# BACTERIAL GRANULOMA IN BROILER CHICKS

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## ABSTRACT

*An ISA-brown flock started with 5000 birds on day 1 of chicken age suffered from high mortality(50% mortality during rearing period) that was characterized by stunting, ruffling of feathers and occasionally corneal opacity. On necropsy, the most pronounced lesions were granulomas in heart, gizzard, pancreas, duodenum and ceccal core. Pathologically, granulomas were represented by caseous necrosis surrounded with macrophages, hepatic coagulative necrosis and separation of corneal epithelium, necrosis of retina and infiltration of iris with macrophages were recognized.*

*Three bacterial agents were recognized and were co-included as etiological agents. These were Salmonella pullorum-gallinarum (identified on serological basis), Proteus mirabilis (identified on morphological basis as well as biochemically using the api 20 E system) and Actinomyces pyogenes.*

*Experimental infection in baby chicks resulted in reproduction of the granulomas mainly with Actinomyces pyogenes and to a small extent with P. mirabilis.*

*It was concluded that the 3 pathogens Salmonella pullorum-gallinarum, Proteus mirabilis and Actinomyces pyogenes cooperated in inducing the field picture of the severe outbreak which may be described as multifactorial granulomatous outbreak in broiler chicks.*

## INTRODUCTION

Recently, special attention to causes of granulomatous diseases in broilers rises with the discovery of broiler affection with avian leukosis J-virus **(Fadly and Smith, 1999)** and the very virulent Marek's disease virus **(Calnek and Witter, 1997)**. Sometimes, white nodules resembling Marck s disease tumors may be present in cardiac muscles, pancreas, gizzard muscles and intestine of broilers infected with Salmonella pullorum **( Shivaprasad, 1997)**. Granuloma in liver,

ceca, duodenum, and mesentery resembling leukosis tumors are sometimes caused by E. coli **(Barnes and Gross, 1997)**. Granuloma in different visceral organs were found to be a sequella of infection with Mycobacterium avium **(Feldman, 1938; Francis, 1958 and Thoen et al., 1984)**, with Actinomyces (Corynebacterium) pyogenes **(Senior et al., 1982; Corrales 1988; Hill et al., 1992)**, with Nocardia **(Okoye et al., 1991; Hill et al., 1992)**, and with Eubacterium tortuosum **(Langheinrich and Schwab, 1972; Arp, et al., 1983; Hafner, et al., 1994)** .

Recently in Dakahlia province young birds in some poultry farms suffered from stunting, ruffling feather and unusual increasing mortality. On necropsy, some birds had whitish nodules in heart and gizzard muscles, in pancreas, intestine and ceccal core. The aim of our work was to investigate bacterial causes of such granuloma in ISA-brown broiler flock that had high mortality during rearing period with granulomatous lesions.

## *MATERIAL AND METHODS*

**Flock history:**

An Isa-brown flock (5000 one-day-old chicks) suffering from high mortality rate from the 2nd day of life and conttnued to have high mortality throughout the rearing period. Only 50% of the flock reached the marketing age (55 days). This flock was hatched from 6 day-stored eggs and chicks were left for 24 hours in hatchery before reaching the poultry farm. During rearing period, a lot of birds suffered from stunting, ruffled feathers and only few birds showed cornealopactty. Necropsy was done on weekly basis where abnormalities were recorded and bacteriological isolation was done. Fourteen blood samples were collected on day 20 and other 14 samples were collected on day 40 of chicken age for serological identification.

**Bacteriological isolation and identification:**

All freshly dead or dying birds showing granulomatous lesions were subjected to bacterial isolation from heart, liver, gizzard muscles and ceccum on Selenite-F broth at 37°C for 18 hours and on brain heart infusion agar at 37°C for 24 hours. Subcultures from selenite-F broth and brain heart infusion agar were plated on Salmonella-Shigella agar (SS agar) and MacConkey's agar plates and incubated at 37°C for 24 hours. Bacterial isolates were purified and identified through staining of bacterial film with Gram stain and through biochemical tests **(Koneman et al., 1992)**. Gram negative bacteria were identified using the identification system of Enterobacteriaceae. Other gram-negative rods were identified through the api 20 E system, bioMerieux Vitek, Inc. Isolates were transferred to trypticase slope agar where it was stored in refrigerator.

