors potentiate pathogenicity of CoNS in infected chickens.

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امراضية معزولات المكورات العنقودية السلبية المتخثر لكتاكتيت الدجاج عمر ١٠ ايام

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تم تقسيم ١٦٠ من كتاكتيت التسمين عمر يوم واحد الى ٤ مجموعات ، في اليوم العاشر تمت عدوى المجموعات ١ -٣ باستخدام ٠,٥ مل تحتوي على $1,5 \times 10^8$ من معزولات *S.xuosis* و *S. sciuri* و *S. lentus* ، على التوالي وكانت المجموعة ٤ ضابطه غير مصابة.

بدأت الاعراض المرضيه العامه في المجموعات المصابة عند اليوم الثاني من العدوى. تلاشت الاعراض بعد ٢٤ ساعة من العلاج بالسيبيروفلوكساسيلين واستمرت إلى ٧ ايام في غير المعالجة.

كان متوسط وزن الجسم المكتسب عند المصابين بالعدوى *S.xylosis* أعلى (٨١٣,٩٠ جم) ، يليه ٧٧٨,٥ جرام في عدوى *S. sciuri* و ٧٧٣,٧٥ في *S. lentus*. كان معدل التحويل الغذائي هو الأعلى في المجموعه الضابطه (١,٦٩ معالجه) و (١,٧٤ غير المعالجه) يليه ١,٩٢ في *S.xylosis* , *S. sciuri* المصابه المعالجه ل وأدنى ١,٩٤ في *S.Lentus* المصابة غير المعالجة.

تمت اعاده عزل *S. sciuri* من الأمعاء والطحال في اليوم الخامس من العدوى ومن الأمعاء في ٧ و ١٠ من العدوى. في حين تم إعادة عزل *S.lentus* من الأمعاء والكبد والطحال في اليوم الثالث من العدوى ؛ من الأمعاء والطحال (٥ من العدوى) والأمعاء في اليوم العاشر.

سجلت الأفة النسيجية المصابة في المجموعة المصابة في شكل بؤرنزفيه و تنكزيه وخريه في الخلايا الليمفاويه وانكماش خلايا الكبد في الكبد ، والتغيرات النخرية في الخلايا الليمفاوية وفجوة الكريات في الطحال. تخلل الخلايا الليمفاويه بين الخلايا الطلائيه وتحت الاغشيه المخاطيه ، نخر وتتركز في الخلايا الطلائيه المبطنه للأمعاء.

في الختام تم التعرف على عدوى تحت اكلينكيه جراء عدوى الكتاكتيت بعترات المكور العنقودى سالبه المتخثر الا انها تحتاج الى المزيد من التجارب التاكيديه.