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 10^7 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 trophoblast in 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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دراسة مرضية لخمج الاجنة داخل الرحم بابواغ *Encephalitozoon cuniculi* في الفئران الحوامل

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الخلاصة

هدفت الدراسة الحالية الى التعرف على امراضية التجريع الفموي بابواغ *E. cuniculi* في اناث الفئران الحوامل واجنتها. استخدم ٤٠ انثى فار حامل في اليوم الأول من الحمل قسمت الى مجموعتين، المجموعة الأولى خمجت فموياً بابواغ *E. cuniculi* بجرعة 10^7 بوغ / انثى فار حامل، اما المجموعة الثانية تركت بدون اية معاملة طوال فترة التجربة، وعند اليوم ١٨ من الحمل تم قتل جميع الاناث في كلا المجموعتين قتلاً رحيماً. أظهرت نتائج الفحص العياني للإناث الحوامل في المجموعة المخمجة وجد احتقان في الكبد والرئة واظهرت الاجنة تضخم الراس والبطن. اما الافات النسجية في الاناث الحوامل المخمجة تالفت من افات ورمية حبيبية غير قيحية في الكبد مع وجود ابواغ *E. cuniculi* في تجمعات لارتشاحات من الخلايا اللمفية، اما افات الرئة تالفت من ارتشاح للخلايا اللمفية مع ابواغ *E. cuniculi*، اما افات الكلية فقد تالفت من تغيرات تنكسية وتنخرية في النبيبات الكلوية، اما افات الدماغ فقد تالفت من ارتشاحات لمفية مع ازدياد في اعداد

الخلايا الدباقية، اما مقاطع الأمعاء النسيجة فقد أظهرت فرط تنسج في النسيج اللمفي مع وجود الفجوات الطفيلية في قمم الزغابات المعوية، اما مقاطع المشيمة النسجية فقد أظهرت تواجد ابواغ *E. cuniculi* مع فرط تنسج الارومة الليفية الغاذية في الزغابات المشيمية، اما مقاطع الانسجة للاجنة بعمر ١٨ يوم من الحمل فقد أظهرت ارتشاحات لمفية حول الحويصلات الهوائية مع فرط تنسج النسيج اللمفي حول القصيبات الهوائية مع فقدان الترتيب السوي للحبال الكبدية فضلاً عن حدوث التفجي في الخلايا الكبدية، كما لوحظ فرط تنسج الخلايا اللمفية في اللب الأبيض للطحال. زودت الدراسة الحالية نظرة عن امراضية الخمج بابواغ *E. cuniculi* في الاناث الحوامل فضلاً عن اجنتها، كما انها دعمت وأثبتت النظرية المتعلقة بحصول الخمج في داخل الرحم بابواغ *E. cuniculi* للاجنة وخلال فترة الحمل.

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 Pastor identified the cause of Pebrine disease in silk worm as one of microsporidian microorganisms (2), at the end of 19th century microsporidia classified as protozoa (3), but after one century of researches 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 rout 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 aviary 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 achieved to cure the infection and described the its pattern in immunocompetent and immunosuppressed hosts (16), Although *E. cuniculi* have been studies more than one century especially on bees and silkworm by many 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.

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 100 adults (male and females) of inbreed Balb/c mice at age of 7-8 weeks, weight 25-45 gram housed (separated male from females) in house of Laboratory Animals, College of Veterinary Medicine, University of Mosul, which given food and water add libitum, mice kept at light and dark cycle of 14-hour light and 10 hours dark, with temperature at 20±3ºC and humidity at 50%±8% (17). The permits of animals housing were sterilized before used and bedding were changed daily to prevent reinfection, also mice were treated orally by albendazol at dose of 25 mg/kg of body weight, after five they treated with oral dose of fumagillin at dose of 0.5 mg/kg of body weight to get rid of microsporidia or other parasitic infections (18).