Gram positive bacteria were identified morphologically and biochemically according to **Koneman et al., 1992 and Quinn et al., 1994**.

**Pullorum Test:**

Nobilis. Sp Antigen, an Intervet antigen was used for detection of infection by both standard and variant strains of Salmonella pullorum and Salmonella gallinarum in blood or serum of chicken using the plate agglutination test. Serological identification was done using sera collected on day 20 and 40 of chicken age. Sera were tested undiluted or diluted to 1/2, 1/4 and 1/8. A drop of serum (undiluted or diluted) was added to approximately equal size drop of well-shaken Nobilis. Sp Antigen on glass slide at room temperature. The drops were mixed with curved Pasteur pipettes and agglutination was recorded within 2 minutes.

**Micro-agglutination test :**

In a 96 well-plate, 2 fold serial dilutions of the pullorum-positive-tested serum samples were made starting from 1/2 dilution up to 1/256. Equal volume (50ul) of 1/3 diluted Nobilis. Sp salmonella antigen was added to each well. Plate was sealed and incubated at room temperature and read after 6 and 20 hour incubations **(Williams and Whittemore 1971 and 1973)**.

**Histopathology:**

Specimens from heart, liver, lung, gizzard, duodenum, pancrease, cecum and the eye-ball of dying birds with granulomatous lesions were fixed in 10% neutral buffered formalin solution. Five micron thick paraffin sections were prepared and stained with Hematoxylin and Eosin **(Lillie and Fulmen, 1976)** for microscopic examination.

**Experimental infection:**

One isolate that was identified as Proteus mirabilis, and another one that was identified as Actinomyces pyogenes were cultivated on typticase soy broth overnight. For each of the isolates, forty 2-day old broiler chicks were inoculated intraperitoneally with 0.1ml of baeterial suspension per chick. Birds were kept in wire-floored pens with continuous lighting and feed and water were supplied at libitum. The observation period was 40 days. Dead or sacrificed birds were necropsied and re-isolations were performed as usual.

## *RESULTS & DISCUSSION*

**Bacteriologically:**

All Selenite-F broth cultures were salmonella negative when transferred to the MacConkey's and SS plates and tested biochemically. The isolated colonies were colorless on MacConkey's and colorless with a black center on SS plates, the isolates were gram negative rods that reacted positively in the api 20 E system with ornithin, sodium citrate, sodium thiosulfate, urea, tryptophane, Kohn s gelatin and glucose and reacted negatively with ortho-nitro-phenyl-B-D-galaetopyranoside, arginine, lysine, tryptophane, sodium pyruvate, mannitol, inositol, sorbitol, rhamnose, sucrose melibiose, amygdalin and arabinose. According to the api 20 E system it was identified as a Proteus mirabilis.

Brain heart infusion agar revealed two types of colonies. One of them was swarming on the media and was identified as Proteus mirabilis. Other colonies were gram-positive bacteria that were identified as Aetinomyces pyogenes.

**Pullorum test:**

Results of pullorum test of a total of 28 serum samples collected from diseased flock on days 20 and 40 of chicken age (14 at a time) are summarized in table 1.

**Micro-agglutination test:**

Results of retesting of pullorum positive sera colleeted from naturally diseased flock on days 20 and 40 of chicken age using the microagglutination test are summarized in table 2.

**Postmortem examination:**

On necropsy of the naturally affected chicks, small whitish nodules started to appear on heart by day 7 of chicken live and continued to appear in about 10% of dead birds up to day 40 where no other postmortem examination was carried out. Nodules became larger that even a complete distortion of heart shape appeared in some birds by day 21 and continued to be found in some dead birds up to 40 days of age. Ceeal core was seen in almost all birds with heart lesion. Similar nodules appeared in duodenum, pancreas and gizzard of naturally infected birds. Experimental infection with isolated A. pyogenes induced similar lesion in heart, gizzard, pancreas and duodenum of 2 birds out of 40 (Fig. 1,2 and 3) by day 18 post-infection. Two birds showed only one small whitish nodule in liver. Mortality started 48 hours post infection and stopped on the 20th

day post-infection, without any treatment. A total of 21 birds died from the 2nd to 20th days post-infection. A. pyogenes was reisolated from heart, liver and gizzard of dead and dying experimentally infected birds.

