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SOME STUDIES ON BACTERIA INDUCTION OF RENAL LESIONS IN CHICKENS

(With 11 Tables and 9 Figures)

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(Received at 2/4/2009)

بعض الدراسات على البكتريا المسببة الإصابة في الكلى في الدجاج

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تم جمع 80 عينة من كلى دجاج نافق حديثاً وبه احتقانات وتضخم بالكلى وامتلاء الحالبين بحمض اليوريك (ولا يعاني من أي مشاكل في التغذية) وذلك لعزل بعض الميكروبات التي تؤثر على الكلى وقد أسفر هذا العزل بعد عمل الاختبارات البيوكيميائية عن تواجد 8 ميكروبات وهي : E.coli بنسبة (75%)-*Staph.aureus* (37.5%) - *Proteus* - (15%) *Klebsiella pneumoniae* – (31.3%) *Corynebacterium* - (18.8%) *Pseudomons* (11.3%) *Perfringenes* *Clostridium* – (8.8%) *Enterococcus faecalis*(6.3%) وتم إجراء عدوى صناعية لكتاكيت بلدي سليمة عمر 11 يوم بميكروب *E.coli* وميكروب *Corynebacterium* (لارتفاع نسبة عزلهم وكمثال لميكروب سالب لصبغة الجرام وآخر موجب لصبغة الجرام). وقد أحدثت العدوى عن طريق الحقن تحت الجلد نفوق بعض الكتاكيت خلال 7 أيام من بداية العدوى بميكروب *E.coli* وميكروب *Corynebacterium* بنسبة 60% ، 20% على التوالي وكانت الأعراض الظاهرية قبل النفوق انكماش وخمول الطيور وعدم القدرة على المشي وأظهر الفحص الداخلي وجود احتقانات وتضخم بالكبد والطحال والكلى والتهابات بالأمعاء مع ترسب حمض اليوريك بالحالبين في بعض الحالات أما العدوى عن طريق الفم فلم تظهر أي أعراض ظاهرية. وتم ذبح كل الطيور الباقية بعد 21 يوماً من بداية العدوى وأوضح الفحص الداخلي نفس الأعراض السابقة وكونت بعض الطيور حصوات بالحالبين مع عتامة الأكياس الهوائية وأمكن إعادة عزل ميكروب *E.coli* وميكروب *Corynebacterium* من الطيور المعداة صناعياً. أظهرت تلك الدراسة أن أهم المظاهر الباثولوجية هي احتقان وأنزفة داخل نسيج الكلى بين الانيبيبات الكلوية كذلك تنكرز تلك الانيبيبات ومعظم مكونات النيفرون .ووجود ارتشاحات كثيفة مابين النسيج الكلوى . وبإجراء اختبار الحساسية لميكروب *E.coli* وجد أن النيوميسين والجينتاميسين والترايميسوبريم هي الأدوية الأكثر تأثيراً عليه أما ميكروب *Corynebacterium* وجد أن الاريثروميسين والريفمبين والجينتاميسين هي الأدوية الأكثر تأثيراً.

SUMMARY

Eighty samples from enlarged congested kidneys were collected from freshly dead chickens from different farms of Assiut Governorate(and didn't suffer from any ration problems). These samples were taken to isolate some bacteria which affect kidneys. After biochemical reactions, the isolation revealed presence of 8 organisms: *E.coli* at a rate of (70%) – *Staph. aureus* (37.5%) – *Corynebacterium* (31.3%) – *Klebsiella* (15%) – *Proteus* (18.8%) – *Pseudomonas* (11.3%) – *Clostridium perfringenes* (8.8%) – *Enterococcus faecalis* (6.3%). Experimental infection of 11 day – old chicken subcutaneously with isolated *E.coli* and *Corynebacterium* showed mortality rates of 60% and 20% respectively within 7 days postinoculation. Before death birds were depressed, huddling together and couldn't move. Postmortem examination revealed congestion and enlargement of kidneys, liver and spleen and the ureters were filled with urate. Birds which inoculated orally, didn't show any clinical signs. 21 days postinoculation all birds were sacrified, P.M examination revealed congestion and enlargement of kidneys and the ureters were distended with urate. Some birds formed stones in the ureters. Reisolation of the 2 organisms from kidneys of experimentally infected birds was successful. Histopathological study reveled that interstitial nephritis in the form of blood vessel congestion and hemorrhage tubular cell degeneration mononuclear cell infiltration and glumerular hypercellularity are the most permanent lesions in bacterial infected chicken. In vitro sensitivity test revealed that neomycin, gentamycin and trimethoprim are the most effective drugs for *E.coli* while erythromycin, rifampin and gentamycin are the most effective for Corynebacterium.

