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PCR-Based Detection of *Toxoplasma gondii* DNA in a Pet Rabbit

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Abstract : A 1-year-old female pet rabbit (Lion head, BW: 1.1 kg) was present to the animal hospital for vaccination of viral hemorrhagic disease. This rabbit was adopted in 4-month-old, and contacted with wild cats in outdoors before adoption. We examined feces for *Toxoplasma gondii* by PCR analysis. We detected the presence of the 497 base pair fragment as a positive result. This is the first detection of *T. gondii* DNA in a pet rabbit from Korea by PCR analysis.

Key words **:** *Toxoplasma gondii*, PCR, pet rabbit.

Introduction

Infection of *Toxoplasma (T.) gondii* is widely prevalent in cats, dogs, pig and rabbits (5). Toxoplasmosis in domestic rabbits (*Oryctolagus cuniculus*) was first described in Brazil as early as 1908 (20), and since then clinical cases of this disease among rabbits have been reported by many authors in various countries (2,6,15,16,18). Fatal toxoplasmosis in three domestic rabbits in USA and the most striking lesions in all three rabbits were necrotic foci in the spleen and liver associated with massive presence of multiplying *T. gondii* tachyzoites (18). The pathological lesions of toxoplasmosis were mainly observed in the liver and spleen (9).

Although some authors treat this role marginally, others place the rabbit as a major source of infection for humans (3,10,12,14,19). Cases of cervical toxoplasmosis transmitted from rabbit to humans have been described previously (11) and very high titers of anti-*Toxoplasma* antibodies in rabbit hunters have been reported (3). Positive *Toxoplasma* in workers at rabbit farms has also been reported (21), therefore suggesting that the rabbit should be considered as a potential source of *Toxoplasma* infection among those who are keeping contact with rabbits. Jones *et al.* (13) reported that the PCR protocol of B1 gene appears to be the most sensitive protocol in the detection of *T. gondii* DNA. The purpose of this report is to detect the *T. gondii* DNA in a pet rabbit.

Case

A 1-year-old female pet rabbit (Lion head, BW: 1.1 kg) was present to the Veterinary Medical Teaching Hospital for vaccination of viral hemorrhagic disease. This rabbit was adopted in 4-month-old and contacted with wild cats in outdoors before adoption. We examined feces for *T. gondii* by PCR analysis, because the owner did not agree to collect blood sample from vein. The availability of polymerase chain reaction (PCR) as a diagnostic technique using the B1 gene of *T. gondii* has been reported previously (4).

The DNA was extracted using the i-genomic stool DNA extraction mini kit (iNtRON Bit Ltd., Korea) according to the manufacturer's protocol from feces samples. In this study, PCR was performed using primers designed to detect the B1 gene of *T. gondii* (13). The sequence of the forward-primer is 5'-AGCAAACACCGAACTCT-3'and of the reverse-primer is 5'-CATGGTTTGCACTTTTGTGG-3' (497 bp amplicon). The targeted B1 gene is highly conserved in all *T. gondii* strains and is multiple copy genes within the *T. gondii* genome. For amplification, the PCR reaction was performed in a 20 μl reaction mixtures, containing 1 μl template DNA, 18.8 μl mixture [13.8 μl of the DW, 10 × Reaction buffer 2 μl, 10 mM dNTPs 2 μl, 10 pmol of the outer primers 1 μl (O-F-Primer 0.5 μl, O-R-Primer 0.5 μl) and 0.2 μl of the prime Tag DNA polymerase (Genet Bio Ltd., Korea). The cycling conditions were 94°C for 5 min, 30 cycles of 94°C for 1 min, 55°C for 30 sec, 72°C for 1 min. and a final extension of 72°C for 4 min. The PCR products were analyzed by

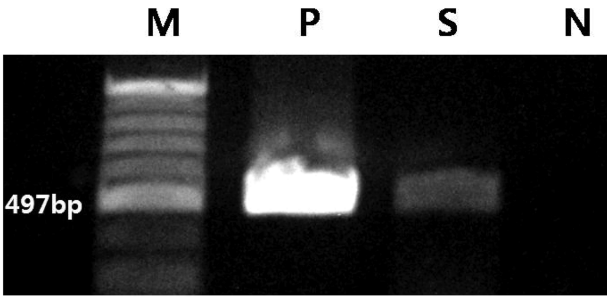

Fig 1. PCR results of pet rabbit feces sample. 497 bp DNA band was amplified in a sample (M: marker, P: Positive control, S: samples, N: Negative control).

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gel electrophoresis using a 2% TBE agarose gel and were visualized using ethidium bromide staining and UV radiation (Fig 1). We detected the presence of the 497 base pair fragment as a positive result.

Discussion

Lindsay *et al.* (17) suggested that sporulated *T. gondii* oocysts can pass the canine intestinal tract and be excreted in the feces in an infectious state. Moreover, the dogs orally inoculated with large numbers of sporulated *T. gondii* oocysts in a previous study (7) did not develop clinical signs of toxoplasmosis and has also been observed in experimentally orally inoculated mice which is other species.

No previous studies have reported *T. gondii* infection in rabbit of Korea. There were several studies for *T. gondii* in blood, tissues of rabbits from Spain (1), and Mexico (8) and 14.2% of wild rabbits in Spain were seropositive by the modified agglutination test (1). Similarly, in 2006, 26.9% of domestic rabbits in Mexico showed seropositive result by the ELISA test (8). The affected rabbits with toxoplasmosis showing different clinical findings include loss of appetite, fever, anorexia, lethargy, respiratory disorders, tremors, uncoordinated gait and diarrhea (8). However, there were no clinical signs in the present case. Thus, we assumed that the case was occult infection of *T. gondii*.

Rabbits are herbivores, the more plausible source of infection is contamination of food with oocysts; hay, straw, and grain that had been contaminated with cat feces have been identified as sources of infection for livestock (22). In the present case, the bedding material (sawdust, straw, hay, or alfalfa) may be contaminated with oocysts from stray cats, thereby infecting rabbits. Also, congenital transmission could not be ruled out as a route of transmission (8). Although there has been no reports in experimentally orally inoculated rabbits, it is supposed that tachyzoites may also pass the intestinal tract of pet rabbits, in common with dogs and mice, and could be excreted in the feces.

In conclusion, this is the first detection *T. gondii* DNA in a pet rabbit from Korea by PCR analysis. Further studies are needed to clarify the molecular characterization and whether tachyzoites are able to pass through the intestinal tract of pet rabbit.

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PCR 기법에 의한 애완용 토끼에서의 톡소플라즈마 유전자 검출

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요 약 : 1세의 암컷 애완용 토끼(라이온 헤드, 체중:1.1kg)가 바이러스성 출혈병에 대한 예방접종을 하기 위해 병원에 내원하였다. 평소 야생고양이와 자주 접촉한 사실을 문진상에서 확인하여 분변을 채취하여 PCR검사를 실시한 결과 톡소플라즈마 B1 유전자를 검출하였기에 이를 보고하고자 한다.

주요어 : 톡소프라즈마, PCR, 애완용 토끼