

DIAGNOSIS OF ENCEPHALITOOZON CUNICULI IN NEW ZEALAND RABITS BY SOME HISTOLOGICAL METHODS

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Abstract: The aim of this study was to determine the histopathological lesions associated with *Encephalitozoon cuniculi* (*E. cuniculi*) infection in 25 New Zealand rabbits randomly selected in a laboratory animal center. Microscopic lesions were evaluated on sample slides stained with HE, Gram, and Immunohistochemistry (IHC) techniques. Mild and moderate lesions included granulomatous inflammation, meningitis, and perivascular cuffing were identified in brain samples; interstitial nephritis and renal fibrosis were identified in the kidneys of 21 out of 25 rabbits. Among the 21 rabbits, the percentage of spore identification by HE and Gram staining methods were 9.5% and 71%, respectively while IHC revealed parasite antibody in 81% of the rabbits. Thus, laboratory New Zealand rabbits in Vietnam might be infected with high prevalence of *E. cuniculi*. Postmortem diagnosis with HE technique can be used for screening while Gram and IHC staining can confirm the infections. The rabbit husbandry units should consider appropriate methods for infection assessment and control.

Keywords: *Encephalitozoon cuniculi*, New Zealand rabbit, histopathological methods, Vietnam

1. Introduction

E. cuniculi is an obligate intracellular parasite and belongs to Microsporida family. Encephalitozoonosis has been detected in many species of rodents, rabbits, carnivores and horses. Among those, rabbits are the most affected animals (Künzel and Joachim, 2010). Latent infection in rabbits occurs due to ingestion of *E. culicuni* spores (Cox *et al.*, 1979). Common symptoms of infected animals include neurological symptoms and renal failure that may lead to weight loss, dehydration, and anemia. Typical

pathological lesions include granulomatous meningitis and chronic interstitial nephritis, with scarring on the kidney surface (Stephen *et al.*, 2016; Suckow *et al.*, 2012). The disease can be transmitted to humans and is considered dangerous in some countries (Künzel and Joachim, 2010).

Rabbits are one of medical laboratory animals. Experimental individuals must be closely monitored, especially microbiological monitoring (Fujikawa *et al.*, 1993). In Vietnam, Encephalitozoonosis in rabbits was reported (Pham Thi Thuoc *et al.*, 2018). However, there are few data on this disease in rabbits in general and suitable diagnostic methods for the conditions in

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Vietnam in particular. Although there are many diagnosis and monitoring methods of the disease, brain and kidney histopathology is still considered the standard for definitive diagnosis of the disease. This study aims to compare diagnostic results and suggest the appropriate methods for assessment and control of *E. cuniculi* infection in rabbit breeding facilities in Vietnam.

2. Material and Methods

2.1. Samples

Specimens were taken from New Zealand white rabbits being raised at a laboratory animal breeding facility in Vietnam. A total of 25 randomly selected rabbits were euthanized with CO₂, followed by necropsy and microscopic sampling. All samples were fixed in 10% formaldehyde solution.

2.2. Chemicals

Chemicals used in sample preservation and processing include formaldehyde, alcohol, xylene, paraffin. Chemicals used in Hematoxyline Eosin (HE) stain include Hematoxyline, Sodium iodate, Trichloroacetaldehyde Monohydrate, Citric acid monohydrate, Aluminum ammonium sulfate (Wako, Japan), Eosin Y (Merck, Germany). Gram staining chemicals include crystal violet solution, iodine solution, Fuchsin, Picric acid, Acetone (Merck, Germany); Acetic acid (Wako, Japan). Chemicals and antigens used in immunohistochemical staining included 3% H₂O₂ in methanol, PBS (pH=7-8) (POLYVAC, Vietnam), Rat antiserum against *E. cuniculi* (Medicago), Simple Stain Mouse MAX-PO (Rat) (2nd antibody) (Nichirei, Japan), DAB (Diamino Bezidin) (Nichirei, Japan).

2.3. Methods

- The routine HE staining method (Edna et al., 1994) was carried to obtain 3-4 µm thick slices. Assessment of the lesions was based on the density and size of the lesion area and the degrees of inflammation (number of lymphocytes, plasma cells, macrophages) or fibrosis (fibroblasts, collagen fibers). Mild level (+) occurs when there are local lesions, small-sized lesions, slight increase in immune cell infiltration. Moderate level (++) is characterized by more widely distributed lesions, larger lesion areas, and a greater number of infiltrates or fibrosis. Severe level (+++) occurs when the lesions were diffuse and there were a large number of infiltrated cells or severe fibrosis.

- Gram staining: All slices of brain, kidney, and liver tissue of rabbits with typical microscopic lesions on HE stain were stained with Gram according to Edna et al. (1994)

- Immunohistochemical staining (IHC): Using the Histofine Simple Stain kit (Nichirei Bioscience., Japan) to stain brain and kidney tissue slices following deparaffinization with xylene and hydrophilicity through alcohol baths with concentration series (100% - 90% - 80%); reducing peroxidase with 0.3% hydrogen peroxide in methanol at room temperature for 30 minutes; coating overnight at 40°C with primary antibody (Rat anti-Encephalitozoon cuniculi antibody - Medicago, Uppsala, Sweden) at a dilution of 1:50.000; attaching secondary antibody (Peroxidase labeled anti Rat Mouse IgG) at room temperature for 30 minutes; creating a color reaction with 3, 3'-diaminobenzidine, creating a color reaction and staining in

Mayer’s hematoxylin solution. Samples were positive if *E. cuniculi* appeared brown and cell nuclei were purple. Samples were negative if cell nuclei were purple.