Key words: *Abnormal kidney, E.coli, staph. aureus, Corynebacterium, Klebsiella, Proteus, Pseudomonas, Clostridium perfringenes, Enterococcus faecalis experimental infection histopathological study*

INTRODUCTION

Kidney damages either due to infectious agents or non_ infectious factors are the most frequently diagnosed causes of mortality among chickens. Enlargement of the kidney has been reported in a number of common infectious diseases and nephrosis is said to complicate some of them such as pasteurellosis (Fletcher and Moas 1962) and Pullorum disease (Suganuma 1960). Siller (1964) isolated *E. coli* and staphylococci from cases of Pyelonephritis in fowl.

Ginzburg (1975) reisolated *Staphylococcus* from the Kidneys of experimentally infected chicks. Abd_Alla (1981) mentioned that *Klebsiella* species cause renal lesions. Mario Podrom (1989) isolated *Salmonella typhymurium* from congested Kidneys. Jordon (1990) and Randall (1991) stated that Colisepticaemia is associated with nephritis.

Chandra and Singh (1980) reported that nephrosis in poultry is due to infectious agents and nutritional imbalances.

Chandra *et al.* $(1984)_{a,b}$ cited that the neurogenic- adrenergic effect of urea which increases the Permeability of capillaries, is responsible for edema in the body of bird. Dehydration has been considered an important factor in the precipitation of urate, this may be due to nonavailability of adequate water to flush out the urinary system leading to its clogging. Appearance of nervous symptoms may be the combined effect of hyperuricemia and excessive Production of ammonia in the large intestine caused by degeneration of urea by microbial urease.

This work was designed to cover the following points:

1 - Survey about different bacteria which cause renal lesions in chickens.
2 - Experimental infection of healthy chicks with the most prevalent organisms to show their effect on kidneys of chicks.
3 - Study histopathological picture of naturally and experimentally infected Kidneys of chicks.
4- In vitro sensitivity test to show the most effective drugs on these organisms.

MATERIALS and METHODS

Materials

Specimens:

Eighty samples from abnormal Kidneys were collected from freshly dead broiler and balady chickens (4-12 weeks age) from different farms of Assiut Governorate.

Media

Nutrient agar, MacCon key's agar, blood agar, T.S.I agar, urea agar base, semisolid agar, sugar media for (glucose, sucrose, maltose, lactose, mannitol, sorbitol and fructose) and gelatin.

Reagents and solutions: Methyl red – Kovac's reagent – Voges - proskauer, oxidase, urea, 3% hydrogen peroxide – esculin broth with ferric citrate- sodium hippurate and ninhydrin.

Stain: Gram's stain.

Pathogenicity test:

Fifty five, 11 day old balady chicks were used in our experiment. All birds were obtained from the faculty of Agriculture farm in Assiut University.

In vitro antibiotic sensitivity dises:

Danofloxacin (5µg), gentamycin (10µg), trimethoprim (5µg), ampicillin (10µg), streptomycin (10µg), erythromycin (15µg), neomycin (30µg), oxytetracyclin (30µg), penicillin (k10µg), tetracycline (30µg), rifampein (30µg), kanamycin (30µg), and naladixic acid (30µg).

Methods:

Isolation:

Direct swabs were taken from abnormal kidneys (have renal lesions) of freshly dead chickens. Each sample was inoculated onto 2 nutrient agar plates, 2 MacConkey's agar plates and 2 blood agar plates. Inoculated plates were incubated aerobically and anaerobically for 48h, at 37°c. Suspected colonies were subjected to bacteriological examination to identify the organisms by showing (shape – size – colour) of the colonies, typical morphology of the organism by Gram's stain and studying biochemical reactions. Biochemical reactions were done according to Baily & Scott's (1994), Ellen *et al.* (1994), Kirk Skeels (1997), Connie& George (1995), Flacklam and Teixeila (1998) and Wages (2003).

