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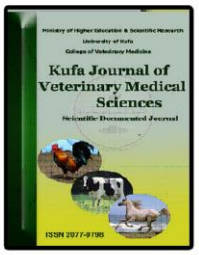

Bacteriological And Pathological Study Of Omphalitis In Broiler Chicks

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Abstract

The aim of this study is to investigate the role of omphalitis in the productive traits of broiler chicks.

The study was conducted from July 2012 to April 2013 in the hatcheries of Al-Fallujah, Al-Anbar province, Iraq to determine the prevalence of yolk sac infection, and to identify yolk sac infection attributed to various bacterial pathogens threatening the area causing economic losses in poultry industry and to take the precautionary measure to minimize such losses .

A total of (116) moribund newly hatched chicks with an age of (1-5)days were received from different hatcheries or farms. The selective chicks were necropsied for bacteriological cultures, histopathological study in addition to investigate their sequel on the health state of the birds.

The study showed that the prevalence of bacteria was in the following order; Streptococcus spp 39%, Escherichia coli 20.6%, Proteus spp 14.2%. Pseudomonas spp. 9.5%, Staphylococcus spp. 6.3%, Pasteurella spp. 4.7% , Klebsiella spp 3.1%, Bacillus (saprophytic) 1.5%.

Our study proved that omphalitis have a vital role in increase mortality during the first week in addition to its direct effect in decrease body weight as well as immunological response to further vaccination because of deprivation of passive maternal immunity that will predispose the bird for the endemic diseases.

دراسة بكتيرية و مرضيّة لحالات التهاب الصرة في افراخ اللحم

الخلاصة:

ان الهدف من هذه الدراسة هو التحقق في تأثير التهاب الصرة على الصفات الانتاجية لافراخ اللحم .
تم اجراء الدراسة للفترة بين شهر تموز 2012 و حتى نيسان 2013 . تم اختيار النماذج من المفاقس و الحقول المحيطة بمدينة الفلوجة / محافظة الانبار / العراق و ذلك لغرض معرفة الجراثيم المرضية و اتخاذ الاجرائات الوقائية التي من شأنها تقلل من هذه الخسائر. احتوت الدراسة على (116) نموذج لافراخ حديثة الفقس او افراخ تراوحت اعمارها بين (1-5) يوم مع التركيز على كون الافراخ تحمل صفات و آفات مرضية. تم اخضاع النماذج الى فحوصات تمثلت بالزراعة الجرثومية و التقطيع النسيجي و متابعة تأثير هذه الجراثيم على الحالة الصحية للطير المصاب.
اوضحت الدراسة بأن الجراثيم الاكثر شيوعا حسب الترتيب الاتي:

39%, Escherichia coli 20.6%, Proteus spp 14.2%. Pseudomonas Streptococcus spp spp. 9.5%, Staphylococcus spp. 6.3%, Pasteurella spp. 4.7% , Klebsiella spp 3.1%, Bacillus (saprophytic) 1.5%.
تم الاستدلال من هذه الدراسة على ان التهاب الصرة له دور فعال في ارتفاع نسبة الهلاكات في الاسبوع الاول كما ان له دور مباشر في انخفاض وزن الافراخ و الاستجابات المناعية للتحصينات اللاحقة بسبب الحرمان من المناعة الأميّة مما يجعله عرضة للامراض المتوطنة في المنطقة.

Introduction:

Omphalitis (yolk sac infection) is a bacterial infection of the navel resulting from its failure to close properly following the drawing of the yolk sac into the abdominal cavity and this infection occurs due to the entrance of bacteria present in the surrounding environment. The infection is aggravated by poor hygiene on breeding farms and faulty management at the hatchery (1). Yolk sac infection (YSI) is the main cause of chick mortality during the first week of post hatching period (2,3) with consequent mortality ranging from 1.7% to 8.6% that would cause high economic losses (4,5). The rate of weight gain by broiler has substantially increased during the last few decades, consequently slaughter age has decreased such that the final carcase weight, attained at 12 weeks of age in the early 1960s, now occur by 5 weeks. This trend is continuing and emphasize the importance of early life of broilers among the major problems during this period is omphalitis (retained yolk sac or YSI) (6).

