Pathological study of intrauterine infection to embryos by Encephalitozoon cuniculi spores in pregnant mice

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Abstract

This study aimed to investigate pathology of oral E. cuniculi infection during pregnancy in pregnant mice and embryos. A total of 40 pregnant mice at first day of gestation were divided into two groups, first group were infected orally by E. cuniculi sores of 10⁷ spores/ mice, second group left without any treatment. At 18th days of gestation all pregnant mice were euthanized. Gross pathology finding in pregnant mice of infected group included congestion of liver and lung, the embryos lesions consisted from enlargement of head and abdomen. Histological lesions in pregnant mice of infected group consisted of hepatic non-suppurative granulomatous lesions with E. cuniculi spores aggregation with lymphocytic infiltration, the lungs lesions consisted of infiltration of lymphocytes with E. cuniculi spores, kidney lesions composed from degenerative and necrotic changes in renal tubules, brain lesions consisted from lymphocytic infiltration with increase in number of glial cells, while intestine tissue sections showed hyperplasia of lymphatic tissue with present of parasitic vacuoles at tips of villi, the placenta exhibited E. cuniculi spores with hyperplasia of trophoblastin chorionic villi, while histological lesions in embryos showed lymphocytic infiltration around alveoli with hyperplasia of lymphatic tissue around bronchioles with absent the normal architecture of hepatic cords and vacuolation of hepatocytes with hyperplasia of lymphocytes in white pulp of spleen. This study provides insight into the pathology of E. cuniculi infection in pregnant mice and their embryos, also supports the hypothesis of intrauterine transmission of E. cuniculi infection to embryos during pregnancy period.

Keywords: Embryos, Encephalitozoon cuniculi, Intrauterine Infection, Pregnant Mice.

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Encephalitozoon cuniculi

دراسة مرضية لخمج الاجنة داخل الرحم بابوغ في الفئران الحوامل

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الخلاصة

دُرَّست الدراسة الحالية على أفراد تم تمييزهم بفميم الفم الفموي بالبواغ E. cuniculi B.10/1 حسبErrorException.1  اثنى فار حامل في اليوم الأول من الحمل وقسمت إلى مجموعتين: المجموعة الأولى حُجمت 200 أثناً بالبواغ E. cuniculi B.10/1. اثنى فار حامل، أما المجموعة الثانية تبقيت بدون أي عاملة معدة فترة التجربة. ومع ذلك اليوم 18 من الحمل تم قتل جميع الإناث في كلا المجموعتين كلهاً رحمياً. أظهرت نتائج التحليل الفيزيائي لإناث الحوامل في المجموعة المحمية وجدت انفجار في الكبد والرئة واضطرابات اضطرابات الأطعمة الشبيهة والبطن. أما الإناث النشبة في المجموعة المحمية تقولت من انفجار. وراثا حبيبة غير قيقة في الكبد مع وجود أعراض E. cuniculi B.10/1، مما أدى إلى تجمعات الارتشاحات من الخلايا اللمفية، الأمر الذي تأثرت من ارتشاحات الخلايا اللمفية مع أبواغ E. cuniculi. اما أعراض الكلية فقد تأثرت من تغيرات تكنولوجية، وخليجية في النتيجة الكلية، أما أعراض الدماغ فقد تأثرت من ارتشاحات لعفية مع ارتفاع في أعداد
Introduction

In middle of 19th century Nägeli described small Schizomycetes pathogen named *Nosema Bombycis* which was the first microsporidian individual that known (1), after that in two decays Louis Pasteur identified the cause of Pebrine disease in silkworm as one of microsporidian microorganisms (2), at the end of 19th century Pebrine disease in silk worm as one of microsporidian that in two decays Louis Pasteur identified the cause of microsporidia classified as protozoa (3), but after one century of research these microorganisms reclassified as fungi depended on phylogenetic analysis of certain protein code in their genome (4,5), these fungi have been reported cause economic losses in silkworm, honeybee and farm animals (6). Microsporidia is obligatory, opportunistic, intracellular, spore forming microorganism that affect wide range of hosts from insect to mammals (7). Microsporidia phylum contained many species and genus pathogenic for human and animals (3), they are eukaryotic pathogens with smallest genome that ever known (8). The life cycle is direct without intermediate hosts and infection occur mainly via oral route also through inhalation, intraperitoneal injection, intratracheal and intrarectal routes by infective stage known as spores (9). The genus Encephalitozoon contained two most important pathogenic species, first one *E. hellem* which cause serious disease in avian hosts, while second species known as *E. cuniculi* which have wide range of hosts from human to animals and caused lethal disease (10-12). *E. cuniculi* common pathogens in immunocompetent and immunosuppressed hosts (13), and its first mammalian microsporidium that was isolated and cultured in vitro (14). The potential zoonotic effect of *E. cuniculi* are widely study (15), and more research are needed to investigate the pathology of infection of *E. cuniculi* in pregnant mice to fully understand the role of intrauterine infection to embryos and pathological changes due to vertical infection in mice embryo. Therefore, the purpose of this study was to fully characterized the pathological lesions from oral infection of microsporidian fungi *Encephalitozoon cuniculi* in pregnant mice and embryos.