3. Results and Discussion

3.1. Postmortem lesions

3.1.1. Macroscopic lesions

Rabbit Encephalitozoon is usually latent. Therefore, detection of specific microscopic lesions or lesions related to infection was the basis for selecting individual rabbits for the definitive diagnosis of the pathogen. The results of lesion evaluation of 25 randomly selected rabbits were presented in Table 1.

Table 1. Postmortem macroscopic lesions in rabbits (n = 25)

Organs	Lesion characteristics	Number of animals
Brain	Meningeal congestion	5
Kidney	Multiple light gray diffusing spots on the surface of both kidneys	11
	Congestion on the renal cortical cross section	
Gan	Abnormal white lesions on the surface and cross-section of liver	1
Liver	Diffusion of gray-white speckles on all lobes	1

The results (Table 1) showed that meningeal congestion (Figure 1A), diffusing light gray spots on kidney surface (Figure 1B), liver damage were detected in five, eleven, and one animals, respectively. No damage in other organs were observed.

Renal surface scarring caused by *E.cuniculi* had been described (Cox and Gallichio, 1978; Csokai et al, 2009a,

Flatt and Jackson, 1970). Those studies suggested that microspores cause chronic interstitial nephritis leading to renal interstitial fibrosis. According to Flatt and Jackson (1970), there were 100 out of 2338 rabbits had brown or pale, concave areas on the surface of both kidneys with a diameter of 0.5 to 5.0 mm.

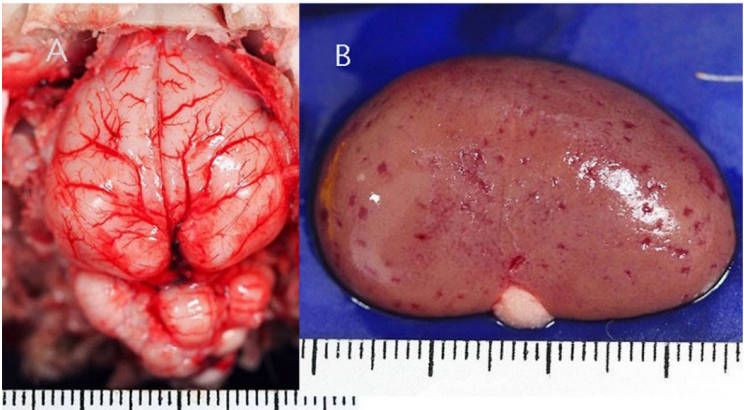


Figure 1. Postmortem macroscopic lesions on the brain (A) and kidney (B) of the rabbits

3.1.2. Microscopic lesions

Out of 25 rabbits, microscopic lesions in the central nervous system and kidneys were observed in 21 animals. Manifestations included granulomatous

encephalitis, meningitis associated with interstitial nephritis. Two animals showed interstitial nephritis lesions and two had no lesions (Table 2).

Table 2. Postmortem microscopic lesions in the central nervous system and kidneys

Organ	Lesion	Number animals			
		-	+	++	+++
Cerebral frontal lobe	Granuloma type 1	20	5	0	0
	Granuloma type 2	0	5	20	0
	Perivascular cuffing	4	18	3	0
	Meningitis	3	19	2	0
Parietal and temporal lobes	Granuloma type 1	21	3	1	0
	Granuloma type 2	12	9	4	0
	Perivascular cuffing	4	13	8	0
	Meningitis	6	16	3	0
Cerebral occipital lobe	Granuloma type 1	21	3	1	0
	Granuloma type 2	14	7	4	0
	Perivascular cuffing	4	18	3	0
	Meningitis	7	17	1	0
Cerebellum	Granuloma	25	0	0	0
	Perivascular cuffing	18	7	0	0
	Meningitis	16	9	0	0
Medulla Oblongata	Granuloma	25	0	0	0
	Perivascular inflammation	17	8	0	0
	Meningitis	24	1	0	0
Tủy sống Spinal cord	Granuloma	25	0	0	0
	Perivascular cuffing	24	1	0	0
	Spinal meningitis	25	0	0	0
Thận trái Left kidney	Interstitial nephritis	2	11	8	1
Thận phải Right kidney	Fibrosis	15	6	4	0
	Interstitial nephritis	2, 4NE	9	9	1
	Fibrosis	13. 4NE	6	2	0

Note: -, No damage; +, Mild level; ++, Moderate level; +++, Severe level; NE, Not evaluation

Microscopic lesions in the brain:

Lesions included granulomatous inflammation, meningitis, and perivascular cuffing. Granulomas type 1 were usually circular in shape and characterized by necrosis of lymphocytes, macrophages, and infiltration of heterophils in the center that was surrounded by epithelial cells and macrophages in the middle layer, and the outermost were lymphocytes (Figure 2A). Granulomas type 2 were aggregates of

epithelial cells, macrophages, lymphocytes, without central necrosis (Figure 2B). Perivascular cuffing was characterized by vasodilation and infiltration of lymphocytes, plasma cells, and some macrophages (Figure 2C). Meningitis was characterized by infiltration of lymphocytes with few plasma cells and very few heterophils, and congestion (Figure 2D).