For the above mentioned points this project was designed to have an overview for such pathogens and to study them microbiologically and pathologically which will contribute to control early mortality in broiler flocks.

Materials and Methods:

Collection of samples:

Newly hatched chicks (n=116) broiler types, were received from different hatcheries and different farms surrounding Alfallujah city, Alanbar province ,Iraq manifesting signs of omphalitis during the period July/2012 to April/2013.

Postmortem (necropsy) examination:

All chicks were subjected to necropsy before sampling for pathological study with special reference to yolk sac infections. Postmortem examination was done according to the procedure recommended for poultry by Chauhan (7).

Bacterial isolation and identification:

All chicks with gross lesions of yolk sac were sampled with sterile cotton swab from the yolk sac. These samples were aseptically cultured on blood agar, MacConky agar and nutrient agar. All the inoculated media were incubated at 37^{o}C and inspected for growth after 48hr. of incubation based on macroscopic and microscopic appearance, the developed colonies were selected from each sample and subcultured on appropriate differential media for further identification. These media include nutrient agar(NA),blood agar(BA), brilliant green agar(BGA), mannitol salt agar(MSA), and triple sugar iron(TSI) agar. Bacterial colonies were investigated after incubation on these media for identification . identification was done on the basis of staining , colony morphology , cultural and biological characters of pure isolated colonies by using standard bacteriological and

biochemical procedures as described by Quinn (8).

Histopathological study:

Microscopic examination of the affected yolk sac was done through preparation of slide histology according to Luna (9)

Statistical analysis:

It was done according to Steel (10) by the use chi square test.

Results and Discussion:

A. Bacteriological findings:

A total number of 116 birds manifesting infected yolk sac (IYS) were necropsied for bacteriological and pathological study. The positive bacterial cultures were 63 sample(54.3%), while the negative cultures were 53 sample (45.6%) that are attributed to non bacterial agents. The isolates of positively ISY were related to 8 bacterial species as shown in Table(1). There was a significant difference ($p > 0.05$) in infection rate between different pathogenic bacteria.

Table (1): The positively cultured bacteria.

Bacterial Isolates	% of infection rate at p<0.05 level
Streptococcus Spp.	39[a]
E-coli	20.6[b]
Proteus (P. vulgaris & P.mirabilis)	14.2[c]
Pseudomonas aeruginosa	9.5[d]
Staphylococcus (S.aureus & coagulase negative spp.)	6.3[e]
Pasteurella (Manhemia hemolytica)	4.7[f]
Klebsiella Spp.	3.1[g]
Bacillus Spp.	1.5[h]

Different letters showed significant difference (P<0.05) in different types of bacteria.

Different bacterial species associated with omphalitis in the present study have been confirmed in previous study(11,12,13,14) but we do not agree with them that E.coli is the highest prevalent pathogen, our study showed that streptococcus species is highly prevalent (39%) as compared to E.coli (20.6%). This result agreed with Rad (15) that streptococcus is the highest prevalent pathogen causing omphalitis.

Other bacterial pathogens include proteus (14.2%) pseudomonas (9.5%) staphylococcus (6.3%) was in the second order and this agreed with Cynthia (16). Pasteurella was in a level of (4.7%), klebsiella (3.1%) and bacillus spp (1.5%) as fixed by other studies particularly those are using fish and fish meal as the major source of animal protein (17) and such isolates may represent chicks which have been necropsied at the age of 4-5 days.

Identification and characterization of bacterial isolates by recording morphology of colonies (size, margin, elevation, color), gram stain as done by Quinn and Merchant (8,18), sugar fermentation , catalase , oxidase , coagulase, indole, KOH test and TSI according to Cheesbrough, Freeman and Jones (19,20,21) (Table2,3).

