A study on bacterial dissemination and pathology of experimental intraperitoneal infection of broiler chickens with Pasteurella multocida

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Summary

Following intraperitoneal inoculcation of 11week – old broiler chicken with 1ml (10^9 cfu/ml) Pasteurella multocida the organisms were disseminated in different organs at specific intervals. The microorganisms presisted in the brain (69.23%) for 19 days postinoculation, in the kidneys and ovary or testis (76.92%) for 23 days, in liver (92.31%), in spleen and lungs (100%) and in heart (84.62%) and for 27 days postinoculation in all of these organs. During the first week postinoculation (acute stage). There were extensive congestion, edema, thrombosis and petichial and eccymotic haemorrhages in the various organs. Infiltration of heterophils, fibrin deposition and acute necrotic areas were also found in different organs such as the brain, meninges, intestine, liver, spleen, kidneys, lungs, heart wall and ovary or testis. During the second week postinoculation (subacute stage), the Infiltration of heterophils, fibrin deposition and necrotic areas in different organs were localized and replaced gradually from the outside by lymphocytes and macrophages to form early pyogranulomas. Also, hyperplasia of peyer's patches and solitary lymphoid nodules in the intestinal wall were predominant. During the 3rd and 4th weeks postinoculation (chronic stage), the early pyogranulomas seen in subacute stage become more localized and chronic, consisting of central areas of necrosis surrounded by lymphocytes, macrophages, and giant cells and on the outside there was fibroblasts proliferation. These chronic granulomas were mostly predominant in liver, lungs, kidney and ovary or testis. Also, lesions of chronic necrotic enteritis were seen in certain cases. The above findings revealed that acute septicemic lesions, followed by pyogranulomas and finally chronic granulomas were persistent lesions associated with Pasteurella multocida infection.

دراسة أنتشار جراثيم Pasteurella multocida والآفات الناجمة عنها بعد حقنها داخل الخلب لأفراخ اللحم وبعمر 11 أسبوع

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الخلاصة

بعد حقن واحد سم3 (910) من جراثيم Pasteurella multocida داخل الخلب لأفراخ اللحم وبعمر 11 أسبوع، تبين أن الجراثيم تتوزع في معظم الأعضاء وفي فترات مختلفة. حيث أنها عزلت من الدماغ بنسبة 69.23% ولمدة 19 يوم بعد الحقن وفي الكلى والمبايض أو الخصيتين بنسبة 76.92% ولمدة 23 يوم، في الكبد92.31%، في الطحال والرئتين بنسبة 100%، في القلب 84.62% ولمدة 27 يوم بعد الحقن في هذه الأعضاء.

أما التغيرات المرضية المصاحبة للخمج بهذه الجراثيم فكانت على ثلاث مراحل:

1- خلال الأسبوع الأول من الحقن (المرحلة الحادة)، هنالك إحتقان شديد و نزف بنوعيه الحبرى والكدمي و خثرات، ووذمة، مع إرتشاح العدلات وترسب الليفين حول المناطق النخرية في كافة الأعضاء كالرئتين و الكبد و الطحال و الكلى و الدماغ و السحايا و المبيض أو الخصيتين وفي الأمعاء.

2- خلال الأسبوع الثاني من الحقن (المرحلة تحت الحادة)؛ أن هذه الأرتشاحات للعدلات المجاورة للمناطق النخرية وترسب الليفين بدأت تحل محلها خلايا لمفاوية وبلاعم كبيرة من الخارج لتكون أورام حبيبية قيحية في هذه الأعضاء كافة. هنالك أيضاً تضخم لطخات باير والعقيدات اللمفاوية المنفردة في جدار الأمعاء.

3- خلال الأسبوع الثالث والرابع (المرحلة المزمنة)؛ تبين أن كافة الأورام الحبيبية القيحية قد تحولت إلى مزمنة ومحصورة جداً في الكبد، الرئتين، الكلى، الخصيتين أو المبايض في حين أختفت من الدماغ والسحايا وجدار القلب والطحال. أما الأمعاء فقد إحتوت على آفات التهاب نخري ومزمن.

من هذه النتائج استنتج بأن Pasteurella multocida تحدث آفات حادة متمثلة بالأنتان الدمـوي ويليها أورام حبيبية قيحية وأخيراً أورام حبيبية مزمنة ومحصورة.

