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Toxoplasmic meningoencephalitis in a stray cat in Korea

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Abstract : A dead stray cat was necropsied for zoonotic feline disease monitoring. Grossly, there were no specific lesions. Major microscopic lesions included lymphocytic meningoencephalitis, malacia, and tissue cysts in the cerebral and cerebellar cortex. The size and shape of tissue cysts were identical to those of Apicomplexa including *Toxoplasma* (*T.*) *gondii*. Bradyzoites in the tissue cyst were strongly positive for *T. gondii* by immunohistochemistry. Electron microscopy revealed that bradyzoites within the tissue cyst were similar to the morphological features of *T. gondii*. Fresh tissue samples were examined by a polymerase chain reaction assay and resulted in a specific band of *T. gondii* only in the brain. Based on the results, this case was diagnosed as toxoplasmosis. This is the first case of toxoplasmic meningoencephalitis in a cat in Korea.

Keywords : bradyzoite, cat, meningoencephalitis, tissue cysts, toxoplasmosis

Introduction

Toxoplasmosis is one of the most common protozoal diseases caused by *Toxoplasma* (*T.*) *gondii*. *T. gondii* is an obligate intracellular zoonotic protozoan belonging to the phylum Apicomplexa with worldwide distribution [5, 8, 10, 14, 15]. Cats and wild felidae play pivotal roles as definitive hosts in the spread of toxoplasmosis because they are the only animals known to shed environmentally resistant *T. gondii* oocysts [4, 7, 10, 14, 23]. Transmission of *T. gondii* can occur via ingestion of oocysts excreted in feline feces, ingestion of the meat of infected intermediate animals containing tissue cysts, or congenital infection via tachyzoites [5, 11, 14]. It is difficult to diagnose *T. gondii* infected cats because they lack characteristic clinical signs [20]. Prevalence of *T. gondii* in Korea was reported in German shepherd dogs [11] and in stray cats [20] by antigen or antibody detection. In human, seroprevalence of toxoplasmosis was detected in Korean pregnant women [17], and in the residents of Cheju Island [24]. However, the evidence of tissue cysts and the detection of the agent in the brain of cats are absent in Korea. The present report describes a feline case of meningoencephalitis with malacia caused by *T. gondii* using several diagnostic methods including immunohistochemistry (IHC), polymerase chain reaction (PCR), and electron microscopy (EM). To the authors' knowledge, the cat represents the first natural case with brain lesions caused by *T. gondii* in Korea.

Case report

In April 2008, a dead stray adult female cat was submitted to the Animal Disease Diagnostic Center, National Veterinary Research and Quarantine Service for zoonotic feline disease monitoring. The animal was captured near the Seoul Grand Park and limited information regarding clinical signs was available. At necropsy there were no gross lesions. Tissue samples

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from all organs were collected, fixed in 10% neutral buffered formalin, and embedded in paraffin wax. Sections (4 μm) were stained with hematoxylin and eosin. Additional sections were immunolabelled with polyclonal rabbit antibody against *T. gondii* (1 : 40 dilution, PU125-UPE; Biogenex, USA). Further IHC was done using a streptavidin-biotin amplification system and a Discovery XT platform (Ventana Medical Systems, USA), with the DAB Map kit (Ventana Medical Systems, USA). For antigen retrieval, tissue sections were incubated for 8 min with protease 1 (Ventana Medical Systems, USA). Then they were incubated with the primary antibody for 32 min, followed by the incubation with LSAB2 (labelled streptavidin-biotin) biotinylated link for streptavidin (K1015; Dako, Denmark) for 12 min. All sections were counterstained with hematoxylin and bluing reagent (Ventana Medical Systems, USA). A tissue sample from the liver of a pig with toxoplasmosis was used as a positive control. For negative control, normal rabbit serum (X0902; Dako, Denmark) was applied as a primary antibody. For EM, a 1-mm^2 thick section was taken from the paraffin block, deparaffined in xylene overnight, routinely processed and then fixed in 1% buffered osmium tetroxide and embedded in epoxy resin. Ultra thin sections were stained with uranyl acetate and lead citrate and were examined using a transmission electron microscope (H-7100FA; Hitachi, Japan). For differential diagnosis of *T. gondii* and *Neospora* (*N.*) *caninum*, PCR was performed using fresh brain, heart, lung, liver, spleen, kidney and muscle samples. The cultured *T. gondii* (RH strain) was used as a positive control and all PCR reagents except sample DNA were included in a negative control. After