Table -2- Cultural characteristics of bacterial Isolates from yolk sacs of chicks suffering from omphalitis.

bacterial isolate		Cultural characteristics on :				
		Blood Agar	MacConky Agar	Nutrient Agar	Mannitol salt Agar	TSI Agar
Streptococcus Spp.		Small pin point colonies and some of them are hemolytic	No growth	Small pin point colonies	*	*
E coli		Small colonies with β hemolysis	Bright pink colonies	Small colonies	*	Yellow slant and butt , no H_2S
Proteus	P. vulgaris	Colonies are flat & white with swarming	Pale colonies with swarming	Colonies are flat & white with swarming	*	Yellow slant and butt , with H_2S
	P. mirabilis	Colonies are flat & white with swarming	Pale colonies with swarming	Colonies are flat & white with swarming	*	Yellow slant and butt , with H_2S
Pseudomonas aeroginosa		Large flat colonies with irregular edge	Pale colonies with green pigment	Sticky colonies with pyocyanin production	*	*
Staphylococcus	S. aureus	Large yellow colonies with B hemolysis	No growth	Large colonies	Large rounded colonies with mannitol fermentation	*
	coagulase negative staph.	Large rounded colonies	No growth	Large colonies	Large rounded colonies with no mannitol fermentation	*
Pasteurella (mannhaemia hemolytica)		Small colorless colonies with narrow zone of hemolysis	Small pin point colonies	Small pin point colonies	*	*
Klebsiella Spp.		Large and sticky colonies	Large and sticky Pink colonies	Large and sticky colonies	*	Yellow slant and butt , no H_2S
Bacillus Spp.		Positive growth	Positive growth	Positive growth	*	*

Keys: *= not done

Table-3- Biological characters of bacterial Isolates associated with omphalitis.

bacterial isolate		Gram stain	KOH test	Catalase test	Oxidase test	O/F test	Coagulase test	Indole test
Streptococcus Spp.		G^+ cocci	-ve	-ve	*	*	*	*
E coli		G^- bacilli	+ve	*	-ve	F	*	+ve
Proteus	P. vulgaris	G^- coccobacilli	+ve	*	-ve	F	*	+ve
	P. mirabilis	G^- coccobacilli	+ve	*	-ve	F	*	-ve
Pseudomonas aeroginosa		G^- bacilli	+ve	*	+ve	O	*	*
Staphylococcus	S. aureus	G^+ cocci	-ve	+ve	*	F	+ve	*
	coagulase negative Spp.	G^+ cocci	-ve	+ve	*	F	-ve	*
Pasteurella (mannhaemia hemolytica)		G^- bacilli	+ve	*	+ve	F	*	*
Klebsiella Spp.		G^- bacilli	+ve	*	-ve	F	*	-ve
Bacillus Spp.		G^+ bacilli	-ve	+ve	*	U	*	*

Keys : G^+ = gram positive , G^- = gram negative, +ve = positive result, -ve = negative result, O=oxidative, F = fermentative, U = non-reactive, *= not done

Samples that were negative for bacterial culture (45.6%) gave us an indication that omphalitis may be due to factors that are other than bacterial pathogens. This result agreed with MuraKami (22) who mentioned that delay of feeding post hatching either due to delay in the transport from the hatchery to the farm or because of different hatching time may result in retained yolk sac because yolk utilization is more rapid in fed than in fasted chick, suggesting that the transfer of yolk into the intestine may be facilitated by the intestinal motility of feed chicks(23). A hatching environment that is not sufficiently humid is often associated with a high incidence of yolk sac infection (24). Other non bacterial agent associated with yolk sac infection is Aspergillus fumigates as reported by Schonhofen (25)