Experimentally infected birds with isolated Proteus mirabilis resulted in 4 dead birds during the observation periods. Tow dead birds (48 hours post-infection) showed no obvious postmortem changes while the others that died in days 12 and 14 post-infection showed military necrotic focci in liver and in one bird there was adhesion between liver, heart and sternum. Proteus mirabilis were reisolated from liver and heart of dead birds.

**Histopathology:**

Microscopically, heart and gizzard were focally replaced by caseous necrosis infiltrated and surrounded with macrophages. The macrophages showed large nuclei with scanty cytoplasm (Fig. 4). Moreover, focal replacement of myocardium with macrophages that were arranged either in sheets or nodules were noticed (Fig. 5). The proventricular and gizard wall were infiltrated and focally replaced by macrophage (Fig. 6 and 7).

Coagulative necrosis of hepatic parenchyma that was infiltrated with few macrophages could be recognized as in Fig 8. Other hepatic areas were replaced with macrophages and few neutrophils.

Massive infiltration of intestinal wall with macrophages besides thickened and sloughed villi were observed (Fig. 9 and 10). The cecal core showed caseous material besides infiltration of the wall with macrophages (Fig. 10).

Focal replacement of pulmonary and pancreatic tissue with macrophages besides cystic dilatation and periductal fibrosis of pancreatic ducts were seen. The spleen showed hyperplasia of reticuloendothelial cells and hyalinization of the wall of splenic artery (Fig. 11).

Vacuolation of the lens, separation of the corneal epithelium and infiltration of iris with macrophages were detected (Fig. 12 and 13). Separation of the retina from chroid with protenaceous fluid and necrosis of inner retinal layers were noticed (Fig. 13).

Microscopical examination of the experimentally infected birds with A. pyogenes revealed nearly similar microscopic changes similar to those of the naturally infected birds beside focal caseous necrosis of the hepatic parenchyma. Coagulative necrosis of hepatic parenchyma besides retrogressive changes and congestion of blood vessels of paranchymatous organs could be detected in birds experimentally infected with P. mirabilis.

There is a number of disease conditions affecting young birds that go undiagnosed with re-

spect to identifying a definite etiological agent either due to lack of specific reagent(s), improper timing of sample collections, previous medication, and others which may contribute to a failure to diagnose the causative agent(s). There have been granulomas of young poultry (turkeys and chickens) which are grossly visible as firm, nearly spherical, pale yellow to white variable sized masses that may be seen in liver, spleen, pancreas, gizzard and intestine. A variety of bacteria were reported including S. pullorum **(Shivaprasad, 1997)**, E. coli **(Barnes and Gross, 1997)**, Mycobacterium avium **(Feldman, 1938; Francis, 1958 and Thoen et al., 1984)**, Actinomyces (Corynebacterium) pyogenes **(Senior et al; 1982; Corrales, 1988; and Hill et al., 1992)**, Nocardia **(Okoye et al., 1991and Hill et al., 1992)**, Eubacterium tortuosum **(Langheinrich and Schwab, 1972; Arp et al., 1983; Hafner et al., 1994)** and staphylococcus **(Langheinrich and Schwab, 1972)**.

In the present work, Salmonella pullorum infection was the main suspect because of the similarities in clinical and pathological lesions.This infection was diagnosed serologically through the plate agglutination test and the microagglutination test. Sera collected from naturally affected flock on the 20th day of age, gave positive agglutination in 3 out of the 14 investigated sera (21.4%). Out of the 3 positive samples one was positive up to the dilution of 1/8 which was also positive with the microagglutination test at the dilution of 1:16. At the age of 40days, samples from the naturally affected flock had 7 positive out of the 14 examined sera. Out of the 7 positive, 4 samples were positive for serum pullorum test at 1:8 dilution. With the microagglutination test, the 7 positives yielded positive reactions at dilution up to 1:128. Even though, no salmonella organisms were recovered from the naturally infected flock. Failure to recover S. pullorum organisms from the lesions of the naturally affected flock could be attributed to the massive usage of antibacterial drugs (Ciprofloxacin and rifamycin) and the following possibility of localization of the microorganisms or its killing. The gross and microscopic lesions as well as the clinical signs were not pathognomonic since the granulomas could be seen in other diseases. Since pathogens other than Salmonella-pullorum-gallinarum were isolated, it could be concluded that S. pullorum-gallinarum was not the only responsible pathogen for the very high mortality in the naturally infected flock. However, it is beyond doubt that it shared in the clinical, pathological changes as well as in the high mortality in the naturally diseased flock.