Material and methods

Ethics Statement

The experimental techniques were conducted in accordance with regulations of the College of Veterinary Medicine, University of Mosul, especially protection animal against cruelty and conducting merciful euthanization procedures.

Experimental Animals

A total of 40 pregnant mice were divided in two groups randomly (n=20 each group): first group (infected group) and second group (control group). A total of 50 female mice (not pregnant) were given at estrus synchronization single intraperitoneal dose of 0.5 µg of cloprostenol and 3 µg of progesterone for each female, at third day of estrus synchronization single intraperitoneal dose of 0.5 µg of cloprostenol to each female, the fertile male were mixed with synchronization female in ratio of 1:1 at third day of synchronization, next day the females were examined for present of vaginal plaque (white plaque) that represented occurrence of meeting with male and considered pregnant at first day of gestation (17).

*E. cuniculi* spores

Spores were obtained from fecal samples of rabbits (that naturally infected in Mosul city – Iraq), which isolated by researchers, further study are need to investigate the pathology of infection of *E. cuniculi* in pregnant mice to fully understand the role of intrauterine infection to embryos and pathological changes due to vertical infection in mice embryo. Therefore, the purpose of this study was to fully characterized the pathological lesions from oral infection of microsporidian fungi *Encephalitozoon cuniculi* in pregnant mice and embryos.

Experimental Design

A total of 40 pregnant mice were divided in two groups randomly (n=20 each group): first group (infected group)
given $10^7$ spores/ pregnant females orally at first day of pregnancy, second group (control group) left without treatment during experiment.

**Fecal Examination**

Fecal samples were collected at 18th day of gestation and smear were prepared and stained with quick Hot-chromotrope stain and Weber green modification trichrome stain (20), then slides were examined for presence of *E. cuniculi* spores in fecal.

**Histopathological Examination**

All pregnant mice from infected and control groups were euthanized at 18th day of gestation by intramuscular injection of ketamine (50 mg/kg of body weight) and zylaxine (40 mg/kg of body weight) at ratio of 1:10 to induce euthanization in pregnant mice and their embryos (21). After euthanization gross examination applied to both pregnant mice and their embryos, the tissue samples were collected from pregnant mice fixed in 10% neutral buffered formalin, while embryos are fixed in bouin’s solution (22), later tissue samples were routinely processed by dehydration in increased concentration of ethyl alcohol, cleared by xylene, infiltrated and embedded by paraffin wax, then blocked at 1.5*1.5*1.5 cm of paraffin cubes, then sectioned at 4-6 micrometer using rotating microtome, later tissue slides were stained by Harris Hematoxylin and Eosin stain (H&E) (23).

**Results**

**Gross Pathology**

The pregnant mice showed congestion of meninges, liver and lungs (Fig. 1), while embryos showed enlargement of head with distension of abdomen in compared with embryos of control group (Fig. 2).

**Histological Pathology**

In pregnant mice the tissue sections of brain showed heavy infiltration of inflammatory cells especially lymphocytes in cerebral and meninges with increase in number of glial and microglial cells (Fig. 3), the liver sections showed infiltration of mononuclear inflammatory cells which accumulated with *E. cuniculi* spores with coagulative necrosis in hepatocytes (Fig. 4), in lung the lesions consisted from presence of *E. cuniculi* spores in alveolar septa, with hyperplasia of lymphatic tissue around bronchioles with vasculitis (Fig. 5), in kidney sections showed perivascular cuffing with congestion in affected blood vessels and hemorrhage in interstitial tissue (Fig. 6), while lesions in intestines consisted from hyperplasia of lymphatic tissue in submucosa layer of small intestine with presence of *E. cuniculi* at the tips of villi with mucinous degeneration (Fig. 7), in placenta tissue the microscopic lesions included hyperplasia of trophoblast with presence of *E. cuniculi* in syncytiotrophoblasts (Fig. 8).