Introduction

Fowl cholera, an avian disease of major economic importance, is caused by infection with Pasteurella multocida. This organism is distributed world wide and infects a variety of avian and non avian animal hosts[1]. The disease often occurs as a lethal septicemia in which Pasteurella endotoxin is considered to play a role in pathogenesis[2]. The outbreaks are characterized by sudden increases in mortality lasting few days. Mortality ranges widely depending on the virulence of the bacterial agents and the effects of environmental stressors [3].

Two forms of the disease are described in avian species: acute septicemic fowl cholera associated with sudden death due to generalized venous congestion is suggestive of shock induced by bacterial endotoxins[2] and chronic fowl cholera, which may follow an acute stage of the disease or result from infection with organisms of low virulence and associated with localized infections involving wattles, sinuses, middle ear, leg joints in different avian species infected with this disease.

Although much research has been undertaken to characterize the bacteria responsible for fowl cholera, relatively little work has been conducted on the bacterial dissemination and pathological findings associated with causative agents of fowl cholera. For this reason, the aims of the present study were:

1- Study the dissemination of Pasteurella multocida in organs of broiler chickens following intraperitoneal route of inoculation.

2- Study the pathological findings associated with this microbial infection, (fowl cholera).

Materials and Methods

Broiler chickens: Twenty-six broiler chicks, provided by Al-Ghalibia poultry industry, Baghdad. The broiler chickens were reared together to check them free from any disease. During the 11th week of age, all the chickens were intraperitoneally inoculated with Pasteurella multocida.

Preparation of inoculum:

A local strain of Pasteurella multocida isolated from layers flock infected with fowl cholera. Isolation and complete biochemical identification of Pasteurella multocida were done [5]. A logarthmic growth of this microorganism was done in brain heart infusion broth, the growth culture was

washed in phosphate buffer saline and the final bacterial saline suspension contained 10^9 colony forming unit (CFU) of Pasteurella multocida /ml. After intraperitoneal inoculation of chicken with 0.5 ml of the bacterial saline suspension (6), 2-3 chickens were euthenised at specific intervals for 27 days postinoculation, looking for bacterial dissemination in different chicken organs.

All gross lesions were recorded and for histopathology, a representative pieces of diseased organs were fixed in 10% neutral buffered formalin, processed routinely, embedded in paraffin blocks; cut at 5 μ thickness and stained with hematoxylin and eosin according to the standard procedures.

Results

Dissemination of Pasteurella multocida in organs of broiler chickens following intraperitoneal inoculation:

During the 27 days postinoculation of Pasteurella multocida. the microorganisms were disseminated in the different chicken organs at different intervals (Table-1). The microorganisms presisted in brain (69.23%) for 19 days postinoculation, in the kidney and ovary or testis (76.92%) for 23 days postinoculation and in liver (92.31%), spleen and lungs (100%) and heart (84.62%) for 27 days postinoculation.

Table –1: The dissemination of Pasteurella multocida in organs of broiler chickens following intraperitoneal inoculation

*PI days Interval	No. Chicks	Heart	Lung	Spleen	Liver	Kidney	Brain	Tastis /Ovary
1	3	3/3	3/3	3/3	3/3	3/3	3/3	3/3
4	3	3/3	3/3	3/3	3/3	3/3	3/3	3/3
6	3	3/3	3/3	3/3	3/3	3/3	3/3	3/3
8	3	3/3	3/3	3/3	3/3	3/3	3/3	3/3
11	3	3/3	3/3	3/3	3/3	3/3	3/3	3/3
15	3	3/3	3/3	3/3	3/3	2/3	1/3	2/3
19	3	2/3	3/3	3/3	2/3	1/3	2/3	2/3
23	3	1/3	3/3	3/3	2/3	2/3	0/3	1/3
27	2	1/2	2/2	2/2	2/2	0/2	0/2	0/2
Total	26	22/26	26/26	26/26	24/26	20/26	18/26	20/26
Percentage %		84.62	100	100	92.31	76.92	69.23	76.92

- postinoculation
- 4 chicken died during first week postinoculation

Pathological findings:

The pathological findings were present in three stages:

1- During the first week postinoculation (acute stage):

There were extensive venous congestion, thrombosis and petichial and ecchymotic haemorrhages in different organs. Heterophils and fibrin infiltration together with acute coagulative areas of necrosis were present in the interstitial tissue of lungs (causing fibrinopurulent pneumonia) (Fig-1), the pericardial sac, the liver and the spleen. Also some of these heterophils

infiltration and edema were seen in interstitial tissue of the kidneys, ovary and testis and in certain cases, cellular infiltration and edema were present in meninges, cerebellar tissue and in intestinal mucosa and submucosa causing sloughing of their lining epithelia.

Four chicken had died during this stage of the disease process and giving similar microbial dissemination and pathological findings.

During the second week postinoculation (subacute stage): The pathological lesions become focal type and distributed in different location sites of organs. All the heterophils and fibrin infiltration together with necrotic areas were gradually replaced by lymphocytes, macrophages and some giant cells from the outside forming an early pyogranulomatous type lesions which were most predominant in the cerebellar tissue (Fig-2), in the lung parenchyma, in white pulp of spleen, in the perisinusoidal and central vein (Fig-3) area of liver, and in the interstitial tissues of kidney and ovary or testis(fig- 4). Also there was hyperplasia of peyer's patches and solitary lymphoid nodules in the intestinal wall together with necrosis of their lining epithelia.

During the 3rd and 4th weeks postinoculation (chronic stage):

The pathological lesions were more localized and some of the granulomatous lesion seen in the 2nd week postinoculation disappeared during this stage except that, some other granulomatous lesions become more chronic and were persisted in liver, lungs, kidneys (Fig-5), and ovary (Fig-6) or testis. These chronic granulomas consisted of central areas of mild necrosis surrounded by excessive number of lymphocytes and macrophages together with giant cells, and on the outside fibroblastic proliferation. Also, chronic necrotic enteritis was predominant in certain cases along cecal wall (Fig-7), during this period of the diseases process.

Fig.1:fibrinopurulent pneumonia

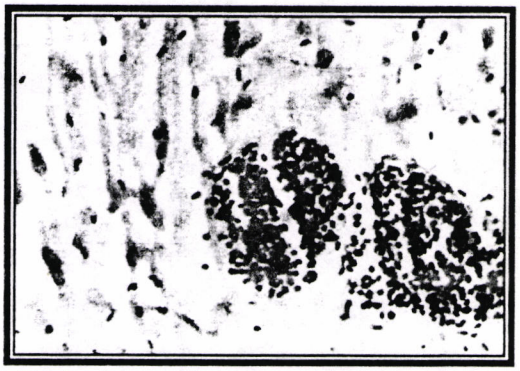

Fig –2: Brain tissue: Observe multifocal early granulomatous reaction consisting of aggregation of lymphocytes and macrophages. At 2nd week postioculation (H & E) × 250

Fig –3: Liver tissue: Note early granulomatous reaction around central vein, consisting of aggregation of lymphocytes and macrophages. At 2nd week postioculation (H & E) × 125

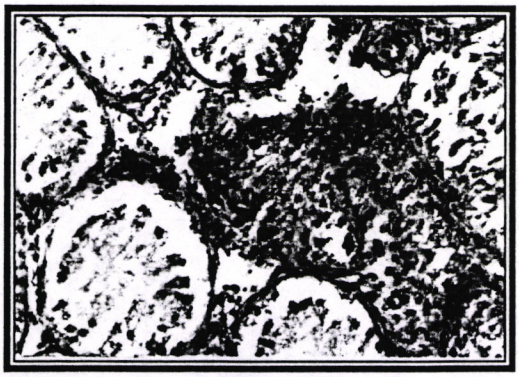

Fig –4: Testicular tissue: Observe chronic granulomatous reaction consisting of aggregation of lymphocytes and macrophages. At 2nd week postioculation (H & E) × 125