A. pyogenes was the second pathogen. It was isolated and identified from the naturally infected flock. This finding is supported by the reports of **Senior et al., 1982; Corrales et al., 1988 and Hill et al., 1992.** These authors described similar infections in young poultry. Further proof was gained from the experimental infection with the isolate to 40 two-day-old broiler chicks. The experimentally infected chickens showed similar clinical signs and lesions, particularly in the heart, gizzard, duodenum and pancreas. High mortality (21 out of 40) occurred in this type of in-

fection within 18 days (day 2 to day 20 post-infection). Successful re-isolation of the A. pyogenes is another proof that this pathogen was at least one of the major causes of the natural infection. From the experimental data, it seems safe to state that this organism could be the main etiological agent.

The third recognized bacterial agent was Proteus mirabilis. This organism was isolated from the naturally infected flock. Identification of the organism was based on its morphological characters as well as the biochemical results obtained from the api 20 E system. Proteus mirabilis was recovered from a low percentage of salpingitis in layers by **Bisgaard and Dam (1981)** and from duck by **Bisgaard (1995)**. Experimental infection with the isolated P. mirabilis resulted in only 10% mortality with miliary necrotic focci in the liver and successful re-isolation of the organism from the liver and heart of dead birds may help one to conclude that this organism can be co-included with A. pyogenes and S. pullorum-gallinarum as multifactorial agents to the bacterial granuloma of the naturally infected broiler flock. In our judgement, the three pathogens cooperated in the high morbidity, high mortality (50%) and in inducing the gross and histopathological changes described in this outbreak. Since no work was done to include or exclude viral infection, no comment could be undertaken, however no caseous necrosis associates the viral infections in general.

**Table 1:** Serum plate agglutination titers to *Salmonella pullorum* and *Salmonella gallinarum*.

| Chicken age | No. of positive serum/14 samples | | | |
|---|---|---|---|---|
| | Undiluted serum | 1/2 diluted serum | 1/4 diluted serum | 1/8 diluted serum |
| 20 days | 3 | 2 | 1 | 1 |
| 40 days | 7 | 7 | 6 | 4 |

Table 2: Serum micro-agglutination titers to *Salmonella pullorum* and *Salmonella gallinarum*.

| **Chicken age** | **No. of positive serum/14 two-fold serally diluted serum** | | | | | | |
|---|---|---|---|---|---|---|---|
| | **1/2** | **1/4** | **1/8** | **1/16** | **1/32** | **1/64** | **1/128** |
| **20 days** | **3** | **2** | **1** | **0.0** | **0.0** | **0.0** | **0.0** |
| **40 days** | **7** | **6** | **5** | **4** | **3** | **3** | **3** |

**Fig. 1-3 :** Granulomatous lesions in heart (1), and gizzard (2) beside pancreas and duodenum (3) of 20 day old broiler chicks experimentally inoculated with Actinomyces pyogenes.

