

## **Study of some bacterial isolates associated with broiler synovitis in Kerbala Province**

**دراسة عن بعض عزلات البكتيريا المرتبطة بالتهاب المفاصل في فروج اللحم في محافظة كربلاء**

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### **Abstract:**

The present study was conducted to identify the prevalence of predominant bacterial microorganisms of synovitis in broilers , a comprehensive study of bacterial isolates from pus/fluid of synovial fluid taken from broiler chicken at 15, 20 and 25 old days from the Kerbala poultry flocks in the Karbala province, Iraq . over a period of three months from April until June 2015 were undertaken. The results revealed that ratio of isolation were 23 (51.1%) among forty five samples of synovial fluid and classified as *Escherichia coli* 33.3% , *Streptococcus spp* 8.8% and *Salmonella spp* 8.8% , on the other hand the study showed that there is no significant difference ( $P>0.05$ ) between the age and isolations month.

### **الخلاصة :**

أجريت هذه الدراسة لتحديد مدى انتشار البكتيريا السائدة في زليل مفصل فروج اللحم ، وكانت الدراسة شاملة لبعض العزلات البكتيرية من القيح / السائل الزليلي للمفصل المأخوذ من فروج اللحم بعمر يتراوح بين (15-25) يوما في عدد من حقول الطيور الداجنة في محافظة كربلاء، العراق . وكانت الدراسة على مدى 3 أشهر من نيسان 2015 ولغاية حزيران 2015. أشارت النتائج ان نسبة العزل البكتيري بلغت (51.1%) من مجموع خمسة وأربعين عينة من عينات المفصل الزليلي وتقسم كالآتي : الاشريشيا القولونية 33.3% ، المكورات السببية 8.8% ، السالمونيلا 8.8% ، من ناحية أخرى أظهرت الدراسة عدم وجود فروق معنوية ( $P>0.05$ ) للعزلات بين عمر الدجاج وشهر العزل.

### **Introduction :**

Lameness or leg weakness threatens the welfare of chickens and causes considerable economic loss (1).Lame birds have difficulty in accessing food and water, and will therefore become dehydrated and die .Anational survey in the U.S estimated that leg problems cost the broilers industry between 80 and 120 million dollars annually (2) .It resulting from several causes include bacteria ,virus and trauma .The disease has been seen in birds ranging from 14 to 70 days of age, but most cases occurred around 35 days old (3).

Bacterial arthritis in poultry after septicemia or localized is reported to be associated with *staphylococci* such as *S.aureus*, *S.hyicus*, *S.xylosus* and *S simulans*, *Escherichia coli*, *Mycobacterium avium*, *Salmonella spp.* and *Enterococcus spp.* (4,5,6and7), in addition to *Erysipelothrix*, *Listeria*, *Mycoplasma*(8) . (9) reported *Staphylococcus spp.* as the most common bacteria isolated from arthritis/tendonitis/osteomyelitis in broilers, he also noted an increase in the incidence of musculoskeletal infection associated with *E. coli* infection.

The incidence of bacterial synovitis is lower in broilers and accounts for 3-4 % and mortality may reach to 5% (10) , affected joints usually the hocks, are hot, swollen , painful and affected birds are usually depressed, lameness and reluctant to walk, the synovial membranes of tendon sheaths

become thickened and oedematous, with fibrinous exudate within and around the tendon sheaths (11).