B. Pathological finding

Putrefactive and offensive odor was observed as characteristic clinical signs of YSI (26,27). Abdomen of chick appear soft and distended with thickened, inflamed and spoiled vent region. Unabsorbed yolk sac can be palpated externally at the caudo-ventral aspect of the abdominal cavity and therefore the name yolk sac retention. Yolk sac contents changed from viscid

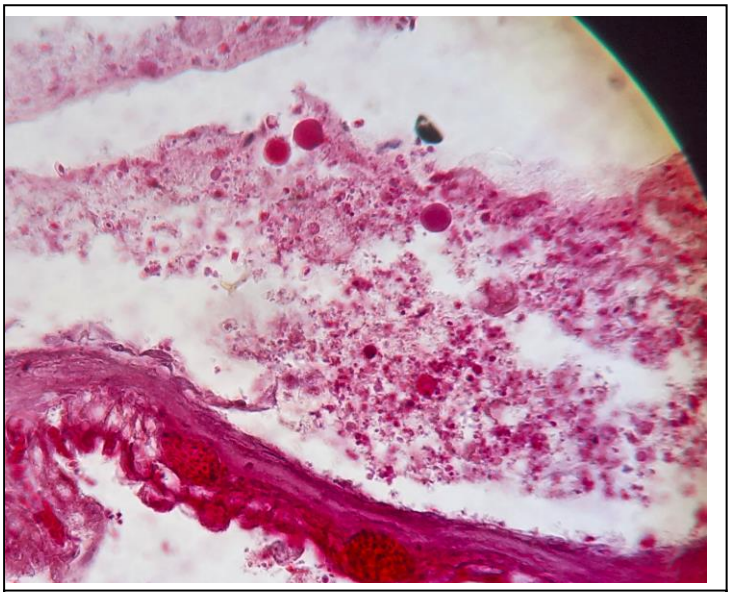
yellow green to watery yellow brown due to denaturation of yolk by bacteria (24,26,27), subcutaneous blood vessels were dilated and engorged with blood (24). Chicks surviving more than four days may develop pericarditis in addition to YSI indicating systemic spread (28). Hemorrhagic spots and peritonitis were agreed with Jordan (24). Sometimes retained YS is caseated (figure 1,2).

In severe cases, the body wall and overlying skin undergo lysis and wet and dirty. The birds are referred to as mushy chicks or poults (29).

Figure (1): Distended abdominal cavity (ascites) with highly inflamed subcutaneous tissue, engorged blood vessels , vent region is occluded with spoiled material.

Figure (2): Yolk sac contents changed from viscid yellow green to watery yellow brown due to denaturation of yolk by bacteria.

Microscopic examination: According to our knowledge, there was no information about histopathological study of yolk sac infection. Inflammatory cells mainly macrophages and heterophils predominate the area (figure-3-), congested blood vessels, foamy cells which represent enterocyte of the yolk that are filled with colliodial material (crysteloid) giving an indication for retained yolk sac. Fibrous tissue infiltrating the fatty layer is indicative for adhesion that will lead to peritonitis as a result of hematogenous spread and finally pericarditis and perihepatitis. Hyperemia, accumulation of edematous fluid with thickened wall is indicative for more severe inflammation.(Figure -3)



Figure(3): YS of one day old chick , proliferation of fibrous tissue , area of adhesion with the abdominal fat , crysteloid material , invasion of inflammatory cell mainly heterophils and macrophages .