Estrus synchronization in mice

A total of 50 female mice (not pregnant) were given at first day of estrus synchronization single intraperitoneal dose of 0.5 µg of cloprostenol and 3 µg of progesterone for each female, at thirds 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 (13), purified pure spores prepared as described by (19).

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. cuniucli* 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 cogaulative 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.

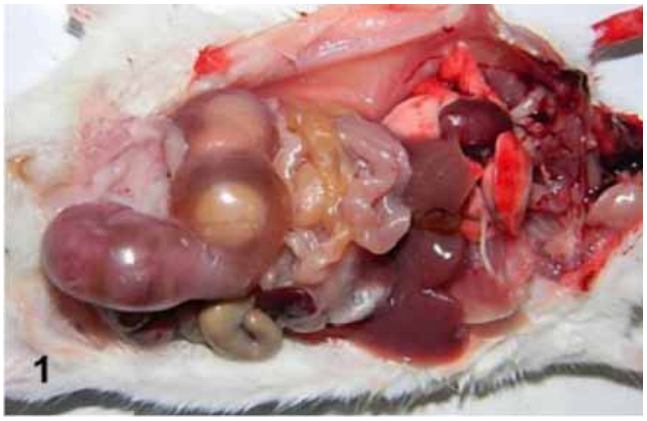

Figure 1. pregnant mice; Infected pregnant mice; Congestion of liver and lung.

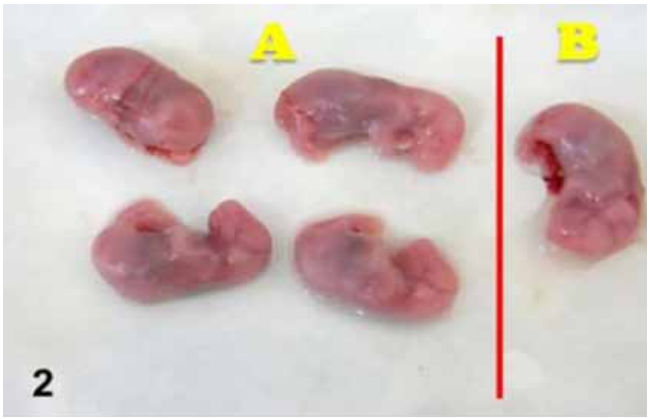

Figure 2. Embryo; (A) Enlargement of head and distension of abdomen (A) embryo from control group.

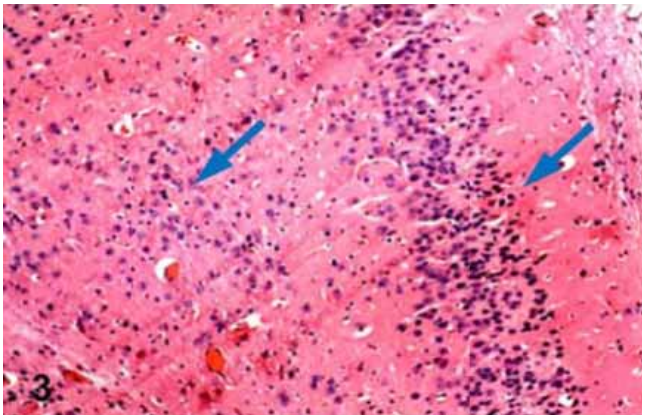

Figure 3. Brain, Infected pregnant mice; (arrow) Heavy infiltration of lymphocytes (100x, H&E).