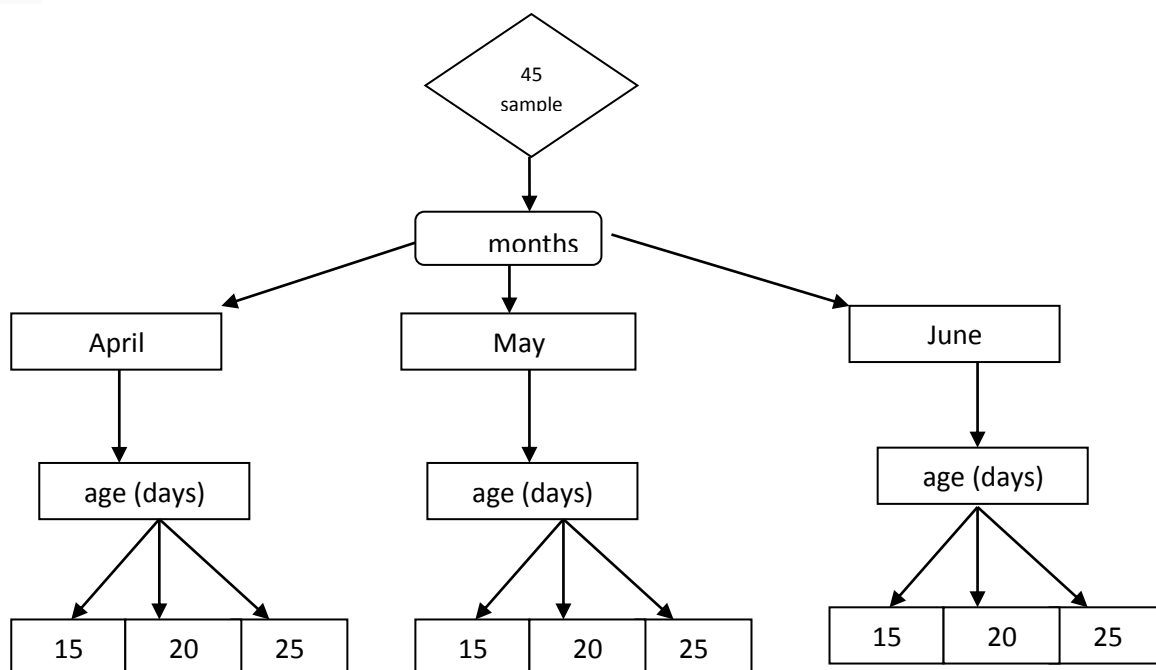
(12) suggest that the most common problems are related to skeletal leg pathology specially Femur Head Necrosis (FHN) among modern hybrid broiler chickens was most commonly caused by osteomyelitis, which was caused predominantly by *E. coli* infection . (5) isolated bacteria from the proximal end of femurs with lesions, indicative of Bacterial Chondro Osteomyelitis (BCO). From the lesions samples, coagulase-positive *staphylococci* (22.2%), coagulase-negative *staphylococci* (11.1%), and *E. coli* or mixed cultures (13.3%) were isolated.

(13) observed concerning increased incidence of musculoskeletal problems associated with *E. coli* infections among broilers. In more than 90% of the bacteriologically examined samples with FHN in association with osteomyelitis, *E. coli* was isolated.

The aim of current research is to diagnose the most bacteria that causes arthritis in broiler chickens.

**Materials and Methods:**

The study conducted over a period of 3 months from April 2015 until June 2015 on many poultry flocks in Karbala province, Iraq. Synovial fluid and synovial pus specimens taken from 45 broiler chickens after opening by sterile sharp knife were inoculated directly in different types of media (Macconkey agar, XLD agar, Blood agar), in addition to confirm the diagnosis of isolates we were using biochemical tests(14), each microorganism has distinctive characteristics that distinguished it from other (15). The study focused on the number of bacterial isolation in three months, each month were collected approximately 15 samples of different ages ranged (15-25) old days. from the same herd of the same month (Figure 1). Results was analyzed by Chi-square test, with level of significance set at a level of  $P > 0.05$  and Least significant difference (LSD) using SAS program.



**Figure 1: Schematic diagram of bacterial isolated according to months and age**

**Results and Discussion :**

The study showed that out of the total samples of 45 synovial joint was 23 bacterial isolates (51.1%), these isolates were *E.coli* (33.3%), *streptococcus spp* (8.8%) and *Salmonella spp* (8.8%) ,the bacterial isolates were distributed according to the three months(Table 1) ,the table show there is no significant difference ( $P>0.05$ ) between the three months, because the convergence of three months within a same year ,in same season and the same climate temperatures in Iraq (16).