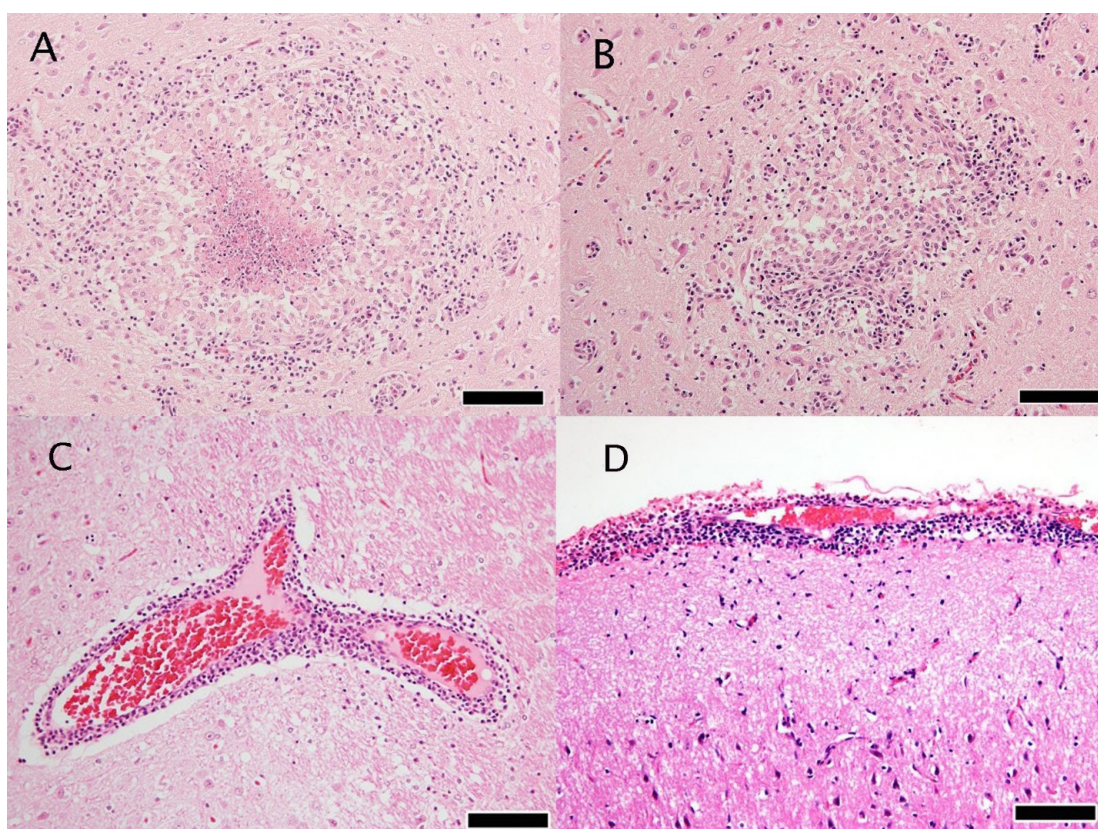


Figure 2. Postmortem microscopic lesions of rabbits

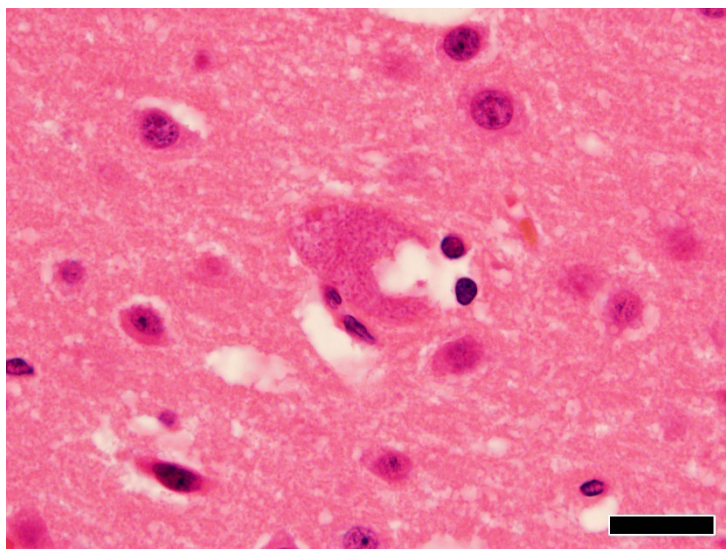
Note: A, HE Granuloma type 1 in brain with central necrosis of animal 10). B, HE granuloma type 2 in brain without central necrosis, animal 10. C, HE Perivascular cuffing in brain of animal 2. D, HE, meningitis and congestion of animal 13. Scale bar = 100 μ m.

Four types of lesions including granulomatous type 1, granulomatous type 2, perivascular cuffing, and meningitis were detected in all brain lobes. The lesion degrees ranged from mild to moderate

severity. No severe lesions were seen. Perivascular and meningococcal lesions appeared in the regions of cerebrum, while granulomatous lesions were distributed predominantly in the cerebral cortex (21

out of 25 animals). Short rod-shaped spores (approximately 1x2 μm) with hematoxylin-staining located in a vesicular structure about 20 μm in diameter were detected in

brains of two among twenty one animals (9.5%). This spore-containing structure was in brain tissue where no inflammatory response was observed (Figure 3).



**Figure 3. Brain (animal 12) showed vacuoles of microspores appeared in cells.
HE, scale bar = 20 μm**

No granulomatous lesions were detected in cerebellum. However, seven animals had mild perivascular cuffing and the other seven showed mild cerebellar meningitis. In the medulla oblongata, no granulomatous lesions were observed; eight rabbits had perivascular cuffing, and one animal showed meningitis. The injuries were all mild. In the spinal cord, there was no granulomatous lesions and spinal cord inflammation in 25 rabbits examined, only one rabbit had mild perivascular cuffing. Microscopic lesions in rabbit brain caused by *E. cuniculi* were similar to that reported by Wesonga and Munda (1992); Miriam et al. (2013).