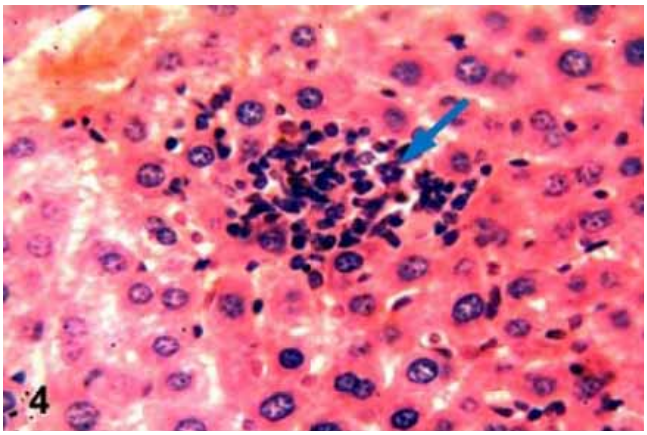

Figure 4. Liver; Infected pregnant mice; (arrow) Heavy infiltration of mononuclear inflammatory cells with *E. cuniculi* spores (400x, H&E).

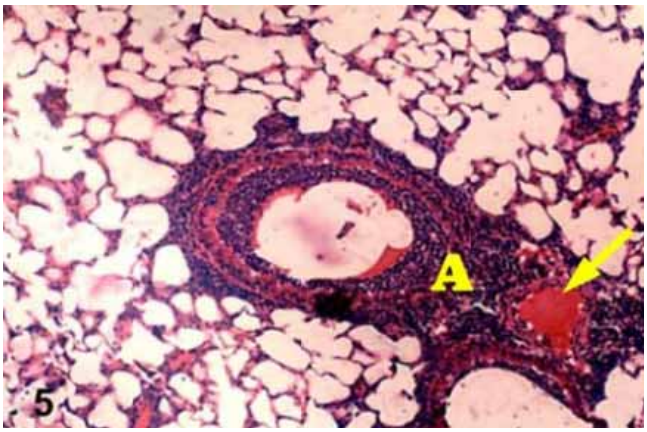

Figure 5. Lung; Infected pregnant mice; (A) hyperplasia of lymphatic tissue around bronchioles (arrow) with vasculitis (100x, H&E).

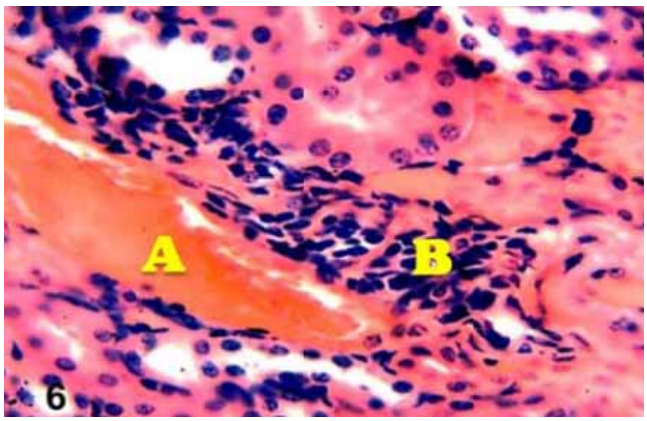

Figure 6. Kidney; Infected pregnant mice; (A) congestion in blood vessels (B) perivascular cuffing of lymphocytes and macrophages (400x, H&E).

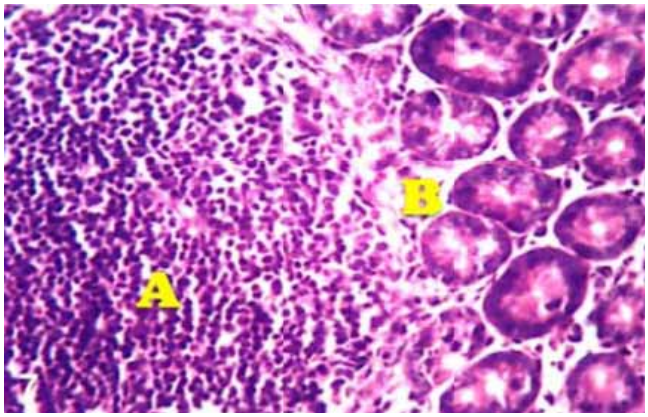

Figure 7. Intestines; Infected pregnant mice; Hyperplasia of lymphatic tissue in submucosal layer of small intestine (100x, H&E).