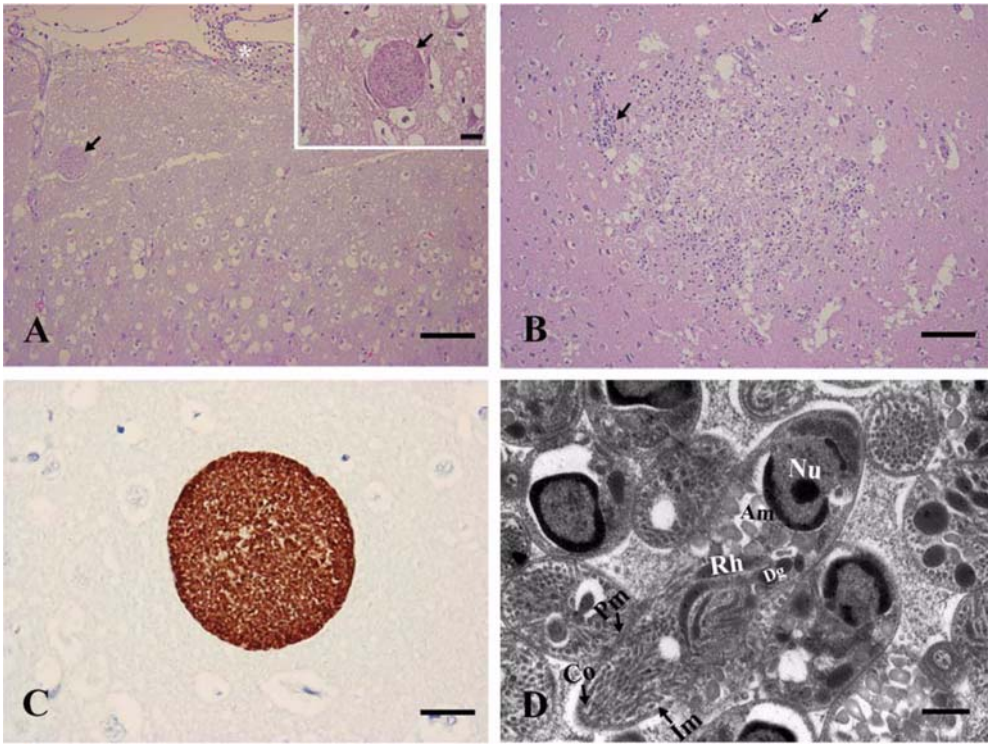

Fig. 1. Lesions and *Toxoplasma* (*T.*) gondii in the brain of a stray cat. (A) Lymphohistiocytic meningitis (*) and intracellular tissue cyst of *T. gondii* (arrow) are observed in the cerebrum. H&E. Bar = 200 μm. Insert: Higher magnification of the tissue cyst. Thin cyst wall (arrow) and bradyzoites with a characteristic single terminal nucleus are seen. H&E. Bar = 40 μm. (B) Microgliosis, malacia (arrowheads) and lymphocytic perivascular cuffings (arrows) are observed in the cerebrum. H&E. Bar = 200 μm. (C) Bradyzoites in the tissue cyst. Immunopositive for *T. gondii* in cerebral cortex. Immunohistochemistry. Bar = 40 μm. (D) Bradyzoites of *T. gondii* in the tissue cyst. The organism is crescent-shaped and its nucleus is located near one pole of the cell. Am, amylopectin granule; Co, conoid; Dg, electron-dense granule; Im, inner membrane complex; Nu, nucleus; Pm, plasmalemma; Rh, rhoptry. TEM. Bar = 500 nm.