Microscopic kidney lesions

There were 21 rabbits with microscopic kidney lesions. However, microscopic lesions were seen in animal with no macroscopic injuries. Microscopic lesions

were characterized by mononuclear inflammatory cell infiltration and renal interstitial fibrosis. The renal interstitial lesions were characterized by infiltration of lymphocytes, plasma cells, and macrophages (Figure 4A). Inflammatory lesions were mainly observed in the cortical renal interstitium. Mild lesions had very few inflammatory foci and inflammatory cells. Moderate lesions had more inflammatory cells, and severe lesions were observed in large areas with many inflammatory cells. Only one animal showed severe interstitial nephritis in both right and left kidneys.

Renal interstitial fibrosis was formed by proliferation of fibroblasts and collagen fibers, mainly observed in the renal cortex (Figure 4B). Lesion degrees including mild, moderate and severe were identified according to number of fibroblasts, collagen

fibers, and small fibrous areas. Eight cases had mild interstitial fibrosis, five cases had moderate lesions and twelve had no fibrosis. Fibrosis was observed in areas

with interstitial nephritis lesions. In addition to inflammation and fibrosis in the renal interstitium, there was regeneration and degeneration of renal tubular epithelial cells.

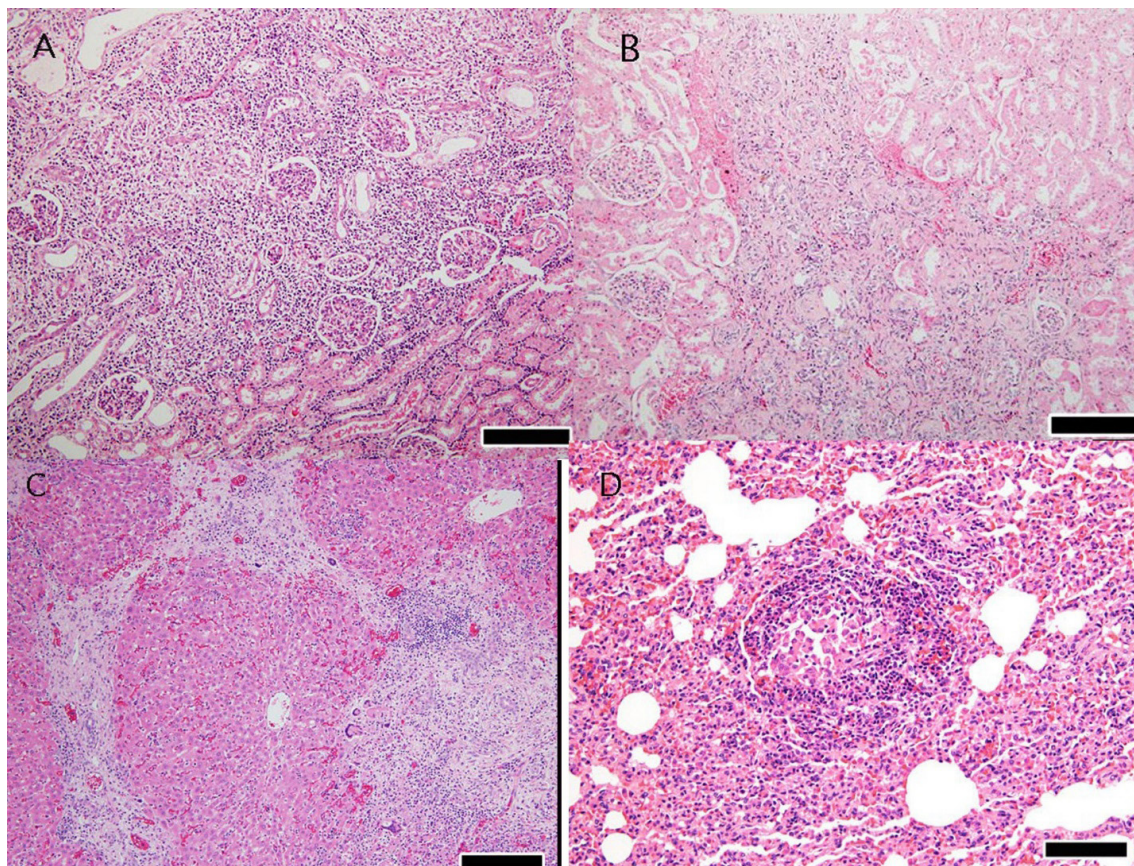


Figure 4. Postmortem microscopic lesions in the kidney, liver and lung

Notes: A, Interstitial nephritis with infiltrating inflammatory cells (HE, animal 5, scale bar = 20 μ m); B, Fibrosis in the renal cortex (HE, animal. 9, scale bar = 200 μ m); C, Severe inflammation and fibrosis disrupting the structure of liver lobes (HE, animal 17, scale bar = 200 μ m); D, Focal granulomatous inflammation - lung (HE, animal 21, scale bar = 100 μ m)

The renal microscopic lesions were similar to the description of Cox and Gallichio (1978), Csokai et al. (2009a), Flatt and Jackson (1970). According to the authors, renal lesions included interstitial nephritis mainly with mononuclear cell infiltration, and chronic interstitial

nephritis with scar tissue formation due to interstitial fibrosis. A study by Wesonga and Munda (1992) showed that rabbits with *E. cuniculi* infection had kidney lesions that were abnormal gray-white depressions on the surface of kidney with microscopic manifestations of interstitial

nephritis characterized by mononuclear cell infiltration and fibrous tissue proliferation. In the cerebrum and cerebellum of infected animals, *E.cuniculi* was detected with or without perivascular cuffing and meningitis.