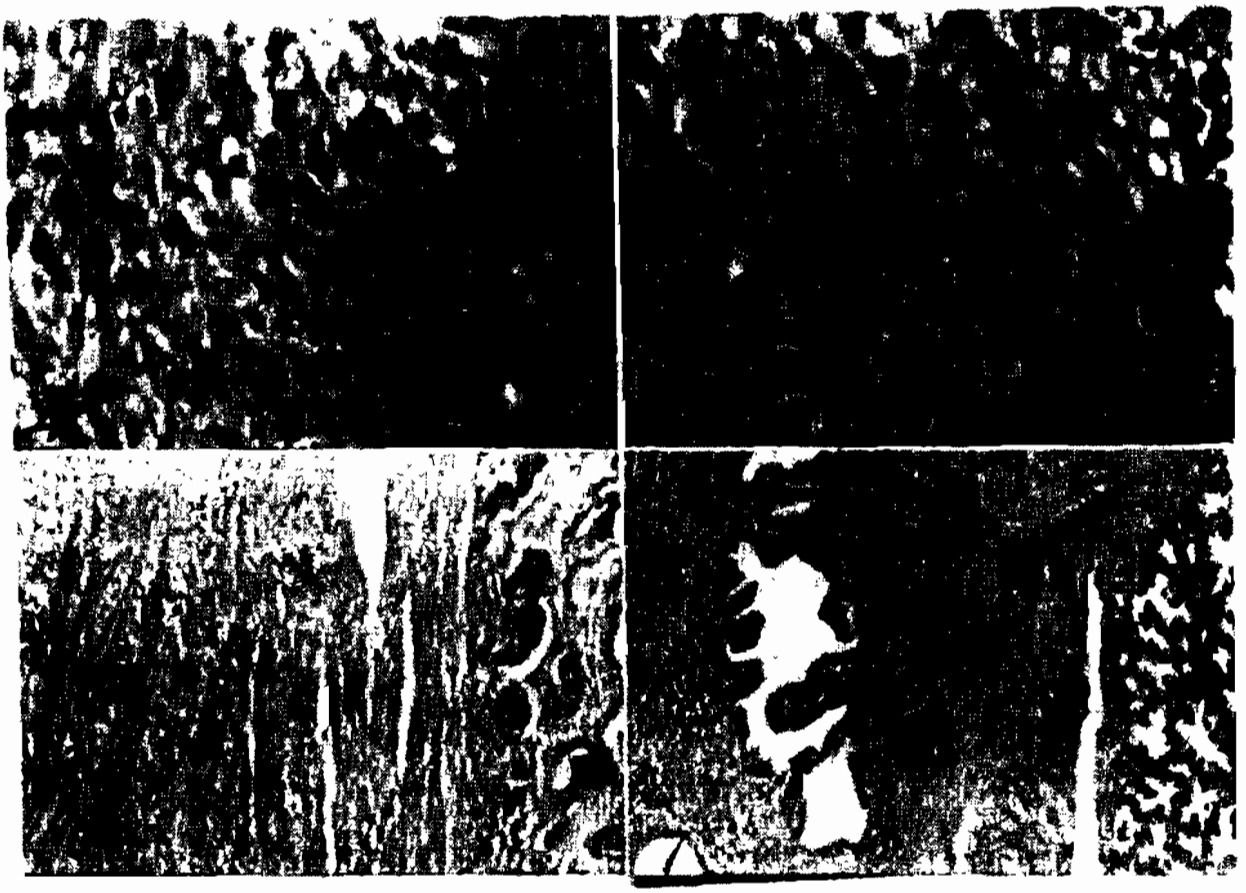

**Fig. 4-7:**

**4:** Myocardium of 20 day old chick showing focal caseous necrosis surround with macrophges. H&E. X 1200.

**5:** Myocardium showing focal replacement of cardiac muscels with macrophges. H&E. X 1200.

**6:** Gizzard muscular-wall showing focal infiltration macrophges. H&E. X 300.

**7:** Proventriculus showing massive infiltration of lamina propria with round cells. H&E. X 300.

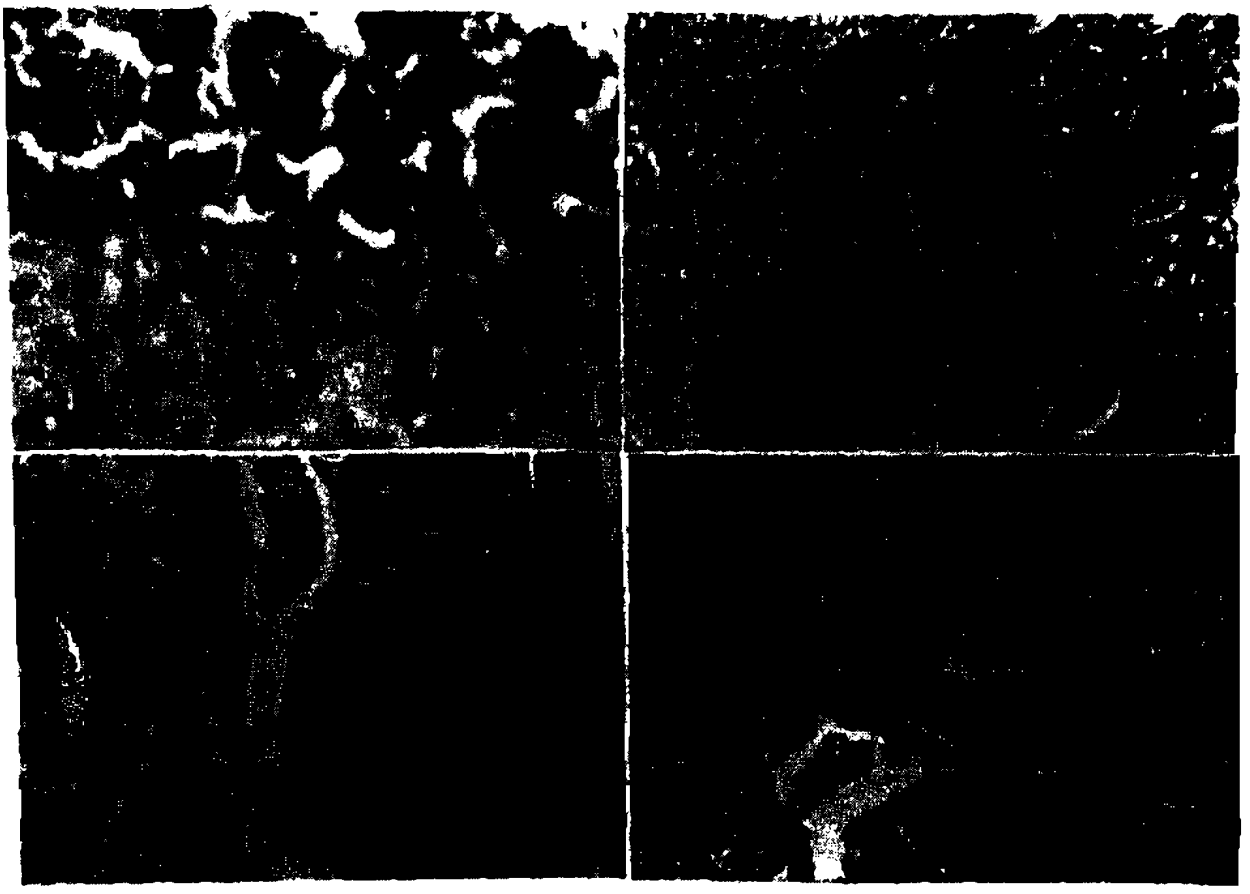

**Fig. 8-11:**

**8 :** Liver showing focal coagulative necrosis. H &E x122

**9 :** Small intestine showing massive round cell infiltration in the intestinal wall. H&E, x 300.

**10:** Cecum showing caseated cecal core. H&E. x 120.

**11:** Spleen showing thickening and hyalinized wall of splenic artery beside hyperplasia of reticuloendothelial cells. H&E., X 300.