DNA extraction with a commercial kit (QIAamp DNA Mini Kit; Qiagen, Germany), PCR was performed as previously described [16, 19, 21]. A forward primer (5′-GGAACTGCATCCGTTCATGAG-3′) and a reverse primer (5′-CAGACGAATCACGGAACTG-3′) were designed for PCR and the amplified product was a 501 bp fragment. Amplification conditions for PCR were as follows: initial denaturation at 94°C for 4 min; 35 cycles at 94°C for 1 min, 52°C for 1 min, and 72°C for 1 min; and final extension at 72°C for 8 min. Amplification products were then electrophoresed on a 2.0% agarose gel, stained with ethidium bromide, and photographed under UV illumination. Additional PCR tests were performed with commercial kits (Intron, Korea) to rule out feline infectious diseases such as feline immunodeficiency virus (FIV), feline leukemia virus (FeLV), feline parvovirus (FPV), feline calicivirus (FCV), and feline coronavirus (FCoV) infections.

Histopathologically, there was mild to moderate lymphohistiocytic meningitis (Fig. 1A) and multifocal encephalomalacia with a few lymphocytes and gitter cells infiltration in the cerebrum (Fig. 1B) and cerebellum. Mild lymphocytic perivascular cuffings in the midbrain and brain stem were also observed. Specially, round encapsulated cyst-like structures up to 135 μm in diameter were multifocally found in the cerebral cortex (Fig. 1A) and the granular layer of the cerebellum. However, cysts or the characteristic pathologic changes of toxoplasmosis were not observed in any tissues or organs other than the brain. The tissue cysts in the cerebrum and cerebellum of the cat were strongly immunoreactive against *T. gondii* (Fig. 1C). Ultrastructurally, the tissue cysts were found intracellularly in brain neural cells, contained numerous bradyzoites of *T. gondii* and had walls that were relatively thin and smooth. The bradyzoites were crescent-shaped and their nucleus was terminally located (Fig. 1D). Rhoptries were homogenously electron-dense in bradyzoites (Fig. 1D). Additionally, conoids, amylopectin granules, and dense granules were observed (Fig. 1D). These ultrastructural features were similar to those of tissue cysts and bradyzoites of *T. gondii* [18]. The *T. gondii*–specific PCR product was detected only in the brain by PCR. However, that was not detected in the fecal sample and other organs including heart, lungs, liver, spleen, kidneys, and muscle. The size of the PCR band was identical to that of the *T. gondii* positive control (Fig. 2). The detections

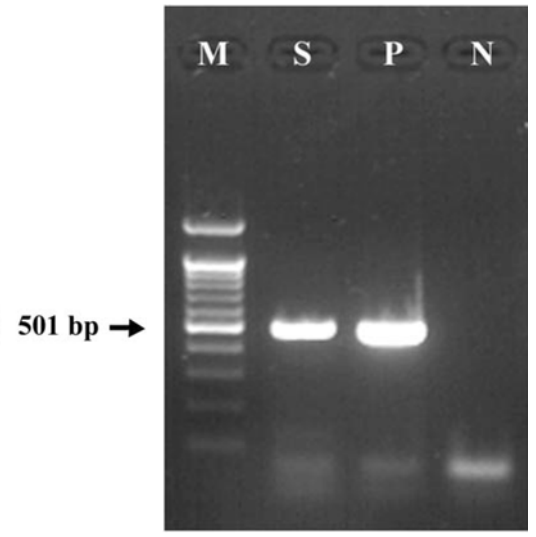

Fig. 2. Results of *T. gondii* PCR analysis using brain sample; cat. DNA marker (lane M), DNA from the brain sample (lane S), positive control (lane P), and negative control (lane N).

for *N. caninum* and other feline diseases including FIV, FeLV, FPV, FCV, and FCoV infections resulted in negative (data not shown).