In embryos, the histological finding in brain consisted from sever infiltration of microglial cells in all layer of cerebral tissue (Fig. 9), while microscopic lesions in liver showed disturbance in the normal architecture of hepatic cords with presence of *E. cuniculi* spores in distended hepatic sinusoids (Fig. 10), in lung tissue lesions consisted from heavy infiltration of lymphocytes in alveolar septa and around bronchioles with alveolar emphysema (Fig. 11), while in spleen tissue histological finding consisted from hyperplasia of germination centers with hyperplasia of lymphocytes in white and red pulp (Fig. 12).

**Detection of *E. cuniculi* in fecal samples**

The result of staining of fecal samples of pregnant mice at 18th day of gestation presence of *E. cuniculi* spores in 20/20 pregnant mice (100%) in infected group, while 0/20 (0.0%) in control group.

![Figure 1. pregnant mice; Infected pregnant mice; Congestion of liver and lung.](image1)

![Figure 2. Embryo; (A) Enlargement of head and distension of abdomen (A) embryo from control group.](image2)
Figure 3. Brain, Infected pregnant mice; (arrow) Heavy infiltration of lymphocytes (100x, H&E).

Figure 4. Liver; Infected pregnant mice; (arrow) Heavy infiltration of mononuclear inflammatory cells with \textit{E. cuniculi} spores (400x, H&E).

Figure 5. Lung; Infected pregnant mice; (A) hyperplasia of lymphatic tissue around bronchioles (arrow) with vasculitis (100x, H&E).

Figure 6. Kidney; Infected pregnant mice; (A) congestion in blood vessels (B) perivascular cuffing of lymphocytes and macrophages (400x, H&E).

Figure 7. Intestines; Infected pregnant mice; Hyperplasia of lymphatic tissue in submucosal layer of small intestine (100x, H&E).

Figure 8. Placenta; Infected pregnant mice; (arrow) presence of \textit{E. cuniculi} between trophoblast and syncitiotrophoblast (400x, H&E).
Discussion

In current study, the spores of *E. cuniculi* of rabbit origin isolated in Iraq were used to infect the pregnant mice with special emphasis was direct on pathology of the infection in pregnant mice and their embryos to investigate the pathology of infection with *E. cuniculi* to embryos through intrauterine route.

The pathological changes were recorded in pregnant mice are more commonly and wide spread in liver and brain after given $10^7$ *E. cuniculi* spores / pregnant mice orally and these changes composed mainly from infiltration of inflammatory cells specially lymphocytes and non suppurative encephalitis, also lesions in other tissues included perivascular cuffing of inflammatory cell in lung tissue with hyperplasia of lymphoid tissue around bronchioles, also there is degenerative and necrotic changes in renal tubule, all these pathological changes are similar to that described in hosts that infected naturally and experimentally by *E. cuniculi* in mice and rabbits (24-27).

The pathological changes recorded by current study in placenta of pregnant mice experimentally infect by *E. cuniculi* spores are similar to other hosts included natural infection in squirrel monkeys (28), alpaca (29), horse and Quarterhouse female (30), these previous studies described lesions composed from necrotic debris and stenosis in placental villi with parasitic vacuoles contain spores of *E. cuniculi* but they didn’t describe embryos infection or abortion.

The lesions recorded by current study supposed that the microsporidian species *E. cuniculi* due to its wide range of hosts and its ability to resistance the environment can cross uterus and placenta to infect embryos with or without abortion and caused embryonic deformities represented grossly by enlargement of heart and abdominal region, while microscopically caused infiltration of microglial cells in cerebral tissue, in liver there is loss of normal architecture of hepatic cords with *E. cuniculi* spores, the lung tissue showed heavy infiltration of lymphocytes in alveolar septa and around bronchioles, in spleen...
histological finding consisted from hyperplasia of germination centers and lymphocytes in white and red pulp. These lesions caused by *Encephalitozoon cuniculi* as a result of their replication in affected tissue also the role of macrophage system which not capable to kill these spores because the composition of spore wall that not affected by their killing mechanisms lead to disseminated these spores into other organs by infected macrophages (31), also the main type of cells that infiltrated to these lesions are mononuclear inflammatory cells and lymphocytes these due to releasing of IL-6 and IL-12 from damaged tissue by *E. cuniculi* in both embryos and placenta (29).

Current study indicated the occurrence of intrauterine infection through placenta when the pregnant mice infected by *E. cuniculi* spores orally result in embryonic deformities. Depended on result of current study we recommended for further investigation on the factor the influence intrauterine transportation of *E. cuniculi* infection in pregnant hosts and the effect of immunity to prevent and overcome the infection.

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