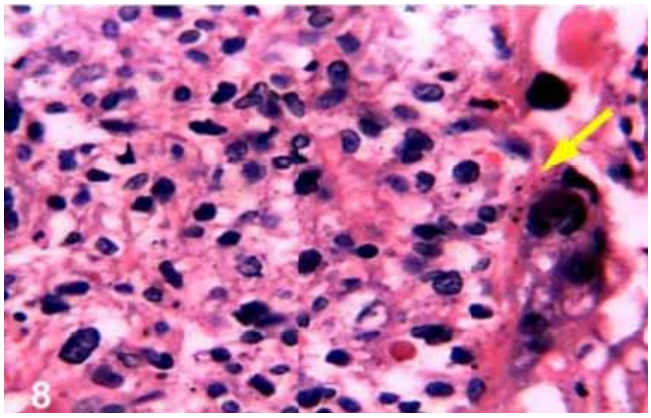

Figure 8. Placenta; Infected pregnant mice; (arrow) presence of *E. cuniculi* between trophoblast and synchtiotrpphoblast (400x, H&E).

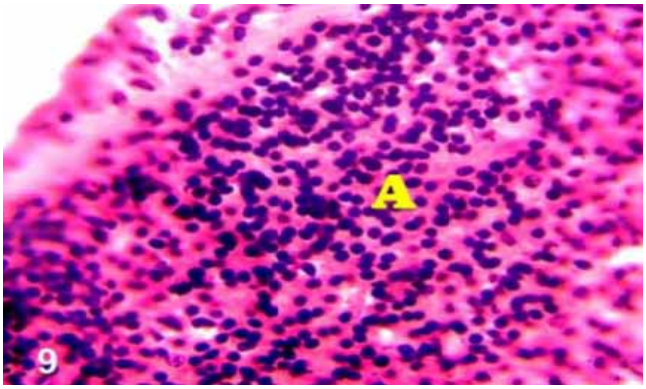

Figure 9. Embryo; Brain; (A) heavy infiltration of microglial cells (400x, H&E).

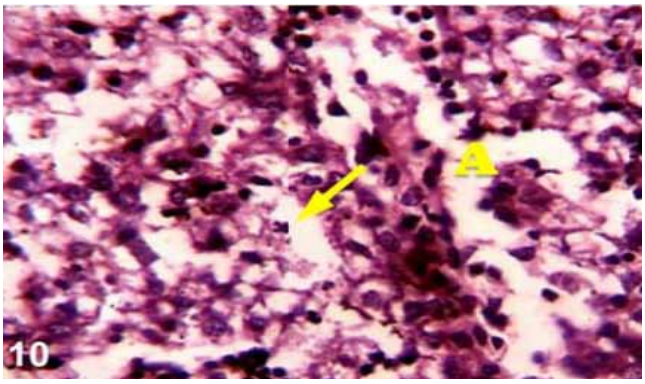

Figure 10. Embryo; Liver; (A) Disturbance of hepatic cords (arrow) present of *E. cuniculi* spores in hepatic sinusoids (400x, H&E).

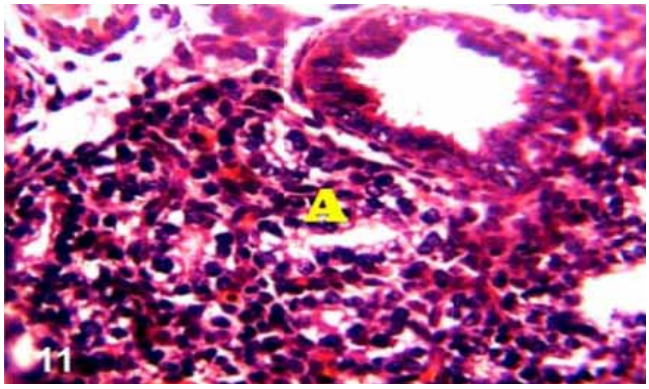

Figure 11. Embryo; Lung; (A) Heavy infiltration of macrophages in alveolar septa near bronchioles (400x, H&E).