Discussion

Feline toxoplasmosis is generally subclinical [4], however this disease has zoonotic potential because abortion or stillbirth could occur in sheep, goats, pigs, and even humans [1, 10, 21]. The prevalence of toxoplasmosis has been documented previously in animals and humans in Korea [10, 11, 17, 20, 24]. Moreover, some reports [10, 11] suggest that the prevalence of *T. gondii* in the stray cat population is as high as 47.2% and that this could be an indicator of the prevalence of the parasite throughout Korea, although there have been no confirmed cases of the organisms in feline tissues of natural case with brain lesions.

In cats, clinical toxoplasmosis has been reported concurrent with infections of immunosuppressive feline diseases such as FIV and FeLV [4]. As a predisposing factor, toxoplasmosis-suspected cats should be checked for those diseases [15]. Therefore, we conducted viral disease screening because of histopathologic evidence of meningoencephalitis. None of the viral agents,

including FeLV, FIV, FPV, FCV, and FCoV, were detected in tissue samples and there was no related histologic evidence of these viral diseases.

Many feline toxoplasmic infections have been reported as brain granuloma or hepatitis [7, 8, 15, 22] and various types of toxoplasmosis have also been described in other species, including black-footed ferrets, farmed mink, wallabies, canaries, and marine mammals such as a harbor seal, a sea otter, dolphins and walrus [2, 3, 6, 9, 12, 13, 23].

Natural *T. gondii* infection shows systemic pathologic changes in feline tissues including meningoencephalitis, iridocyclochoroiditis, interstitial pneumonia, myocarditis, necrotizing hepatitis, pancreatitis, intestinal ulcers, necrosis of lymph nodes, dermatitis, and so on [5]. Encephalitis or meningoencephalitis with the absence of gross lesions is especially characteristic of feline CNS toxoplasmosis [4, 5]. In the present case, no systemic histologic lesions were observed and we found lesions only in the brain, including meningoencephalitis and focal malacia. In addition, the chronic inflammatory foci were usually not associated with cysts and those histological lesions corresponded with those previously described [22].

For differential diagnosis between *T. gondii* and *N. caninum*, PCR and DNA sequence analyses have been considered more accurate than IHC and EM [14]. Even though EM is known to be useful for the differentiation of these two organisms, they are sometimes indistinguishable because their tachyzoites, bradyzoites and tissue cysts share many ultrastructural characteristics [14, 18]. Regarding IHC, low specificity and a cross-reaction of antibodies against these two organisms have already been described [1, 14]. Therefore, we conducted not only IHC and EM, but also additional PCR to differentiate between the two organisms. In addition, PCR using cerebrospinal fluid has been done as an ante-mortem diagnostic method to support the diagnosis of CNS toxoplasmosis [19]. However, even the sensitive nature of PCR does not allow for differentiation between acute or chronic subclinical infections [5]. Therefore, various diagnostic tools have to be used when making a precise diagnosis of toxoplasmosis in a suspected case.

Tachyzoites start to disappear from visceral tissues and may be localized as tissue cysts by approximately the third week after infection [5]. *T. gondii* oocysts are hardly found in routine fecal tests because cats usually shed them for 1 or 2 weeks after ingesting tissue cysts [5, 7]. In the present study, there were no tachyzoites in tissue sections, but tissue cysts with bradyzoites were found. Histological lesions were found only in the brain. The organism was not detected in fecal sample even by PCR and *T. gondii* oocysts were not found by the fecal test. Therefore, this case is not an acute infection but thought to be a chronic or latent case.

It is very accessible for stray cats to contact other infected wild animals because they occupy the top of the urban food chain. Therefore, this cat was presumably thought to become infected from ingesting an intermediate host infected with *T. gondii*. In Korea, many stray cats are often found in residential areas. Their numbers have increased gradually and they pose a pubic heath risk for animals and humans [10, 11]. The present finding encourages careful examination even though some cats may have no neurological signs or gross lesions. Moreover, control programs for toxoplasmosis are needed to prevent zoonotic transmission to animals and humans.

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