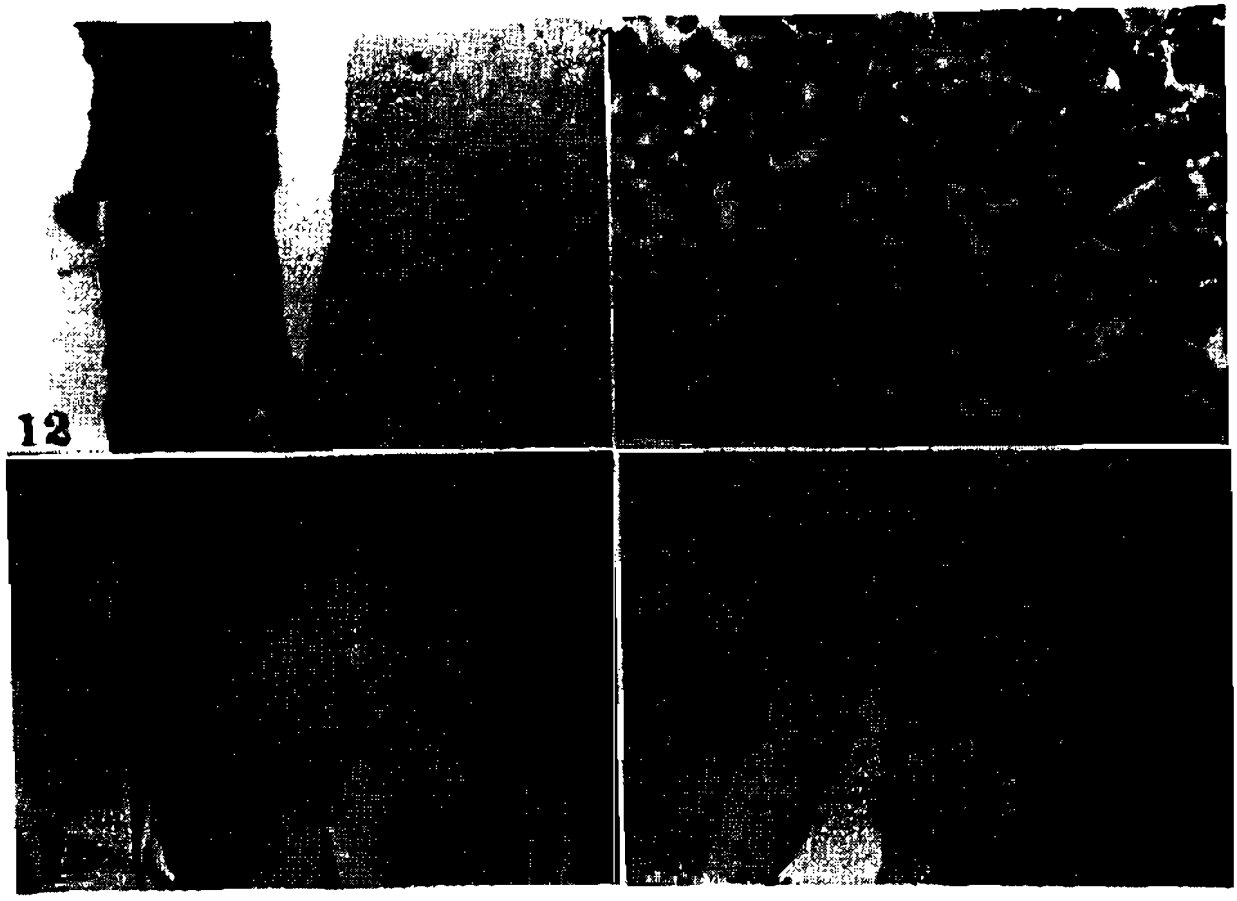


**Fig. 12-15**

**12:** Cornea showing separated anterior epithelium and Bowman s membrane from the substantia propria. H&E, x 1200

**13:** Iris showing massive infiltration with round cells.H&E, x1200.

**14:** Lens showing vacuolations. H&E, x120.

**15:** Retina showing caseous necrosis of the inner layer and separation from chroid with proteinacious fluid. H&E, x300.

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*الملخص العربى*

# الجرانيـــولومـــا البكتيريـــة فى دجـــاج اللحـــم

*المشتركون فى البحث*

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كلية الطب البيطرى - جامعة المنصورة

تم دراسة المسببات البكتيرية المسؤلة عن حدوث أورام فى الأعضاء الداخلية لقطيع تسمين ايزا برون و التي تسببت فى حدوث نسبة نفوق مرتفعة ( ٥٠٪ ) أثناء مرحلة التربية .

تميزت التغيرات الباثولوجية بظهور أورام فى القلب . القونصة . البنكرياس والاثنى عشر والأمعاء الدقيقة وكذلك وجود سدة متجبنة فى الأعورين وعتامة فى القرنية واظهر الفحص الميكروسكوبي للأنسجة المصابة وجود تنكرز متجبن فى القلب القونصة مع وجود الخلايا الالتهامية ( ماكروفاج ) بأنسجة عضلة القلب القونصة وكذلك وجود تنكرز متخسر فى الكبد .

تم التعرف سيرولوجياً على إصابة القطيع بعدوى السالمونيلا بللورم ـــ جللينا رم وكذلك تم عزل وتصنيف ميكروبات الاكتينوميسيس بيوجينس والبروتيس ميرابيلس .

أدت العدوى التجريبية بميكروب الاكتينوميسيس بيوجينس فى كتاكيت التسمين الى حدوث جرانيولوما أما العدوى بميكروب والبروتيس ميرابيلس فقد أحدثت بعض التغيرات الباثولوجية بالكبد.

خلاصة هذه النتائج تدل على أن الثلاثة ميكروبات )السالمونيلا بللورم -جللينارم ,الاكتينوميسيس بيوجينس والبروتيس ميرابيلس ) متعاونة معاً فى إحداث الصورة الحقلية الشديدة لهذا الوباء والتى توصف بالجرانيولوما متعددة الأسباب البكتيرية فى قطيع التسمين.