According to the study of Miriam et al. (2013), similar histopathological lesions in the brains and kidneys of rabbits infected with *E. cuniculi* were observed. Luis et al. (2017) showed that, due to small size of the microspores, it was difficult to identify the pathogen on HE stain samples from kidney.

Liver lesions

Liver lesions were seen in 23 rabbits. Among those, 21 showed mild infiltration of inflammatory cells and fibrosis in the portal triads. In some samples, there was inflammatory cell infiltration in the liver lobules. Infiltrating cells comprised lymphocytes, a few plasma cells, macrophages, sometimes with multinucleated giant cells. The fibrous lesion in the portal triads was characterized by a slight proliferation of fibroblasts and collagen fibers. Animal n° 4 had coccidia oocysts in the portal triads with many inflammatory and fibrous cells. Infiltrating inflammatory cells included lymphocytes, plasma cells, a few heterophils. Some enlarged bile ducts showed degenerated and necrotic epithelium and containing many coccidia oocysts. There was infiltration of macrophages, epithelial cells, multinucleated giant cells, inflammatory cells. The bile duct wall was thickened due to inflammation, fibrosis, and the appearance of new bile ducts. Rabbit no. 17 had fibrosis and severe inflammation in the portal and intralobular bile ducts. Infiltrating inflammatory cells were mainly lymphocytes, plasma cells, giant cells and

few heterophils. The fibroblast area was full of fibroblasts and collagen. The severe fibrotic lesions led to disruption of liver lobules (Figure 4C).

Lung lesions

Granulomas localized in the diaphragmatic lobe of the right lung with epithelial macrophages, lymphocytes, and plasma cells were observed (Figure 4D).

Thus, 21 out of 25 animals showed histopathological lesions of *E. cuniculi* infections characterized by granulomatous encephalitis associated with interstitial nephritis. Among those, two and one animals showed only interstitial nephritis and lung lesions, respectively.

3.2. Microscopic lesions on Gram-stained specimens

Microspores (about 1x2 µm) were seen in brain samples of 13 (among 21) animals. Twelve showed microspores in the cerebral tissue and one had microspores in the medulla oblongata. Gram-positive staining spores existed in vacuoles or free forms. Microspores in vacuoles appeared near granulomas or glial areas where no lesions were present (Figure 5A). Free-form microspores were seen scattering in the necrotic area of granuloma type 1 (Figure 5B).

Renal lesions: Six (among rabbits) with interstitial nephritis showed Gram positive spores (about 1x2 µm). The vacuolar spores were concentrated in the renal tubular epithelial cells of the medulla and cortex, particularly on the collecting duct epithelium of the renal medulla (Figure 5C). One animal had free-form spores in the renal tubule lumen (Figure 4D); another one showed free spores in the Bowman's sinus (Figure 5E).

A study (Flatt and Jackson, 1970) showed that rod-shaped Gram-positive spore clusters appeared in the renal tubular epithelial cells, usually the collecting duct epithelium. The spores were about $1 \times 2 \mu\text{m}$ in size, appearing in groups filled with vacuoles in the cytoplasm. There is no inflammatory response associated with these parasite vacuoles. Occasionally,

the lumen of renal collecting tubules contained cellular debris and small groups of microorganisms. In addition, Luis et al. (2017) described the spores of *E. cuniculi* as a dark green oval structure against a very clear background color, but the internal structure of these spores was difficult to observe. No microspores were observed in the liver and lungs of examined rabbits.

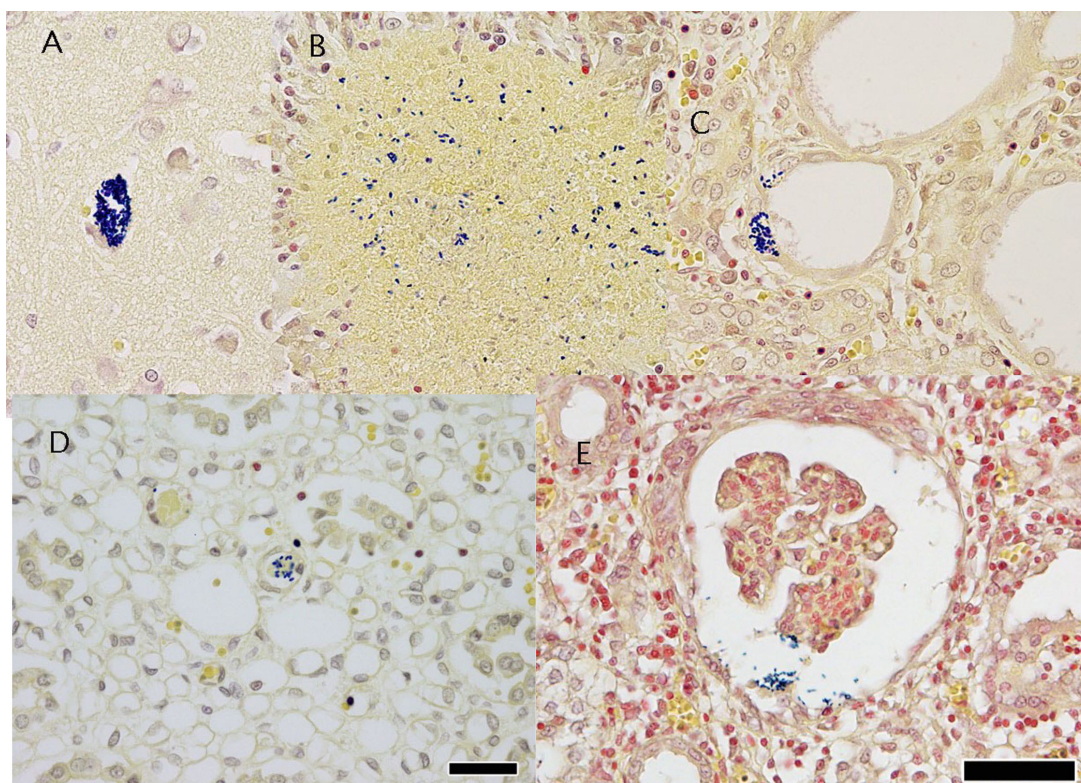


Figure 5: Microscopic lesions in some organs of rabbits suspected to be *E. cuniculi* infected