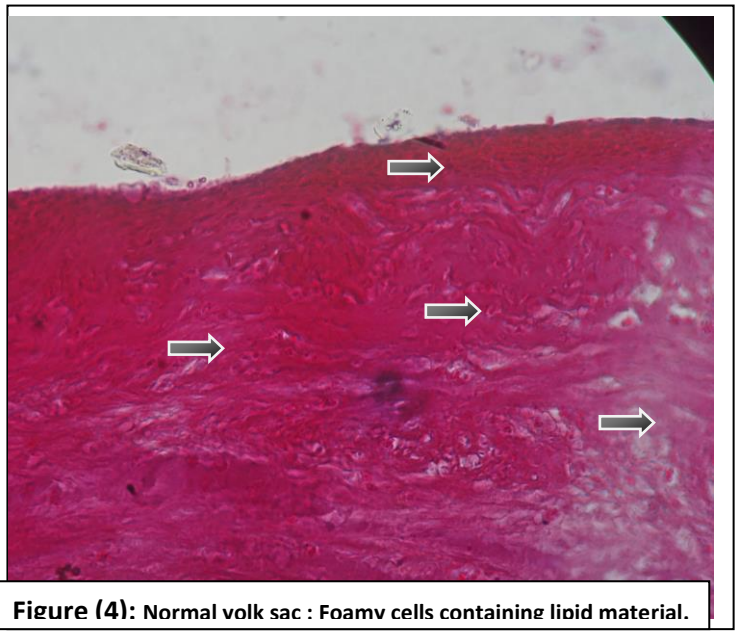


Figure (4): Normal yolk sac : Foamy cells containing lipid material.

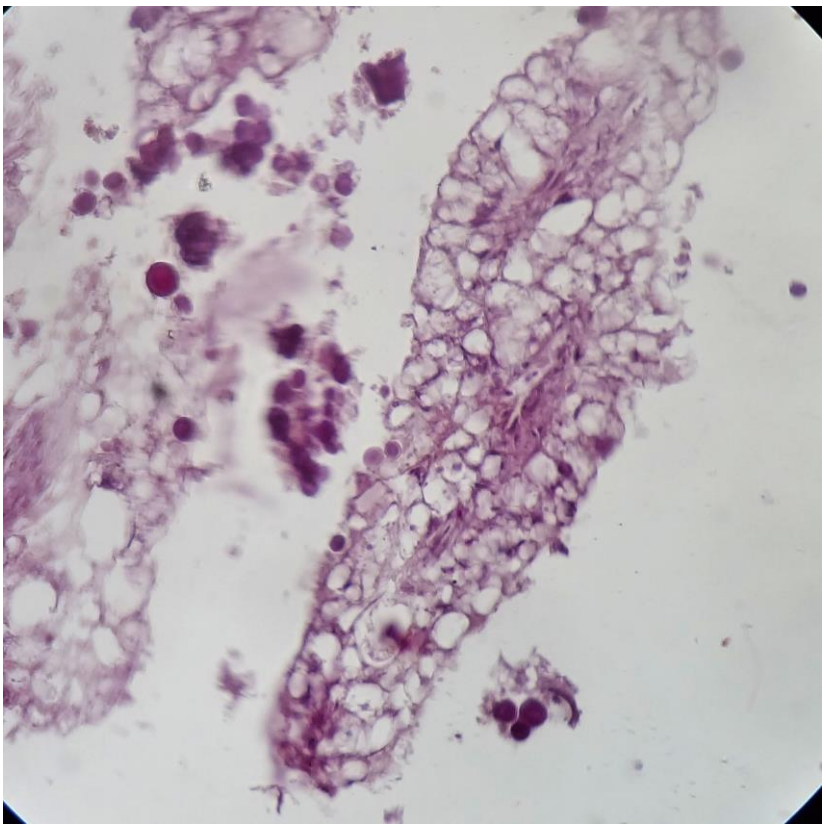


Figure (5): Normal YS villus , the foamy cells are distended with foamy material . (X 40)

The yolk sac is regarded as a diverticulum of the small intestine, this means that material is absorbed directly from the sac into the blood stream (30), consequently retained yolk sac will predispose for deprivation of nutrient and maternal antibodies, absorption of toxins and spread of E. coli by extension into the body (29).

From the above mentioned points, we concluded that retained yolk sac plays an important role on body weight, mortality during the first week of age and on the passive maternal immunity.

We recommend strict hygienic measures for receiving new chicks as well as strict hygienic measures in the hatcheries and further investigations for non bacterial causes of omphalitis should be done.

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