Pathological change in mice after oral administration of egg raw from experimentally infected chicken with Listeria monocytogenes

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Abstract:
This study was done to determine the role of egg in transmission of Listeria monocytogenes, for this reason layer were infected orally with Listeria monocytogenes and after 30 days the microorganism appear in the eggs, then thirty (30) mice were obtained and split to two groups the first (20) mice were administrated 0.2 ml egg row that contain $10^6$ CFU Listeria monocytogenes orally, and the second group (10) mice were administrated 0.2 ml sterile normal saline orally, Then animals sacrificed in 6 hr, 12 hr, 18 hr, 24 hr, then 2, 3, 4, 6, 8, 10, 13 days post administration.

The results shown that the Listeria monocytogenes isolated from the intestine and also from other internal organs like liver, spleen and kidney, and the pathological results revealed that severe inflammatory cells infiltration with degenerative changes in all examined organs.

We concluded that the Listeria monocytogenes was virulent and infect the mice and give a subclinical disease that approved by the isolation of the microorganism and pathologic feature give the indication that Listeria monocytogenes transmitted by egg and still virulent and have the ability to multiply and invade the internal organs.

Listeria monocytogenes

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الخلاصة:
هذ هذه الدراسة إلى تحديد دور البيض في نقل بكتريا L.M، وبعد 30 يوم من الأصابة ظهرت الجرثومة في البيض.
قسمت مجموعة من الفئران إلى مجموعتين. الأولى مشرّعة بـ 3.00 مل من البض الحاوي على CFU من Listeria monocytogenes وتم قتل الورمونات بعد 6، 12، 18، 24 ساعة. والثانية جرعت بـ 3.00 مل من المحلول الملح المعقم. تم قتل الحيوانات بعد (6، 0، 4، 8، 10، 13) يوم من الاصابة.

النتائج: نشرت نتائج الدراسة و szer bela Listeria monocytogenes قد عزلت من الامعاء والاعضاء الداخلي_loctive العضلات مثل الكبد، الطحال، الكلى. أما التغييرات المرضية الشبيهية أظهرت ارتفاع شديد للخلايا الالتهابية وتشنجات في كل الاعضاء المفحوصة. Listeria monocytogenes عينت نتائج من هذه الدراسة أن بكتيريا Listeria monocytogenes شديدة الضرأوى وأصابت الفئران Listeria monocytogenes المصابه. ودل هذا على أن Bacteria Listeria monocytogenes انتقال كلم الشبيه على الإصابة.

Introduction:
Listeria monocytogenes is a gram positive bacteria that cause severe infection of immunocomprasmised patient (1). This microorganism commonly found in the environment and initially isolated from laboratories and domestic animals (2).

Clinical feature of listeriosis in human are strikingly similar to those found in animals population and incloud sepsis, meningitis and during pregnancy chorioamnionitis (3، 4). While listeriosis has always been considered a zoonosis, the mode of transmission from animals to man has been obscure, some evidence have suggested that both epidemic and sporadic cases of listeriosis in human are primarily due to food borne infection(5; 6).

Natural infection with Listeria monocytogenes results from ingestion of contaminated food such as soft cheese, chocolate, milk and meet (7).Food products that contain chicken meet and egg and contaminated with Listeria monocytogenes specially that stored in refrigerating or freezing consider an important source for infection, Listeria monocytogenes isolated from these products and there is an increase in the meat contamination up to 65% from refrigerated chicken meet contaminated with L. monocytogenes (8). Also the bacteria isolated from egg and the products that contain egg (9)indicating that there is an important role to the infected chicken and the egg produced in transmission of infection.

Epidemiological investigation demonstrate that epidemic listeriosis is a food borne disease and can affect immunosuppressed and apparently immunocompitan human (1). Asymptomatic fecal shading of Listeria monocytogenes has been reported in healthy people, suggesting that the gastrointestinal tract may be the human reservoir of the organism (10).

In order to clarify some of these questions and to know the role of eggs in the outbreak of listeriosis, we administer the mice egg-raw from
experimentally infected chickens with Listeria monocytogenes.

**Materials and methods:**
- **Bacterial strain:** Listeria monocytogenes isolate obtained from zonosis unit \ Baghdad University.
- **The layer infected with L. monocytogenes 10^5 CFU orally, and after period of 30 day the egg examined bacteriologically to the presence of L. monocytogenes, and its count.**
- **White mice obtained from collage of veterinary medicine.**
- **Egg row made by mix the egg in a sterile container and count the number of bacteria in it, then the dose determined and given to the animals.**
- **Bacterial count were done according to Miles and Misra (11).**
- **Histopathological slides were done according to (12)**

**Experimental design:**
30 white mice were divided into two groups randomly
- **The 1st group (20) mice administrated 0.2 ml egg row contain 10^6 CFU Listeria monocytogenes orally.**
- **The 2nd group (10) mice administrated 0.2 ml sterile normal saline orally.**

Then animal sacrificed in 6 hr, 12 hr, 18 hr, 24 hr, then 2, 3, 4, 6, 8, 10, 13 days post administration, and samples for bacteriological and other for pathological examination were taken.

