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Intestinal toxoplasmosis in cats treated with Procox (case report)

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Abstract. *Toxoplasma gondii* is a widespread zoonotic protozoan that infects most, if not all species of mammals and birds. Felids are the definitive host and they get infected by the ingestion of cysts that are located in various tissue of the intermediate hosts, and less common by ingestion of the oocysts.
The present study evaluates the therapeutic efficacy of emodepside/toltrazuril (Procox® Bayer) for the treatment of intestinal toxoplasmosis in two cats of one and eight years-old respectively. Both animals presented apathy and diarrhea after the consumption of beef and sheep raw meat purchased from market. During coproparasitological examination *T. gondii* like-oocysts were identified. The presence of *T. gondii* was confirmed by PCR and bioassay in mice. The cats were treated with Procox® (Bayer) (off label) for three days consecutively. Fecal samples were collected at the end of treatment and one week later to evaluate the presence of *T. gondii* oocysts by flotation technique and PCR. After the three days of treatment both cats were negative for *T. gondii.* One of the two *T. gondii* isolates was identified as genotype II.

Keywords: *Toxoplasma gondii;* Cat; Treatment; PCR; Emodepside/Toltrazuril.

Toxoplasmoza intestinală la pisici tratate cu Procox (raport de caz)

Rezumat. *Toxoplasma gondii* este un protozoar zoonotic larg răspândit, care infectează majoritatea, dacă nu toate speciile de mamifere şi păsări. Gazdele definitive sunt reprezentate de felide, care se contaminează prin ingerarea chisturilor, care se găsesc în diferite ţesuturi ale gazdei intermediare şi mai puţin frecvent prin ingerarea oochisturilor.
În acest studiu s-a evaluat eficacitatea terapeutică a emodepsidului/toltrazurilului (Procox® Bayer) în tratamentul toxoplasmozei intestinale la două pisici de unu şi, respectiv, opt ani. Ambele animale au prezentat apatie şi diaree după consumul de carne de vită şi de oaie achiziţionate din supermarket. La examenul

coproparazitologic s-au identificat oochisturi de *T. gondii/H. hammondi*. *T. gondii* a fost confirmată prin PCR și bioproba pe șoareci. Pisicile au fost tratate cu Procox® (Bayer) timp de trei zile consecutiv. La sfârșitul tratamentului și o săptămână mai târziu au fost recoltate probe de fecale pentru a evalua prezența oochisturilor *T. gondii* prin tehnica de flotaţie și PCR. După cele trei zile de tratament, ambele pisici au fost negative pentru *T. gondii*. Unul dintre cele două izolate de *T. gondii* a fost identificat ca genotipul II.

Cuvinte cheie: *Toxoplasma gondii;* pisici; Tratament; PCR; Emodepsid/Toltrazuril.

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Introduction

Toxoplasma gondii is an obligate intracellular coccidian that infects most species of warm-blooded animals and humans worldwide. Sexual reproduction takes place in the guts of felids that are the definitive hosts, causing excretion of oocysts in cat feces (Frenkel et al., 1969; Dubey, 1986; Frenkel et al., 1987). Cats are more frequently infected through ingestion of tissue cysts through consumption of prey species and/or raw/undercooked meat, and less commonly by ingestion of oocysts.