Pathogenicity test:

Fifty five, 11 day old balady chicks were used, five from them were tested before the experiment and proved to be free from renal lesions. The other fifty birds were divided as follow:

1 - 1st group: was ten – 11 day old chicks, they were inoculated subcutaneously with 10^9 of bacterial suspension of the isolated *E. coli* (according to Sokkar *et al.,* 1998)

2 - 2nd group: was ten -11 day old chicks, they infected with 3×10^9 of bacterial suspension of the isolated *E. coli* orally.

3 - 3rd group: was five - 11day old chicks, were left as control.

4 - 4th group: was ten - 11day old chicks, were infected subcutaneously with 10^9 bacterial suspension of the isolated *Corynebacterium pyogenes*.

5 - 5th group: was ten - 11day old chicks were inoculated orally with 3×10^9 bacterial suspension of isolated *Corynebacterium pyogenes*.

6 - 6th group: was five, 11-day old chicks were left as control.

(according to Sokker *et al.,* 1998).

In vitro antibiotic sensivity test:

Susceptibility tests were done using different sensitivity discs against the isolated *E. coli* and isolated Corynebacterium pyogenes.

Histopathological examination

Small pieces of Kidneys from naturally and experimentally infected chickens were fixed in 10% buffered formalin embedded in paraffin and stained with haematoxylin and eosin.

RESULTS

Postmortem examination of collected chickens with renal lesions revealed enlarged, congested kidneys and ureters were distended with urate.

Bacteriological isolation revealed different colonies in aerobic and anaerobic condition. Gram's stain revealed gram negative bacilli, gram positive bacilli and cocci. From the characters of colonies (shape, size and color which illustrat in Table 1) and gram's stain, we could isolate 8 suspected types of bacteria: *E coli*. *Staph* sp, *Corynebacterium* sp, *Proteus* sp, *Klebsiella* sp, *Pseudomonas* sp, *Clostridium* sp and *Streptococcus* sp. Biochemical reactions which done to identify these organisms are illustrated in Tables 2-9.

According to the cellural and colonial morphology and biochemical reaction, the frequency of the isolates were:

- *E coli* 60/80 isolates (75%)
- *Staph.aureus* 30/80 isolates (37.5%)
- *Corynebacterium pyogenes* 25/80 isolates (31.3%)
- *Klebsiella pneumoniae* 12/80 isolates (15%)
- *Proteus mirabilis* 15/80 isolates (18.8%)
- *Pseudomonas aeruoginosa* 9/80 isolates (11.3%)
- *Clostridium perfringenes* 7/80 isolates (8.8%)
- *Enterococcus faecalis* 5/80 isolates (6.3%)

So we used *E. coli* and *Corynebacterium pyogenes* for Pathogenecity test because they were more prevalence and as an example for gram - positive organism and gram – negative organism.

Pathogenicity Test

All birds which were inoculated subcutaneously with the isolated *E. coli* and Corynebacterium pyogenes showed mortality rates of 60% and 20% respectively within 7 days postinoculation (PI). Before death, birds were depressed, huddling together and couldn't move. Postmortem examination (PM) revealed turbidity of air sacs, enteritis, congestion and enlargement of liver, spleen and Kidney. The ureteres were filled with urate (Fig, 1 and 2). Birds, which inoculated orally, didn't show any clinical signs. 21 days PI all birds were sacrificed, PM examination revealed congestion and enlargement of Kidneys and the ureters were distended with urate. Some birdes formed stones in the ureters (Fig.3).

There was no sings, lesions or death in control birds.

Reisolation of *E. coli* and *Corynebacterium* from Kidneys of experimentally infected birds was successful.

The pathological changes in the kidneys of both natural and experimentally infected birds were the same.

Interstitial nephritis with very prominent dilatation of blood sinuses in between the renal tubules. Congestion and hemorrhage were dominant picture.