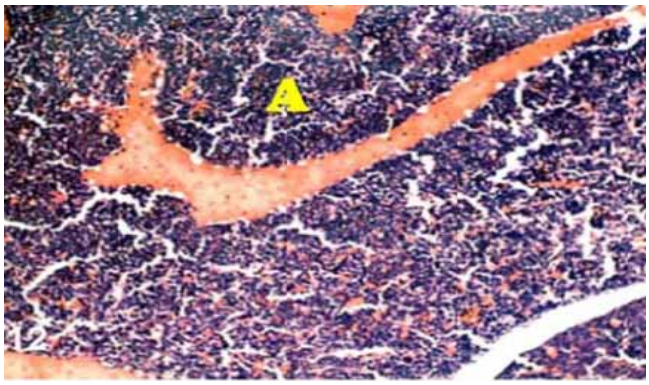

Figure 12. Embryo; Spleen; (A) Hyperplasia of germination center and lymphocytes (100x, H&E).

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 Ouarterhouse 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 *E. 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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References

1. Franzen C. Microsporidia: a review of 150 years of research. Open Parasitol J. 2008;2(1):1-34.
2. Casal G, Clemente SCS, Matos P, Knoff M, Matos E, Abdel-Baki. Redefining the genus Spraguea based on ultrastructural and phylogenetic data from *Spraguea gastrophysus* n. Sp. (Phylum Microsporidia), a parasite found in *Lophius gastrophysus* (Teleostei) from Brazil. Parasitol Res. 2012;111(1):79-88.
3. Didier ES, Weiss LM. Microsporidia current status. Current Opinion Infec Dis. 2006;19(5):485-492.
4. Lee SC, Corradi N, Doan S, Dietrich FS, Keeling PJ, Heitman J. Evolution of the sex related locus and genomic features shared in microsporidia and fungi. Vet Parasit. 2010;5(5):137-139.
5. Peyretaillade E, Alaoui HE, Diogon M, Polonais V, Partisot N, Biron DG. Extreme reduction and compaction of microsporidian genomes. Res Microbiol. 2011;162(6):598-606.
6. Mathis A, Weber R, Deplazes P. Zoonotic potential of the microsporidia. Clin Microbiol Rev. 2005;18(3):423-445.
7. Vavra JL, Ronny JID. Structure of microsporidia. In: Weiss LM, Bencel KJL, eds Microsporidia: Pathogens of opportunity, 1st ed. Iowa, USA: John Wiley and Sons, 2014.
8. Keeling PJ. Slamovits CH. Simplicity and complexity of microsporidian genomes. Eukaryotic Cell. 2004;3(6):1363-1369.
9. Jordan CN. *Encephalitozoon cuniculi*: diagnostic test and methods of inactivation. An M.Sc. thesis submitted to the Faculty of Virginia Polytechnic Institute and State University in partial fulfillment of the requirements for the degree of Master of Science, July 14, 2005, Blacksburg, Virginia, USA, 2005.
10. Brasil P, de Lima DB, de Paiva DD. Clinical and diagnostic aspects of intestinal microsporidiosis in HIV infected patients with chronic diarrhea in Rio de Janeiro, Brazil. J São Paulo Inst Trop Med. 2000;42(6):299-304.
11. Deplazes P, Mathis A, van Saanen M, Iten A, Keller R, Tanner I. Dual microsporidial infection due to *Encephalitozoon intestinalis* and *Encephalitozoon hellem* in a patient with AIDS. Clin Infec Dis. 1998;27(6):1521-1524.
12. Ferreira MS. Infections by protozoa in immuno-compromised hosts. Memorias do Instituto Oswaldo Cruz. 2000;95(1):159-62.
13. Al-Sadi HI, AL-Mahmood SS. Microsporidial infection in some domestic and laboratory animals in Iraq. International J Basic Appl Med Sci. 2013;3(3):78-91.
14. Shadduck JA. *Nosema cuniculi*: in vitro isolation. Sci. 1969;166(3904):516-517.