Note: A, Gram-positive spores in the vacuole of E. cuniculi in the neural network (brain, animal 1, scale bar = $30 \mu\text{m}$); B, Free Gram-positive staining spores in the necrotic area in the granulomas (brain, animal 3, scale bar = $30 \mu\text{m}$); C, Gram-positive spores in the vacuole in the epithelial cells of the renal tubule, (animal 5, scale bar = $30 \mu\text{m}$); D, Gram-positive spores in vacuoles in epithelial cells of renal tubules (animal 5, scale bar = $30 \mu\text{m}$); E, Gram-positive spores freely appeared in Bowman's sinus (animal 5, scale bar = $50 \mu\text{m}$).

3.3. Central nervous system microscopic lesions on IHC staining samples

Brain tissue of 13 out of 21 rabbits showed the presence of *E. cuniculi* antigen on IHC staining samples. The two spore forms confirmed in Gram staining were observed with IHC method (Figure 6). The spores appeared concentrating in the vacuoles of glial cells (Figure 6A) or

near granulomas (Figure 6B) and trending near areas of necrotic granulomas (Figure 6C). Not only in the cerebrum, free spores also appeared in the granular layer of the cerebellum (Figure 6D). *E. cuniculi* antigens were distributed in the gray matter area of the cerebral cortex (12 of 13 animals), the medulla oblongata (2 of 13), and the cerebellum (one of 13 animals).

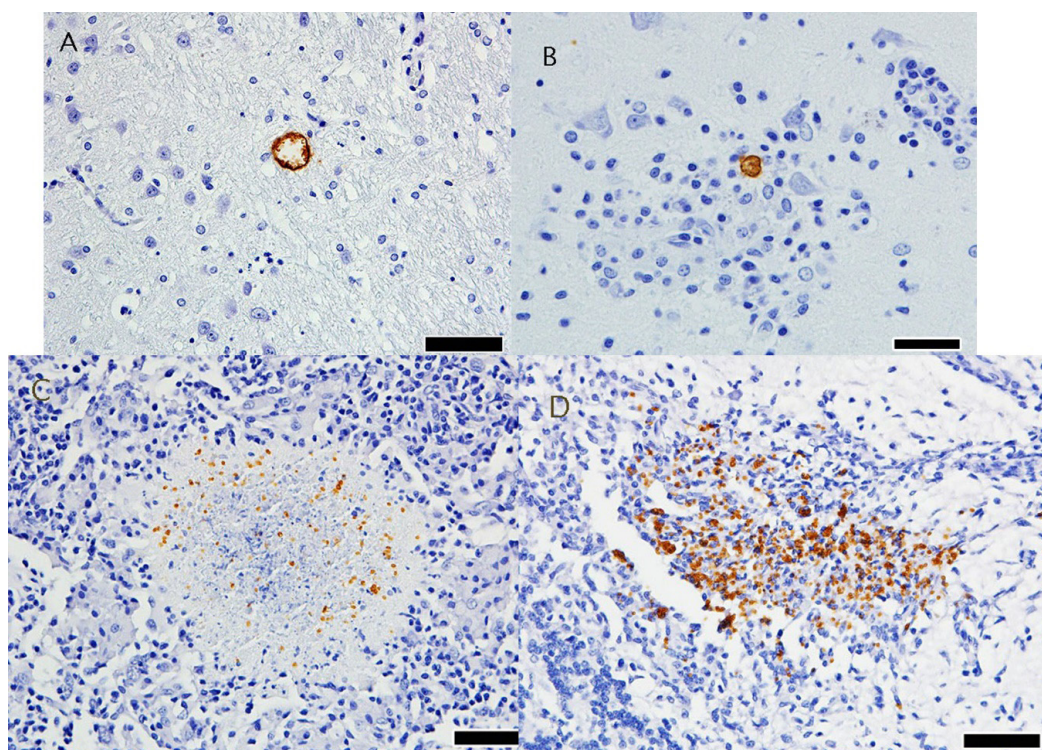


Figure 6: Microscopic lesions associated with *E. cuniculi* infection in IHC brain tissue

*Note: A, Spores in vacuole were *E. cuniculi* antibody-positive in the glial area (animal 2, IHC, scale bar = 30 μ m); B. Spores in the vacuole were *E. cuniculi* antibody-positive near the granuloma (animal 23, IHC, scale bar = 30 μ m); C. *E. cuniculi*-positive free spores in necrotic areas in granulomas of the cerebrum (animal 3, IHC with DAB colorant, scale bar = 50 μ m); D, *E. cuniculi*-positive free spores in the cerebellar gray matter area (animal 2, IHC staining with DAB colorant, scale bar = 50 μ m)*

In renal tissue, vacuoles containing microspore were located in renal tubular epithelial cells and vascular epithelial cells, especially in the collecting tubular epithelial cells in the renal medulla (Figure

7A). The second form freely occurred in the renal tubule lumen and in the central of interstitial inflammation area (Figure 7B). In some cases, vacuoles were detected in the vascular epithelium of renal tissue

(Figure 7C). Nine of 23 rabbits had antigens to *E. cuniculi*, mainly in the renal medulla (8 rabbits), and in both renal medullary and cortex (in one animal).

