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Short communication

# Diagnosis of the *Encephalitozoon cuniculi* infections in pet rabbits with neurological symptoms

J. Ziętek<sup>1</sup>, Ł. Adaszek<sup>1</sup>, B. Dzięgiel<sup>1</sup>, M. Kalinowski<sup>1</sup>, M. Bartnicki<sup>1</sup>,  
A. Kalinowska<sup>2</sup>, Ł. Jarosz<sup>1</sup>, S. Winiarczyk<sup>1</sup>

<sup>1</sup> Department of Epizootiology and Infectious Diseases, Faculty of Veterinary Medicine, University of Life Sciences, Głęboka 30, 20-612 Lublin, Poland

<sup>2</sup> Department of Ophthalmology, Medical University, Chmielna 1, 20-079 Lublin, Poland

## Abstract

The purpose of the study was the *in vivo* diagnosing of *E. cuniculi* invasions in pet rabbits with neurological symptoms using the Real-Time PCR, and determination of the rate of invasion, in this group of animals. The study involved 103 pet rabbits with neurological symptoms. Parasitic invasions were diagnosed using Real-Time PCR. The DNA of the parasites for molecular tests was isolated from the urine of the diseased animals. Out of the 103 tested DNA samples, the presence of the *E. cuniculi* genetic material was detected in 27 samples (26.21%). The melting temperature ( $T_m$ ) of all products was 77.5°C. The presence of parasitic DNA in the urine of 26.21% of examined animals indicates that *E. cuniculi* infections occur widely in pet rabbits in Poland and are a significant cause of neurological disorders in those animals.

**Key words:** *Encephalitozoon cuniculi*, pet rabbits, Real-Time PCR

## Introduction

*Encephalitozoon cuniculi* is an obligatory intracellular microsporidian parasite that can infect a wide range of mammals, including rodents, rabbits, horses, carnivores and humans (Ziętek et al. 2013).

The main host for *E. cuniculi* is the rabbit, and seroprevalence rates are usually high in pet rabbit with 37% to 68% of the population (Ewringmann and Gobel 1999, Ebrecht and Muller 2004). In wild rabbit populations the parasite is less prevalent, probably due to the lower animal density (Wilson 1979, Cox

and Ross 1980). In veterinary practice, encephalitozoonosis is a common cause of neurological disease in pet rabbits. However, a definitive diagnosis in living animals is difficult and treatment protocols for the disease are still nonuniform (Kunzel and Joachim 2010, Ziętek et al. 2013).

As the parasite can pose a threat to human health and previous works did not explore the determination of the frequency rate of *E. cuniculi* invasions in pet rabbits in Poland, the purpose of the study was to use Real-Time PCR for *in vivo* diagnosis of *E. cuniculi* invasions in rabbits with neurological symptoms, as

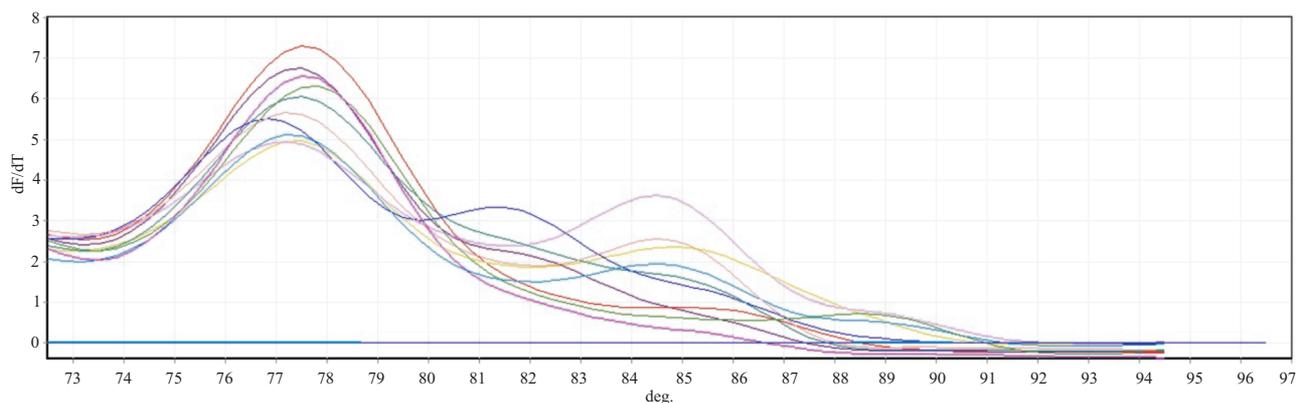


Fig. 1. The analysis of the *E. cuniculi* amplicon melting curve obtained in the Real-Time PCR reaction. The melting temperature ( $T_m$ ) of the obtained products was 77.5°C.

well as determination of the rate of infection caused by the pathogen in this group of animals.