15. Malcekova B, Halanova M, Sulinova Z, Molnar L, Ravaszova P, Adam J. Seroprevalence of antibodies to *Encephalitozoon cuniculi* and *Encephalitozoon intestinalis* in humans and animals. Res Vet Sci. 2010;89(3):358-361.
16. Gannon J. The course of infection of *Encephalitozoon cuniucli* in immunodeficient and immunocompetent mice. Lab Anim. 1980;14(3):189-192.
17. Pallares P, Gonzalez-Bulnes A. A new method for induction and synchronization of oestrus and fertile ovulation in mice using exogenous hormones. Lab Anim. 2009;43:295-299.
18. Didier PJ, Phillips JN, Kuebler DJ, Nasr M, Brindley PJ, Stovall ME. Antimicrosporidial activities of fumagillin, TNP-470, Ovalicin, and Ovalicin derivatives in vitro and in vivo. Antimicrobial Agents Chemotherapy. 2006;6:2146-2155.
19. Garcia LS. Clinical microbiology procedures handbook. California: USA, LSG & Associates, 2007.
20. Weber R, Bryan RT, Qwen RL, Wilcox CM, Gorelkin L, Visvesvara GS. Improved light – microscopical detection of microsporidia spores in stool and duodenal aspirates. New England J Med. 1992;326(3):161-166.
21. Cooney KA, Chappell JR, Calle RJ, Connally BA. Veterinary Euthanasia Techniques: A practical guide. California: USA: A John Wiley & Sons, 2013.
22. Suvarna SK, Layuton C. Bancroft JD. Bancroft's theory and practice of histological techniques. New York, USA: Churchill Livingstone Press, 2013.
23. Luna LG. Manual of histological staining methods of the army forces institute of pathology division. New York, USA: McGraw Hill book company, 1968.
24. Lallo MA, Hirschfeld MPM. Encephalitozoonosis in pharmacologically immunosuppressed mice. Exp Parasitol. 2012;131(3):339-343.
25. Wasson K, Peper RL. Mammalian microsporidiosis. Vet Pathol. 2000;37(2):113-128.
26. Al-Sadi HI and Al-Mahmood SS. Pathology of experimental *Encephalitozoon cuniculi* infection in immunocompetent and immunosuppressed mice in Iraq. Pathol Res Int. 2014;857036:1-6.
27. Al-Mahmood SS and Al-Sadi HI. The first unequivocal report of *Encephalitozoon cuniculi* in rabbits and *Encephalitozoon intestinalis* in cattle in Mosul city – Iraq. Iraqi J Vet Sci. 2015;29(1):19-26.
28. Zeman DH, Baskin GB. Encephalitozoonosis in squirrel monkeys (*Saimiri sciureus*). Vet Pathol. 1985;22(1):24-31.
29. Webster JD, Miller MA, Vemulapalli R. *Encephalitozoon cuniculi* – associated placentitis and perinatal death in an alpaca (*Lama pacos*). Vet Pathol.2008;45(2):255-258.
30. Patterson – Kane JC, Caplazi P, Rurangirwa F, Tramontin RR, Wolfsdorf K. *Encephalitozoon cuniculi* placentitis and abortion in a Quarterhorse mare. J Vet Diag Inves. 2003;15(1):57- 59.
31. Khan IA, Moretto M, Weiss LM. Immune response to *Encephalitozoon cuniculi* infection. Microbes and Infection. 2001;3(5):401-405.
32. Williams BAP. Unique Physiology of host – parasite interactions in microsporidia infections. Cellular Microbiol. 2009;11(11):1551-1560.