These results are similar to the study of Miriam et al. (2013) which detected antigens of *E.cuniculi* mainly in the brain and kidney in 3 forms including vacuolar form, granular or clustered chromatin form, and granular chromatin form in the necrotic center of the inflammatory foci. Giordano et al. (2005) showed that spores reacted with anti-*E.cuniculi* antiserum in all 4 rabbits with *E.cuniculi*-associated choroiditis and presented in macrophages and vitreous epithelial cells. The authors also suggested

that IHC staining was a useful tool to determine the presence and distribution of *E. cuniculi* spores in tissue samples. Flatt and Jackson (1970) suggested that spores clustered together in the renal tubular epithelial cells and were released when the vacuole was ruptured, then invaded the interstitium and initiated inflammation; or might enter lumen of the renal tubule, and follow the renal tubule and infect other epithelial cells; or possibly entered the urinary tract. After passing out in the urine, the spores could infect a new host. When digested by the new host, the microorganism invaded the lining of the gastrointestinal tract and then different parts of the body.

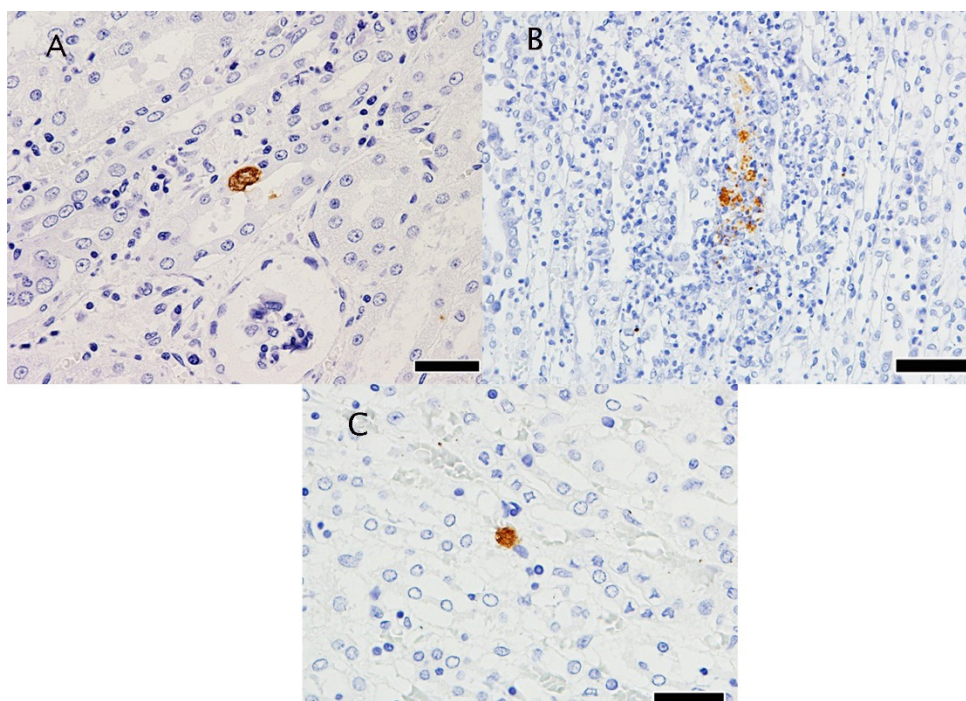


Figure 7. Renal micromorphology of rabbits infected with *E. cuniculi*

Note: A., Spores in the vacuoles in renal tubular epithelial cells (animal 5, IHC, scale bar = 30 μ m); B, Free spores in the cytoplasm of co-inflamed cells (animal 6, IHC, scale bar = 50 μ m); C, Spores in vacuoles in the vascular epithelium of renal tissue (animal 13, IHC staining, scale bar = 30 μ m)

3.4. Comparative evaluation of the diagnostic methods for *E. cuniculi* infection

Table 3. Determination of *E.cuniculi* and related lesions

Animal no	Brain			Kidney		
	HE	Gram staining	IHC	HE	Gram staining	IHC staining
1	+	-	+	+	-	-
2	+	+	+	+	-	-
3	+	+	+	+	-	-
4	+	+	+	+	-	-
5	+	+	+	+	+	+
6	+	+	-	+	-	+
7	+	+	+	+	+	+
8	+	-	-	+	-	-
9	+	-	-	+	+	+
10	+	+	+	+	+	-
11	+	-	+	+	-	-
12	+, +	+	+	+	-	+
13	+	+	+	+	-	+
14	+	-	-	+	-	-
15	+	+	+	+	-	-
16	+	-	-	+	-	-
17	+	-	-	+	-	-
18	-	0	0	-	0	0
19	+	-	-	+	-	-
20	+	+	+	+	+	+
21	-	0	0	+	0	0
22	+	+	-	+	-	+
23	-	0	0	-	0	0
24	-	0	0	+	0	0
25	+, +	+	+	+	+	+
Tổng số	21/25	13/21	13/21	23/25	6/21	9/21

Notes - : no characteristic lesions or no spores/*E. cuniculi* antigens; +: with typical lesions/spores/*E. cuniculi* antigens; 0 = no evaluation

The results (Table 3) showed that the HE staining detected 21 animals with typical lesions of Encephalitozoonosis, of which only 2 (animals 12 and 25, accounting for 9.5%) showed *E. cuniculi* microspores. Both of these rabbits showed positive results on Gram-stained kidney and brain tissue. Gram staining method detected spores in 15 rabbits (detection rate was 71%). The number of rabbits with samples positive for IHC staining on brain and kidney tissue were 17 (accounting for 81%).