The majority of cats infected with *T. gondii* will not present any symptom, but in young and/or immunosuppressed adult cats the signs of disease can be present, especially in cats infected with feline immunodeficiency virus (FIV), feline leukemia virus (FeLV) or other concurrent infections (Jaggy and Platt, 2010; Platt and Olby, 2014). The clinical manifestations of feline toxoplasmosis are variable; the protozoan is able to cause gastrointestinal, respiratory, ophthalmological and neurological disorders. Nonspecific signs as anorexia, lethargy, depression, fever, and weight loss can be also noticed (Gunn-Moore and Reed, 2011; Lorenz et al., 2010; Platt and Olby, 2014). Neurological signs may be observed alone or along with digestive, pulmonary or ophthalmological symptoms (Gunn-Moore and Reed, 2011; Lorenz et al., 2010). Toxoplasmosis can be suspected based on the history and clinical signs, and confirmed by different laboratory tests and bioassay. The diagnostic techniques used for the diagnosis of digestive form of toxoplasmosis are represented by coproparasitological examination for oocysts identification, serological tests to detect specific IgG antibodies, PCR techniques and mouse bioassay. IgM antibodies reflect an acute infection, while IgG antibodies show a chronic phase (Platt and Olby, 2014; Maggs et al., 2012). *T. gondii* oocysts are morphologically similar to those of *Hammondia hammondi* (Dubey, 2010). Moreover, the oocysts are shed in the feces only for a short period of time, therefore the use of the coproparasitological examination as the only diagnosis method is not recommended. The cats usually excrete the oocysts 3 to 10 days after the infection, and may continue shedding up to 20 days (Dubey, 2010; Jaggy and Platt, 2010). Extraintestinal toxoplasmosis in cats is treated with clindamycin, sulfonamide associated with trimethoprim and azithromycin for long period of time (Elmore et al., 2010; Lappin, 2010). The present paper evaluates the results of the off-label treatment with Procox®, in two cats diagnosed with intestinal toxoplasmosis.

Materials and methods

Case report

Two cats were presented to our department with apathy and diarrhea with hematochezia. The first feline patient was a 1-year-old female, European breed, kept indoor and fed with commercial diet. The second cat was an 8 year-old male, European breed, living predominantly indoors but known to hunt outdoors. In case of both animals, the owners reported a recent change in the diet; the first

cat received raw beef while the second one, was fed with raw sheep meat one week before the onset of the clinical signs. In both cases athe meat was purchased from supermarkets.

Fecal samples were collected and examined by flotation technique with saturated salt solution (Mircean et al., 2010). Both cats presented *T. gondii*-like oocysts (figure 1), that were identified by PCR (Homan et al., 2000) (figure 2) as *T. gondii* oocysts. The cats were off-label treated with 0.5 ml/kg Procox® (Bayer) for three consecutive days (day 1-3).

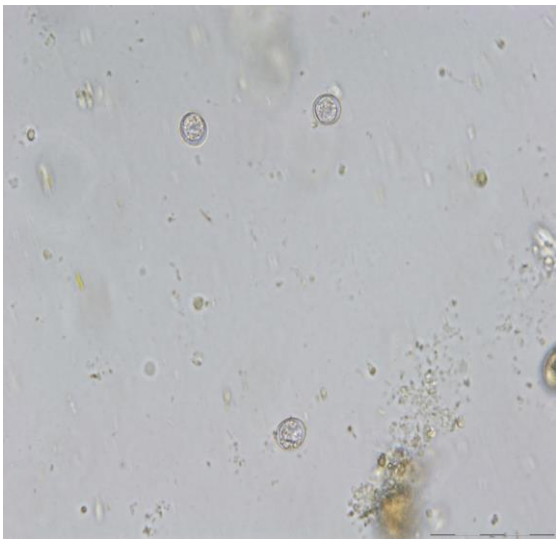

Figure 1. *T. gondii*-like oocysts found to flotation technique (x40)

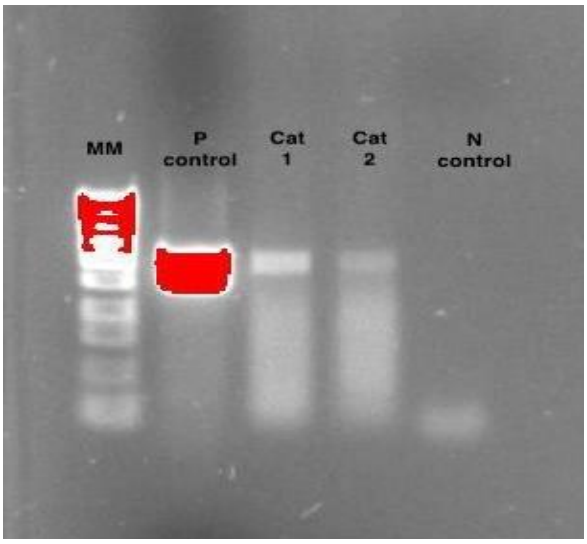

Figure 2. Detection of *T. gondii* by PCR.
Line 1 – molecular marker (529 bp), line 2 – positive control, lines 3-4 – the samples from the cats, line 5 – negative control