There were foci of mononuclear cells in the intertubular space around the glomeruli and blood vessels.

The epithelial cells of some renal tubules showed degenerative changes from cloudy swelling to necrosis. The tubular lumen appeared either empty or contain eosinophilic amorphous material. The glomerular lesions were observed in the form of degeneration of the tufts or hypercelularity.

In vitro sensitivity test

The effect of the different antibiotics to the isolated *E. coli* and Corynebacterium is illustrated in tables 10 and 11.

Table 1: Showing bacteriological examination:

Susbected colony NO	*colony on nutrient agar*	*Colony on blood agar*	*Colony on MacCon key's agar*	*Gram's stain*
The organism grows aerobically				
1-	Smoth, white round colony	Grey round colony	Rose-Pink colony with characteristic coliform smell	Gram-negative bacilli
The organism grows aerobically				
2-	Pigmented white to orange	Smoth, round haemolytic colony	---------- -------	Gram- Positive cocci found in clusters
The organism grows aerobically and an aerobically				
3-	Opaque white pin point colony	Haemolytic grey pin point colony	---------- -------	Gram-Positive pleomorphic bacilli and tendency to form clumps palisade arrangement are frequently observed
The organism grows aerobically				
4-	Greenish diffusible pigment with fruity smell	Produce beta haemolysis	Blue green flat round colony	Gram-negative rod
The organism grows anaerobically				
5-	Small white colony	Small , nonhaemolytic colony	White pin point colony	Gram-Positive spherical bacteria occurlng singly , in pairs or short chains
The organism grows anaerobically				
6-	Small flat colony with irregular edge	Haemolytic colony with double zone	---------- -------	Short to intermediate gram-positive rods
The organism grows aerobically				
7-	Grey Colony	No hoemolysis	Large pink mucoid colony (lactose fermenter)	Gram - negative bacilli
The organism grows aerobically				
8 -	Swarming appearance on the surface with fishy smell	Non- haemolytic colony and turns blood agar brown	Compact pale non - lactose fermenter and edges are irregular	Gram - negative rod

Tables (2-9) showing the results of biochemical reactions:

Table 2: For organism No.1

Biochemical tests	Result	Suspected organism
- H2S production	_	E. coli
- Indole	+	
- Methyl red	+	
- Voges - proskauer	_	
- Urea	_	
- Simmone's citrate	_	
- Gelatin liquefaction	_	
- Sugar fermentation		
• Glucose	+	
• Lactose	+	
• Mannitol	+	
• Sucrose	+	
- Motility	+ / -	

Table 3: For organism No.2

Biochemical tests	Result	Suspected organism
- Coagulase	+	Staphylococcus aureus
- Voges - proskauer	+	
- Catalase	+	
- Gelatin – liquefaction	+	
- Fermentation of sugars:		
• Sucrose	+	
• Glucose	+	
• Lactose	+	
• Fructose	+	
• Mannitol	+	
• Maltose	+	

Table 4: For organism No.3

Biochemical tests	Result	Suspected organism
- Catalase	_	Corynebacterium-pyogenes
- H2S production	_	
- Indole	_	
- Voges - proskauer	_	
- Methyl red	_	
- Gelatin – liquefaction	+	
- Litmus milk	Acidified and coagulated	
- Fermentation of sugars:		
• Glucose	+	
• Lactose	+	
• Maltose	+	
- Motility	-	

Table 5: For organism No.4

Biochemical tests	Result	Suspected organism
- Catalase	+	Pseudomonas aeruginosa
- Oxidase	+	
- Urea	+	
- Methyl red	_	
- Voges - proskauer	_	
- Indole	_	
- Litmus milk	+	
- Glucose Fermentation	+	
- Motility		

Table 6: For organism No.5

Biochemical tests	Result	Suspected organism
- Esculin _ hydrolysis	+	Enterococcus faecalis
- Catalase	-	
- Fermentation of sugars:		
• Lactose	+	
• Mannitol	+	
• Sucrose	-	
• Sorbitol	+	
• L. arabinose	-	
- Motility	-	
- Groth at 10^0c and 45^0c	+	