HE staining was still considered a routine staining method for testing rabbit tissue samples infected with *E.cuniculi*. The micropathological lesions (primarily non-purulent granulomatous encephalitis and interstitial nephritis) were indicative of encephalitozoonosis in rabbits. However, this method was difficult to identify spores

because the agent was very small in size and appeared from pink to purple in color (Luis et al., 2017) that easily blended with the background color.

With Gram staining, spores of *E.cuniculi* appeared with a dark green oval structure standing out on amber background color. La'toya et al. (2014) also suggested that *E. cuniculi* spores could be identified in the parasite vacuoles and Gram staining method gave the best detection. In addition, Gram staining could also be used in the differential diagnosis of *E. cuniculi* from *T. gondii*, *Sarcocystis* spp., *Pneumocystis* spp., *Cryptosporidium cuniculi* and *Neospora caninum*. These agents could cause similar histological lesions, but without discoloration of Gram (La'toya et al., 2014). The study by Luis et al. (2017) also used 14 histochemical staining methods (including alcian blue, calcofluor white, Giemsa, Gram, Grocott, HE, Luna, Luxol fast blue, Masson trichrome, modified trichrome stain, periodic acid Schiff reaction (PAS), Van Gieson, Warthin Starry and Ziehl – Neelsen) to identify *E. cuniculi* spores and demonstrated that Gram staining is one of the three best staining methods. In IHC staining, *E. cuniculi* was observed with a high percentage on brain and kidney tissue. This method also determined the localization of this agent.

4. Conclusion

New Zealand rabbits raised in experimental animal facilities are at high risk of *E. cuniculi* infection. The HE staining method showed microscopic lesions of *E. cuniculi* infection in 21 (among 25) rabbits. Granulomatous meningoencephalitis associated with interstitial nephritis in which granulomatous inflammation, meningitis and encephalitis perivascular cuffing in parts of the brain were

commonly observed. Granulomatosis occurs only in the cerebrum. Lesions range from mild to moderate. Renal microscopic lesions include interstitial nephritis and renal fibrosis.

Gram staining method identified *E. cuniculi* spores in 75% of rabbits. Immunohistochemistry has the highest ability to detect *E. cuniculi* (81% of rabbits). In the brain, there are two spore types including vacuolar and free forms. In the kidney, the vacuolar spores were detected in the tubular epithelial cell of the medulla and cortex, the collecting duct epithelium of the medulla, or freely in the lumen and Bowman's sinus.

The appropriate postmortem method for Encephalitozoonosis should be considered based on the conditions of each breeding facility and the purpose of disease surveillance.

References

- [1]. Künzel F. and Joachim A. (2010). Encephalitozoonosis in rabbits. *Parasitol Res.* 106. 299-309.
- [2]. Cox J. C., Hamilton R.C., Attwood H. D. (1979). An investigation of the route and progression of Encephalitozoon cuniculi infection in adult rabbits. *The Journal of Protozoology.* 26(2): 260-265.
- [3]. Stephen W. B., Stephen M. G., Dean H. P. (2016). *Pathology of laboratory rodents and rabbits.* Fourth edition. Wiley-Blackwell. 293-295.
- [4]. Suckow M. A., Stevens K.A. and Wilson R.P. (2012). *The Laboratory Rabbit, Guinea Pig, Hamster, and Other Rodents.* 1st Edition. The American College of Laboratory Animal Medicine. 424-426.
- [5]. Fujikawa T., Hovel G. J. R, Hänninen O. K., World H.O. (1993). *Veterinary Public Health Unit. Guidelines for breeding and care of laboratory animals.* World Health

- Organization. 169: 65-68.
- [6]. Phạm Thị Thuộc, Ikeda Manabu, Nguyễn Đăng Hiền, Nguyễn Thúy Hương, Ngô Thu Hương, Nguyễn Thị Nguyệt, Phạm Hữu Tiến (2018). Nhiễm trùng *Encephalitozoon cuniculi* trên thỏ thí nghiệm tại một cơ sở chăn nuôi ở Việt Nam năm 2015. Tạp chí y học dự phòng. 28(1): 68-76.
- [7]. Cox J. C. and Gallichio H. A. (1978). Serological and histological studies on adult rabbits with recent naturally acquired encephalitozoonosis. Research in Veterinary Science (UK).
- [8]. Flatt R. E. & Jackson S. J. (1970). Renal neseomatosis in young rabbits. Path Vet. 7: 492-497.
- [9]. Wesonga H. O. and Munda M. (1992). Rabbit encephalitozoonosis in Kenya. Laboratory animals. 26(3): 219-221.
- [10]. Luis R.-T. L. E., Villarreal-Marroquín A., Nevárez-Garza A.M. (2017). Histochemical study of *Encephalitozoon cuniculi* spores in the kidneys of naturally infected New Zealand rabbits. J Vet Diagn Invest. 29(3): 269-277.
- [11]. Giordano C., Weigt A., Vercelli A., Rondena M., Grilli G., Giudice C. (2005). Immunohistochemical identification of *Encephalitozoon cuniculi* in phacoclastic uveitis in four rabbits. Veterinary ophthalmology. 8. 4: 271-275.
- [12]. La'toya V. L., Charles W. B., Nicole R. W. (2014). *Encephalitozoon cuniculi* in pet rabbits: diagnosis and optimal managemen. Veterinary Medicine: Research and Reports. 5: 169-180.