Procox® (Bayer) is an oral suspension for dogs containing 0.9 mg/ml emodepside and 18 mg/ml toltrazuril. It is approved for the treatment of puppies and young dogs with suspected or confirmed mixed infections caused by round-worms (*Toxocara canis*, *Uncinaria stenocephala*, *Ancylostoma caninum*) and *Isospora (Isospora canis*, *Isospora ohioensis*-complex). The efficacy of treatment was evaluated at the end of treatment (day 4) and one week later (day 11) by flotation and PCR. The owners of both cats expressed their consent to the use of the therapy.

Mouse bioassay

Mouse (CD1 line) bioassay was performed to genotype the *T. gondii* isolate from the second cat. Two mice were orally inoculated with 500 µl of sporulated oocysts isolated from feaces by sedimentation technique. The oocysts were sporulated in 2.5% potassium dichromate using standard procedures (Raether, 1995). After 6 weeks, the mice were euthanasied and the brain recovered. The experiment was approved by the Animal Ethics Committee of our institution (protocol no. 93/20.12.2017).

PCR

T. gondii identification was performed by conventional PCR on the 529 bp DNA fragment using the specific primers Tox4 (5'-CGCTGCAGGGAGGAAGACGAAAGTTG-3') and Tox5 (5'-CGCTGCAGACACA GTGCATCTGGATT-3') (Homan et al., 2000). The DNA extraction from feaces was performed using the commercial ISOLATE Fecal DNA Kit (Bioline), and following the manufacturer's instructions. Amplification of the *T. gondii* 529 pb DNA fragments was performed in a final volume of 25 µl containing 5 µl PCR Master Mix (12.5x Green PCR Master Mix), 0,5 µl Tox4 primer (10 µM/µl), 0,5 µl Tox5 primer (µM/µl), 4 µl of DNA sample and 15 µl of ultrapure water. Positive (RH strain) and negative controls (ultrapure water) were used. Amplification was performed with the C1000TM Thermal Cycler (Bio-Rad). The amplification program consisted of one initial initiation cycle at 95°C (5 min); 37 cycles of denaturation at 95°C (30 sec) followed by hybridization (annealing) at 60°C (30 sec), extension at 72°C (1 min) and one final extension cycle at 72°C (5 min).

Genotyping of T. gondii isolate

The genotyping was performed using the multiplex nested PCR-RFLP technique. *T. gondii* DNA was extracted from the brain cysts of inoculated mice followed by PCR. The following markers were amplified: GRA6, altSAG2, BTUB, APICO, C22, C29-2, PK1 and CS3 (Su et al., 2006; Khan et al., 2005). PCR products were digested with appropriate restriction enzymes for the different markers. The products obtained after digestion were visualized by electrophoresis using a 3% agarose gel.

Results

After the start of the treatment, the owners reported progressive improvement in health status of the cat, and absence of the clinical disorders previously described. *T. gondii* oocysts were absent in both cats on days 4 and 11 after initiation of the treatment regardless of the method used (coproparasitological examination and PCR). *T. gondii* was successfully isolated on mice from both cats (figure 3). Based on the results of the RFLP analysis, the isolated strain was type II (table 1).