## Materials and Methods

The study involved a group of 103 pet rabbits, aged 2 months to 8 years, with neurological symptoms (paralysis, paresis, torticollis, urinary and faecal incontinence). For 38 of the animals, the interviews revealed that they have had accidents (hits, being trampled by other animals at home, injuries during play etc.). For the remaining 65 animals the primary cause of neurological disorders at the moment of admittance to the clinic was not known.

The animals were clinically examined, including x-ray, haematological and biochemical examination. Faeces samples were taken for parasitological tests, and urine samples to obtain DNA for molecular tests to detect the *E. cuniculi* infection. The DNA obtained from the urine was isolated using the Genomic Mini kit (A & A Biotechnology, Poland). The isolated DNA was amplified using the Real-Time PCR reaction.

The Real-Time PCR reaction was carried out using the Corbett apparatus. The set of primers: mps3 (5' GGAATTCACACCGCCCGTCACTAT 3'), msp4a (5' CCAAGCTTATGCTTAAGTCCAAGGGGT 3'), and msp4b (5' CCAAGCTTATGCTTAAGTCCAGGGAG 3') were used in the reaction. The polymerase chain reaction in real time with the SYBR Green 1 dye was carried out in thin-walled test-tubes with a capacity of 100  $\mu$ l. A DYNAMO HS SYBR Green qPCR Kit (Finnzymes) was used in the assay allowing to conduct a highly specificity reaction.

The reaction mixture with a volume of 20  $\mu$ l consisted of the following components: 2  $\mu$ l of the DNA

matrix, 6.8  $\mu$ l of water, 0.4  $\mu$ l of each primers (the final concentration of 50 pM), 10  $\mu$ l of the Master Mix containing a hot start version of the modified polymerase Tbr (*Thermus brockianus*), buffer for the Tbr polymerase, dNTP, MgCl<sub>2</sub>, and the intercalating SYBR Green 1 dye. The optimised Real – Time PCR reaction consisted of 50 cycles, each of them consisting of three stages: denaturation at 92°C for 60 seconds, annealing at 58°C for 60 seconds, and extension at 72°C for 90 seconds.

The measurement of the reaction mixture and the determination of the Ct indicator value (the number of amplification cycles, after which the fluorescence intensity of the formed product is higher than the background fluorescence) was carried out in real time at the stage of elongation of a helix complementary to the DNA matrix. In order to prove the specificity of amplification, the melting temperature of the PCR products was defined (melting curve) by a gradual increase of the reaction mixture temperature from 70°C to 95°C while continually measuring the fluorescence.

## Results and Discussion

Out of the 103 tested DNA samples, the presence of the *E. cuniculi* genetic material was detected in 27 samples (26,21%). The Ct values read from the amplification curve fluctuated around 25 cycles for all the examined samples. The melting temperature ( $T_m$ ) of all products was 77.5°C (Fig. 1). The protozoan DNA was not found in the urine of injured animals. The results of clinical examinations and radiological tests in those animals confirmed that their injuries caused the neurological symptoms. In another 12 animals the neurological disorders resulted from gastric/intoxication problems, in 17 animals invasions by *Coccidia*

were observed, whereas in the remaining 9 animals the underlying cause of the disease was not found.

To our best knowledge, this is the first report on the detection of *E. cuniculi* DNA from pet rabbits in Poland. The presence of the parasitic DNA in the urine of 26.21% of the studied pet rabbits, in comparison with results of study performed in Japan, where the DNA of parasites was detected in urine of 7.78% animals (Kimura et al. 2013) indicates that *E. cuniculi* infections occur widely in pet rabbits in Poland and are a significant cause of neurological disorders in those animals.

The presence of infected animals in the rabbit population indicates the need for screening for *E. cuniculi* carrier rabbits, especially considering the potential zoonotic risk. Because *E. cuniculi* spores are regularly excreted into urine, persons should avoid contact with the urine of infected or healthy animals and always use good personal hygiene when handling animals (Kunzel and Joachim 2010).

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