**Table (1): The Percentage of bacterial isolates for three months .**

Months	Total Number	Number of isolates	Percentage (%)
April	15	10	66.6%
May	15	8	53.3%
June	15	5	33.3%
Total	45	23	51.1%
Statistical analysis	$X^2 = 3.379$ , degree of freedom= 2, $P>0.05$		

Regarding, the presence of bacterium species and it relationship with months (Table 2), The study explained that *E.coli* was more significant than other bacterial isolates (*Salmonella spp.* and *Streptococcus spp.*).(17) because it natural habitat of *E.coli* among animal/ host association or open/ non host association in all condition(18).It recently suggested that ‘the more particular species adapt to broiler chicken was *E.coli* In addition to its ability to survive in nutrient-limited open environments(19). Our results agree with(12),which found the most of FHN cases (75%) with present osteomyelitic lesions and with evidence of infectious agent (mostly *E.coli*) are a serious precondition to assume an infectious cause. It is believed that the infection spreads via the blood circulation from the respiratory or the alimentary tract . This is further confirmed by the concurrent signs of colisepticemia (polyserosites,cellulitis) in about 1/3 of chickens with FHN and osteomyelitis. Also the skin injury around the joint may play a very important role in the development of bacterial chondronecrosis or arthritis (20). The results is similar to (13)that his data could be summarized that in broiler chickens, *E. coli* had a primary role in the etiology of osteomyelitis and FHN unlike the growing broiler parents where *Staphylococcus. aureus* was mainly involved.. In more than 90% of the bacteriologically examined samples with FHN in association with osteomyelitis, *E. coli* was isolated.

Not surprisingly, *Salmonellosis* does not play an important role in arthritis, therefore, the study showed that isolation rate was 3 and 1 isolate in April and May respectively, there is no isolates in June, these results were approached with (21.).

**Table (2): Number of different bacterial isolates distributed on three months .**

Type of bacteria and number	April		May		June	
	Number of synovial joint	Number of isolates	Number of synovial joint	Number of isolates	Number of synovial joint	Number of isolates
E.coli (15)	15	6	15	5	15	4
Streptococcus spp (4)	15	1	15	2	15	1
Salmonella spp (4)	15	3	15	1	15	0
Total	45	10	45	8	45	5
LSD	1.63		2.57		2.3	

With others, the study showed that higher rate isolate of *streptococcus* bacterium (two isolates in May compared with the other months) and there is no significant differences (LSD 2.57) with other group of isolates, these findings agree with rare studies which performed on *streptococcus* role in broiler arthritis (22) Apparently, most problems result from mixed bacterial infections including the common coliforms and various species belonging to the genera Staphylococcus, Streptococcus, Proteus and others. synovitis can usually be traced to faulty of incubation, chilling/overheating, vaccination and stressful condition (23).

When the study discusses the age of infected birds, the table (3) shows the rate of bacterial isolates and their relationship with the age. Although, the higher rate of bacterial isolates were at 15 days but there is no significant differences between them with their other age factors. A previous study showed that number of bacterial isolates increased in the 15 days (24) .

**Table(3): shows the relationship between the bacterial isolates with age.**

Months	15 days		20 days		25 days	
	Total Number of bacterial isolates	Number of isolates	Total Number of bacterial isolates	Number of isolates	Total Number of bacterial isolates	Number of isolates
April	10	3	10	4	10	3
May	8	4	8	2	8	2
June	5	4	5	0	5	1
Total	23	11	23	6	23	6
Statistical analysis	$X^2=1.847$ , P value= 0.76, Degree of freedom= 4, P> 0.05					

In conclusion, our knowledge is the most extensive studies to date providing information for how many bacterial isolates that we have obtained during the research period from synovial joint, in addition, the most ratio of bacterium was *Escherichia coli*.