Table 7: For organism No.6

Biochemical tests	Result	Suspected organism
- Litmus milk reaction	+	Clostridium perfringenes
- Gelatin Liquefaction	+	
- sugar Fermentation:		
• Glucose	+	
• Lactose	+	
• Sucrose	+	
- Motility	_	

Table 8: For organism No.7

Biochemical tests	Result	Suspected organism
- Simmon's citrate	+	Klebsiella pneumoniae
- H2S production	-	
- Indole	-	
- Methyl red	+	
- Voges - proskauer	+	
- Urea	+	
- Sugar Fermentation:		
• Glucose	+	
• Lactose	-	
- Motility	-	

Table 9: For organism No.8

Biochemical tests	Result	Suspected organism
- Methyl red	+	Proteus mirabilis
- Urea	+	
- Indole	-	
- Vogus - proskauer	-	
- H2S production	+	
- Gelatin Liquefaction	+	
- Glucose Fermentation:	+	
- Motility	+	

Table 10: Illustrate in vitro sensitivity test of the isolated E.coli

Antibiotic discs	Sensitivity of E.coli isolates
Neomycin	+ + +
Gentamycin	+ + +
Trimethoprim	+ + +
Kanamycin	+ +
Naladixic acid	+
Tetra cycline	-
Streptomycin	-
Ampicillin	-

Table 11: Illustrate in vitro sensitivity test of the isolated Corynebacterium

Antibiotic discs	**Sensitivity of Corynebacterium isolates**
Erythromycin	+ + +
Rifampin	+ + +
Gentamycin	+ + +
Penicillin	+ +
Tetracyclin	+ +
Oxytetracycline	+ +
Danofloxacin	-
Kanamycin	-

LEGENED OF FIGURES

Fig. 1: H and E stained kidney section from natural case Show Blood vessel engorged with blood

Fig. 2: H and E stained kidney of experimentally infected case show inter tubular blood vessel congestion and hemorrhage.

Fig. 3: H and E stained kidney section from natural case Show Tubular necrosis cytoplamsmic vaculation with pcknotic nucleuses.

Fig. 4: H and E stained kidney of experimentally infected case show tubular necrosis

Fig. 5: H and E stained kidney of experimentally infected case show inter tubular mononuclear cell infiltration

Fig. 6: H and E stained kidney of experimentally infected case show glumerular hypercellularity

DISCUSSION

In our study we could isolate 8 orgnisms from renal lesions: *E. coli*, *Corynebacterium*, *Staph. aureus*, *Klebsiella pneumoniae*, *Proteus mirabilis Pseudomonas aeruginosa*, *Clostridium perfringems* and *Enterococcus faecalis*. We somewhat in agreement with Sokkar *et al.* (1998) who isolated only *E. coli*, *Staph. aureus* and *Corynebacteriun* from chickens with renal lesions. But Siller (1964) isolated *E. coli* and *Staph*. only, while Ginzburg (1975) reisolated *Staph*. from the kidneys of experimentally infected chicks. On other hand, Jordan and Randal (1991) isolated *E.coli* only. We differ with Mario Modrom (1989) who isolated *Salmonella typhymurium* from congested kidneys and Fletcher and Moas (1962) who said that nephrosis is complicated of pasteurellosis and pullorum disease.

Experimental infection of 11- day old chicks subcutaneously with isolated *E. coli* and *Corynebacterium* revealed enlargement congested kidneys and ureters dilated and distended with urates. This result is similar to that observed by Sokkar *et al.* (1998) but in our experiment there was mortality in birds within 7 doys P.l and we also noticed formation of stones in the ureters of some birds.

The microscopic changes in the kidney of all infected birds either experimentally or naturally were mainly interstitial nephritis. These finding are similar to those of Siller (1964) and Randal (1991). Similar histopathologic picture were recorded by many other investigators for some bacterial species other than those used in our study. For instance Sugnama (1960) claimed that in pullurum disease there were interstitial nephritis. Rahamathulaand Mohyudeen (1973) described interstitial nephritis with tubular degeneration and necrosis in pullet disease. Randal (1991) showed that chlamydiosis causes glomerulonephritis.