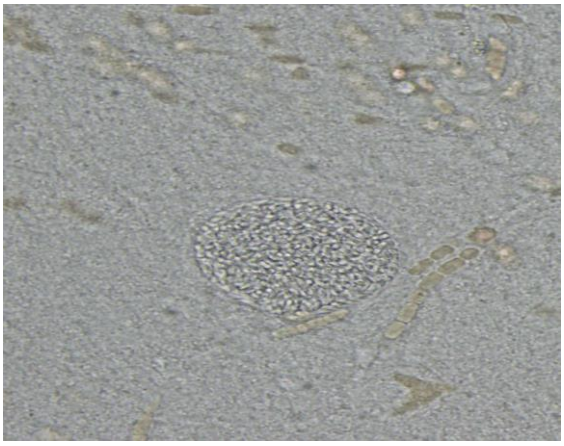

Figure 3. *T. gondii* cyst in the brain of mouse following bioassay (6 weeks post-infection)

Table 1. The strain isolated from the first cat (type II)

Isolate	Genetic markers								Genotype
	GRA6	alt-SAG2	BTUB	C22-8	C29-2	PK1	CS3	APICO	
Cat 2	II	II	II	II	II	II	II	II	II
Reference strains	**Genetic markers**								**Genotype**
	GRA6	**alt-SAG2**	**BTUB**	**C22-8**	**C29-2**	**PK1**	**CS3**	**APICO**	
Me49	II	II	II	II	II	II	II	II	II

Discussion

The large majority of cats infected with *T. gondii* do not develop detectable clinical signs. Manifestation of clinical toxoplasmosis depends on a variety of factors relating to host's immunity and genetic factors as host susceptibility and resistance, along with the inoculum dose of the parasite, the infective stage and the genetic background of the parasite strain (Lappin, 2010; Maubon et al., 2008). It is thought that infection with one genotype of *T. gondii* ensures immunity to all genotypes, but this phenomenon has not yet been demonstrated in cats, only in mice (Dao et al., 2001). Most cats only shed oocysts once in their lives, following infection with infected tissue cysts or more rarely with oocysts. There is a possibility which may lead to cats re-sheding oocysts. The infection in cats with severe immunosuppression (naturally or induced) could potentially have this effect (Malmasi et al., 2009; Zulpo et al., 2018). Most likely, the 8-year-old cat is in the situation previously described, presuming that until this age he had the opportunity to be infected considering that he had access in the environment and a well-developed hunting behavior. However, as the confirmation of a previous diagnosis does not exist, it is impossible to determine if the present

intestinal toxoplasmosis is primary or a reactivation of a latent infection.

The results of the PCR-RFLP revealed that the isolate from cat 2 was type II. Also, other studies from Romania showed that genotype II is predominat being identified in humans, pigs, kids and cats (Costache et al., 2013; Balea, 2016; Paştiu et al., 2015).

We treated the cats with Procox® (Bayer) that is an oral suspension for dogs containing 0.9 mg/ml emodepside and 18 mg/ml toltrazuril approved for the treatment of cystoisosporosis. Petry et al. (2011) demonstrated a good efficacy of toltrazuril in an experimental infection, in cats, infected with *Isospora felis* and *Isospora rivolta.* Beside the indications listed above for Procox® (Bayer), the coccidiocidal active toltrazuril, has been proven effective against *Eimeria* infections in poultry and other animals (Haberkorn, 1996; Mundt et al., 2006; Gjerde et al., 2009). Only a few studies have approached the effect of toltrazuril against *T. gondii* infection and the results are not conclusive so far. Toltrazuril decreases the number of *T. gondii* cysts from the skeletal musculature and brain of sheep with approximately 50% (Kul et al., 2013). It is known that there is no therapy to cure toxoplasmosis, but there are options to control the infection and reduce the symptoms. Treatments that include anti-*Toxoplasma* drugs such as clindamycin, sulfonamide associated with trimethoprim, and azithromycin are known to improve the general status of the patient (Elmore et al., 2010; Lappin, 2010; Bresciani et al., 2016). However, an effective and fast therapeutic response was obtained with the administration of Procox®, for 3 days of treatment. The efficacy and high palatability of Procox® oral suspension, offers a suitable treatment option for cats affected by intestinal toxoplasmosis.

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