**References :**

- Riddell, C.(1992). Non-infectious skeletal disorders of poultry: an overview. In C.C. Whitehead (Ed.), Bone Biology and Skeletal Disorders in Poultry. Poultry Science Symposium No 23 (pp. 119–145). Abingdon, UK: Carfax Publishing Company.
- Morris, M.P. (1993). National survey of leg problems. Broiler Industry, May, 20– 24.
- McNamee, P.T. and Smyth, J.A.(2000). Bacterial chondronecrosis with osteomyelitis(femoral head necrosis) of broiler chickens: a review. Avian Pathology.2000;29(5):477-495.
- Reece, R.L.(1992). The role of infectious agents in leg abnormalities in growing birds. In C.C. Whitehead (Ed.), Bone Biology and Skeletal Disorders in Poultry. Poultry Science Symposium .No. 23 (pp. 231–263). Abingdon, UK: Carfax Publishing Company.
- Thorp, B.H.;Whitehead, C.C.; Dick, L.; Jones, J.M. and Wood, A.(1993). Proximal femoral degeneration in growing broiler fowl. Avian Pathology, 22: 325-342.
- McNamee, P.T. (1998). Investigation of bacterial chondronecrosis with osteomyelitis in broiler chickens. Doctor of Philosophy Thesis. Belfast, Northern Ireland: The Queen’s University of Belfast.
- McNamee, P.T.; McCullagh, J.J.; Thorp, B.H.; Ball, H.J.; Graham, D.G.; McCullough, S. J.; McConaghy, D. & Smyth, J.A. (1998).Study of leg weakness in two commercial broiler flocks. Veterinary Record, 143, 131–135.
- Mohan,K.; Shroeder-Tucker, L.C.; Karenga, D.; Dziva, F.; Harrison, A. and Muvavarirwa,P.(2002).Unidentified Coryneform Bacterial strain from cases of polyarthritis in Chickens ;phenotype and fatty acid profile. Avian Diseases.46:1051-1054.
- Riddell, C. (1997). Leg problems still important. Poultry Digest. 56: 28– 31.
- Raj Manohar,G.; Omprakash,A.V.; and Kanagaraju ,P.(2015). Leg weakness in commercial broiler chicken-overview. International Journal of Science, Environment and Technology, Vol. 4, No 2: 482 – 487.
- Jordan, F.; Pattison, M.; Alexander. D.andFaragher, T.(2002)Poultry diseases. 5<sup>th</sup> ed. London:W.B.Saunders.163p.
- Dinev ,I.(2012). Leg Weakness Pathology in Broiler Chickens. J. Poult. Sci., 49: 63-67.
- Dinev, I. (2009).Clinical and morphological investigations on the prevalence of lameness, associated with femoral head necrosis in broiler chickens. British Poultry Science, 3: 284-290.
- Brooks,G.F.;Butel,J. S. and Mores,S.A.(2010).Medical and adelbergs medical microbiology .27<sup>rd</sup>ed.,Printed in Singapore .

15. Baron, E. and S. Finegold. (1990). Bailey and Scott's diagnostic microbiology, 8th ed. The Mosby Company, St. Louis, MO.
16. Thomas, P.; Sameh, W.; Malic, H.; Nadia, A.; Hermann, E. & Broder, M. (2014). "Discovery of a living coral reef in the coastal waters of Iraq. [www.nature.com/Scientificreports](http://www.nature.com/Scientificreports).
17. Hudault, S.; Guignot, J. and Servin, A.L. (2001). "*Escherichia coli* strains colonising the gastrointestinal tract protect germfree mice against *Salmonella typhimurium* infection". *Gut*.49:47-55.
18. Savageau, M.A.(1983).*Escherichia coli* habitats, cell types and molecular mechanisms of gene control. *Am. Nat.*122 :732–744.
19. Ihssen, J. and Egli, T. (2005). Global physiological analysis of carbon- and energy-limited growing *Escherichia coli* confirms a high degree of catabolic flexibility and preparedness for mixed substrate utilization. *Environ. Microbiol.* Oct. 7(10):1568-1581.
20. Wei, H.J.; Cheng, K.Y.; Cheng, C.P. and Nin, Z.L. (1995). Experiments on portal of infection of arthritis type *staphylococcosis* in chicken. *Journal of Hunan Agricultural College*, 21: 89-92.
21. Oh, J.Y.; Kang, M.S.; An, B.K.; Song, E.A.; Kwon, J.H and Kwon, Y.K. (2010). Occurrence of purulent arthritis broilers vertically infected with *Salmonella enterica* serovar Enteritidis in Korea. *Poult Sci.* Oct.89(10):2116-22.
22. Peckham, M. C. (1966) An outbreak of *streptococcosis* (apoplectiform septicemia) in white rock chickens. *Avian Diseases*, 10:413-421 and
23. Monsuru, O. A.; Kabir, B. O. and Titilayo, E. A. (). Growth, Mineral Deposition, and Physiological Responses of Broiler Chickens Offered Honey in Drinking Water during Hot-Dry Season. *International Journal of Zoology*.
24. Naser, R.; Malidareh, S.; Firouzi, N.; Ranjbar, M. and Hassan, H. (2013). In vitro and in vivo susceptibility of *Salmonella* spp. isolated from broiler chickens. *Comp. Clin. Path.* 22(6) :1065–1068.