In vitro sensitivity test we found that neomycin gentamycin and trimethoprim are the most effective drugs for *E. coli* this result is similar to that observed by Sokkar *et al.* (1998) while erythromycin, rifampin and gentamycin are most effective for Corynebocterium we in agreement with Lynda (2008) in this result.

In our study we concluded that the gross pathological lesions observed in the kidneys and microscopic changes were not related to the inoculated organism. So hygienic measures ore necessary to decrease infectious organism and try to reduce bacterial contamination in drinking water and avoid overcrowding and stress factors.

REFERENCES

Abd_Alla, O.A.M (1981): Histopathological studies on poultry following artificial infection by Klebsiella M.V.SC. Clinical Pathology. Cairo University.

Bailey and Scott's (1994): Differentiation among selected Enterococcus spp and Streptococci and related genera In: Diagnostic Microbiology. Section 26P. 345, 9th Edition. Edited by Ellen. J. B., lance, P.R. and Sydney, F.M.

Chandra, M. and Singh, B.L. (1980): Incidences and pathology of nephritis in domestic fowl in punjob. Indian J. Poult. Sci. 15: 125-131.

Chandra, M.; Singh, G.L. and Ahuja, S.P. (1984a): Renal and biochemical changes produced in broilers by high-protein, high calcium, urea-containing and vitamin-A deficient diet. Avian Dis. 28: 1-11.

Chandra, M.; Singh, G.L. and Ahuja, S.P. (1984b): Hematological changes in Nephritis in poultry induced by diets high in protein, high in calcium, containing urea, or deficient in vitamin A. Poult. Sci. 63: 710–716.

Connie, M.R. and George, M. (1995): Classification of strepto coccus and Enterococcus In: Diagnostic Microbiology P. 343-344.

Ellen, J.O.; Baron lance, R; Peterson and Finegold, S.M. (1994): Diagnostic Microbiology. P. 321-331 9th Ed.

Facklam, R.R. and Teixeira, L.M. (1998): Enterococcus In: Topley & Wilson's in Microbiology and Microbial infections. Chapter 29.P. 674 Vol. 2 Edited by: Leslie Collier, Balows, A. and Sussman, 9th Edition.

Fletcher, R.D. and Maas, H.J.L. (1962): Acute fowl choler in a connecticut flock. Description of the isolation of Pasteurella multocida. Tijdschrift Voor Diergeneeskunde 87, 1125-1128.

Ginzburg, V.V. (1975): Differentiation of staphylococci isolated from fowls. Vet. Moscow 8, 167.

Jordan, F.T.W. (1990): Poultry Diseases. 3rd Ed. Bailliere Tindail and cox. London.

Kirk Skeeles, J. (1997): Staphlococcosis In: Diseases of Poultry 10th Ed. Edited by calnek, B.W. lowa State univ. press, Ames, IA PP. 247-253.

Lynda, A.F. (2008): Corynebacterium infections. MD, Associated Clinical Professor, Department of internal Medicine, University of California at San Contributor.

Mario Modrom, M. (1989): An outbreak of paratyphoid in laying hens (case report). Avian Disease. 34, 221-223.

Rahmathulla, P.M. and Mohyudeen, S. (1973): Studies on gouty nephritis in poultry. Indian Vet. Journal 50, 841-844.

Randall, C.J. (1991): A color atlas of diseases and disorders of the domestic fowl and turkey. Wolfe Medical Publication, London.

Siller, W.G. (1964): The pathology of pyelonephritis in the fowl. Vet. Sciences 5, 323-331.

Sokkar, S.M.; Mohamed, M.A. and Atawia, M. (1998): Experimental induction of renal lesions in chickens. Berl. Munch. Tierarztl. Wschr. 111, 161-163.

Suganuma, Y. (1960): Histological studies on serositis of pullorum disease. Japanese J. of Vet. Science 22, 175-182.

Wages, D.P. (2003): Enterococcosis In: Diseases of Poultry. Chapter 24. P. 809-811. Edited br calnek B.W. John Barnes H., Mcdouglad, C.W. and Saif.Y.M.

Fig 1&2: Showing congested, enlarged kidney and the urerers are filled with urate in experimentally infected chick.

Fig 3: Experimentally infected chick formed stones in the ureter.