**Result:**
The bacteriological examination:
The bacteria Listeria monocytogenes ) isolated from all the internal organs in varying degree, mainly from intestine, lung, liver from the 1st stage of the infection at the 24 hr post infection, while the kidney at 2 days post infection, and also from the brain in the later stage of infection at 2nd and 3rd days post infection.

**The pathological examination:**
Liver: the histopathological examination of the liver at the 8-15 hr post infection showed severe congestion of blood vessels and also central vein, while at 24-31 hr the liver parenchyma revealed acute cellular degeneration in addition to the congestion, and also there is severe inflammatory cells infiltration around the central vein and in the portal area composed mainly from neutrophils and lymphocyte and at 48 hr there is vacuolation of the hepatocyte with inflammatory cells aggregation as foci scattered in the liver parenchyma (fig: 1), in the 3rd days post infection the lesion characterized by multifocal granulomatous reaction composed of lymphocyte and macrophage with few neutrophils (fig: 2).

Lung: lung examination at 15 hr post infection revealed edematous fluid filled the alveolar lumen with congested capillaries (fig: 3) and at 24hr there is increase in the thickness of the alveolar wall due to edema and inflammatory cells
infiltration mainly neutrophils and the alveoli filled with edema in addition to emphysema (fig: 4) and the same changes in the later periods but more severe.

Intestine: at 24hr there is moderate goblet cells proliferation with mucine in the lumen of the intestine and in the other parts of the intestine revealed mild inflammatory cells infiltration in the sub-epithelial layer and in the lamina propria (fig:5, fig:6) and at the 2nd day post infection there is mild infiltration of inflammatory cells in the submucosa and between mucosal glands (fig: 7) while at 3 days post infection there is severe goblet cells proliferation with mucine in the lumen and also atrophy of the epithelial lining cells which become rounded and scattered mucine attached to the tissue (fig: 8).

Brain: there is moderate neutrophil infiltration around the blood vessels with perivascular edema in the robenson space. As well as moderate proliferation of asterocyte with focal aggregation (fig: 9) 1326 at 48 hr post infection, while at 3-4 days post infection there is focal gliosis in the brain tissue (fig: 10).

Kidney: there is only acute cellular degeneration in the renal tubule.

Heart: the endocardium infiltrated with inflammatory cells with hyperplasia of the endothelial cells in the endocardium at 3 days post infection (fig: 11).

Discussion:

The isolation of Listeria monocytogenes from most examined organs at 24hrs post infection indicated that these microorganisms are virulent and rabid transmitted from the intestine to the blood circulation and cause bacteremia.

Listeria monocytogenes have the ability to invade the epithelial and endothelial cells and reach the target organs. This idea was agreed with these described by Mandel and cheers(13) Mengaud, et al., (14) and Greiffenberg, et al., (15).

The present study that give indication that the infected poultry and eggs play a role in Listeria monocytogenes infection.

The pathological lesion of the intestine indicate that the microorganism proliferate in the epithelial cells of the intestine and induce inflammatory response.

Our study revealed that mild pathological changes occur in the intestine that shown as hyperplasia of the goblet cells with inflammatory cells infiltration and also lymphoid tissue proliferation, indeed, a recent study using a rat ilial loop model of intestinal infection, Pron, et al., (10) has shown that Listeria organism are translocated to deep organ very rapidly, demonstrating that crossing of the intestinal barrier occur in the absence of a prior intraepithelial replication. And also Listeria monocytogenes establish an active local infection in the lymphoid structure of the intestine like Peyer's patches.
While the liver reveals a inflammatory cells infiltration mainly neutrophils in the 1st two days and later the lymphocyte infiltration and multifocal granulomatos lesion, during the early steps of liver colonization, neutrophils are recruited at the site of infection forming discrete micro- abscess, neutrophils have been shown to play an important role in controlling the acute phase of Listeria infection (16), and in mediating the distraction of the Listeria infected hepatocytes in vivo (17).

Hepatocyte respond to Listeria infection by neutrophils chemo-attractant and exhibiting an increase in the adhesion to the neutrophils, resulting in micro-abscess formation (18), they also responds by inciting an apoptosis program that may be critical for removing Listeria infected cells from the liver tissue at early stage of infection (19; 20).

Two to four days post infections neutrophils are gradually replaced by blood derived mononuclear cells together with lymphocyte to form the characteristic granulomas (21; 13). These granulomas are the histomorphological correlate of cell-mediated immunity and preassembly act as true physical barriers that confine the infectious foci, impeding further, bacterial dissemination by direct cell-to-cell passage (22).

Brain: revealed a neutrophils infiltration as well as focal gliosis in the brain tissue. Jose et al., (23) revealed that human lesion in Listerial meningioencephalitis are typical and very similar in human and animals, they consist of perivascular cuffs of inflammatory infiltrates composed of mononuclear cells and scattered neutrophile and lymphocyte. Depletion experiments in mice using neutrophils specific monoclonal antibody have shown that neutrophiles play a critical role in eliminating Listeria monocytogenes from infectious foci in the brain (24).

References:


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