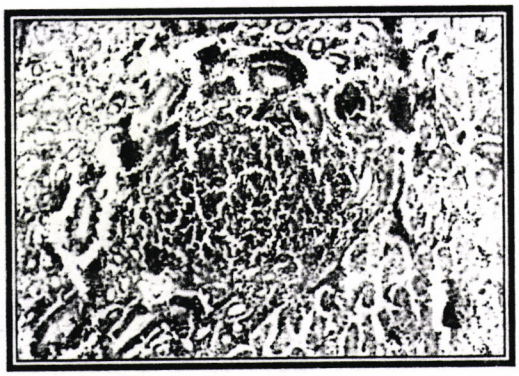

Fig –5: renal tissue: Note focal early granulomatous reaction consisting of aggregation of lymphocytes and macrophages. At 3rd week postioculation (H & E) × 125

Fig –6: Ovarian tissue: Observe chronic granulomatous reaction consisted of aggregation of lymphocytes and macrophages surrounding central area of caseous necrosis on the outside fibroblasts proliferation. At 3rd week postioculation (H & E) × 125

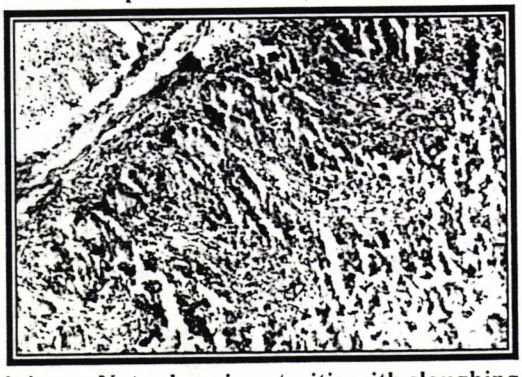

Fig –7: intestinal tissue: Note chronic enteritis with sloughing of lining epithelium. At 4th week postioculation (H & E) × 125

Discussion

Following the intraperitoneal inoculation of the broiler chicken with Pasteurella multocida, an excessive multiplication and proliferation of this microbe occurred in the peritoneal cavity and through the lymphatic and blood vessels the microorganisms disseminated to the different organs of chicken with different infectivities and at specific intervals. The dissemination of these microorganisms was detected previously [4,7] in the lungs, liver, spleen and in subcutaneous tissue of turkeys with facial cellulitis. Also, a similar related microorganism (Pasteurella hemolytica) was isolated from heart and liver of infected pullents and laying hens[8]. The dissemination of Pasteurella multocida in these organs and in other organs of chicken such as brain, kidneys, ovary and testis in the present study, may indicate that a state of bacteremia occurred following the bacterial inoculation, through which the organisms multiply, suggesting primarily intravascular rather than intracellular multiplication in the blood, liver and spleen and in other organs and induction of the pathological lesions [7].

The pathological lesions were initiated as an acute septicemic lesion characterised by extensive congestion, vascular thrombosis, fibrin deposition and heterophils infiltration together with acute necrosis in different chicken organs. The ability and virulence of the Pasteurella multocida to produce these acute pathological lesions was through the endotoxins produced by these microorganisms causing vascular endothelial damage and resulted into heterophil liberation outside the blood vessels and fibrin [9]. Also, the Pasteurella endotoxins were responsible for initiation of vascular fibrinous thrombosis, haemorrhage and acute necrosis in the different organs especially around the blood vessels[10].

During the 2nd week postinoculation of chicken with these microorganisms. All the necrotic areas infiltrated with heterophils were transformed into early pyogranulomatous type lesions which were mostly predominant in brain, kidney, heart wall, spleen, liver, lungs and ovary or testis. The transformation of lesions into granulomas were considered to be a state of cell mediated immunity against the Pasteurella antigens and even against living vaccine[11]. Cell mediated immunity has also been found to be essential to the immunity of chickens to the Pasteurella multocida from active immunization[12], using surgically thymectomised chickens in which no level of cell mediated immunity was detected and all thymectomised birds did not survive the Pasteurella multocida infection. Also, during the 3rd and 4th weeks postinoculation these granulomatous lesions became more developed and chronic and mostly localized in liver, lungs, kidneys and ovary or testis.

Similar granulomatous lesions were reported previously [2,13] in the different avian species infected naturally or experimentally with fowl cholera. Other lesions, such necrotic enteritis, meningitis and even granulomatous encephalitis which were mostly predominant in the chickens in the present study also, were reported previously in the avian species especially in turkeys naturally and experimentally infected with fowl cholera [13,14].

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