

Case Report

CAN PLANT TOXICITY ACCELERATE MORTALITY IN A PET RABBIT (*ORYCTOLAGUS CUNICULUS*) INFECTED WITH *ENCEPHALITOZOON CUNICULI*?

Ana PEŠIĆ¹, Kristina SPARIOSU², Mirjana MILOVANOVIĆ^{3*},
Darko MARINKOVIĆ⁴, Miloš VUČIĆEVIĆ¹

¹University of Belgrade, Faculty of Veterinary Medicine, Department of Equine, Small Animal, Poultry and Wild Animal Diseases, Belgrade, Serbia.

²University of Belgrade, Faculty of Veterinary Medicine, Department of Pathophysiology, Belgrade, Serbia.

³University of Belgrade, Faculty of Veterinary Medicine, Department of Pharmacology and Toxicology, Belgrade, Serbia.

⁴University of Belgrade, Faculty of Veterinary Medicine, Department of Pathology, Belgrade, Serbia.

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Abstract

As grazing herbivores, pet rabbits are known to ingest household plants, some proven to cause intoxication. A previously healthy 2-year-old intact female rabbit was presented with inappetence that lasted about 12 hours. The rabbit was described as free-roaming and had been nibbling on leaves of a new plant over the last month. It was identified as the Madagascar dragon tree (*Dracaena marginata*), a saponin-containing plant, known to be toxic in other companion animals. Following a clinical examination, complete blood and urine analyses were performed, revealing azotemia and tubular proteinuria. Additionally, the rabbit tested positive for IgM and IgG antibodies against *Encephalitozoon cuniculi*. Ultrasonographic examination showed bilaterally enlarged kidneys with unclear demarcation of cortex and medulla, and a hyperechoic zone in the renal pelvis. The day after presentation, the patient died. The necropsy revealed petechial hemorrhages in the lungs, congestion of the spleen, and degenerative changes in the kidneys, liver, and heart. Histopathological examination of the kidneys indicated tubular necrosis

*Corresponding author – e-mail: miram@vet.bg.ac.rs

and interstitial nephritis. Non-purulent encephalitis with perivascular infiltration of lymphocytes and histiocytes was also found. Based on the detected IgM and IgG antibodies against *E. cuniculi*, it is possible that the active infection contributed to the worsening of the patient's health, with a consecutive death after prolonged ingestion of the toxic plant, or *vice versa*. Additionally, to the best of authors' knowledge, this is the first (laboratory) confirmed case of *E. cuniculi* infection in a pet rabbit in Serbia, which raises the awareness of the presence of this zoonotic pathogen.

Key Words: *Dracaena marginata*, encephalitozoonosis, saponins, tubular necrosis

INTRODUCTION

Being grazing herbivores, pet rabbits (*Oryctolagus cuniculus*) enjoy to nibble on household plants. While many ornamental plants are harmless when consumed, some contain toxic compounds that can cause symptoms of poisoning. As species-specific toxic properties of most well-known poisonous plants are not described so far, the plants that lead to alimentary intoxication in other animal species are considered to have similar effects in rabbits (Johnston, 2008). A study performed in Italy reported three cases of Madagascar dragon tree (*Dracaena marginata*) intoxication, one of which was in a pet rabbit (Caloni et al., 2013). To the best of the authors' knowledge, no prior published case studies have described in detail alimentary intoxication by this plant in pet rabbits.

Encephalitozoon cuniculi is an obligate intracellular microsporidium of pet and wild rabbits. The importance of this zoonotic pathogen relies upon its ability to evade host defense mechanisms, causing a chronic disease that predominantly manifests on the central nervous system and/or kidneys. However, ocular and gastrointestinal forms are also recognized (Latney et al., 2014). Although IgM and IgG antibody levels are important from diagnostic aspects, the host response against *E. cuniculi* is primarily cell-mediated and has a significant role in preventing death caused by this pathogen (Doboši et al., 2022).

This report presents the clinical signs, laboratory, macroscopic and microscopic findings in a pet rabbit with active *E. cuniculi* infection and after *D. marginata* consumption, and raises the possibility of plant intoxication to contribute to the clinical manifestation and the lethal outcome of this parasitic disease.

CASE PRESENTATION

Clinical examination

A previously healthy 2-year-old intact female mixed breed domestic rabbit was presented for inappetence that lasted for approximately 12 hours. The rabbit had been described as free roaming. According to the owners, urination and defecation, as well as the appearance and consistency of both urine and stool, were normal. However, the

rabbit appeared somewhat lethargic. Additionally, a slight weight loss had been noticed over the past month, but no other changes were observed until the presentation day, when the pet had completely lost its appetite. The owners mentioned that the rabbit had been seen nibbling on leaves of a new plant, for almost a month. Based on the pictures and the plant's common name, it has been identified as *D. marginata*.

During the clinical examination, the rabbit was apathetic but responsive. Mild dehydration (5%) was observed. Abdominal palpation revealed enlarged kidneys of abnormal shape and texture. Ultrasonographic examination was performed with Vetus 8 (Mindray, Shenzhen, China) using C11-3s Convex Ultrasound Transducer, 10 MHz. It revealed bilaterally enlarged kidneys ($4.5 \times 3.0 \times 3.0$ cm), significantly larger than expected for a rabbit of this size (Banzato et al., 2015). Unclear demarcation of cortex and medulla was observed, and a hyperechoic zone in the renal pelvis without posterior shadow (Figure 1). The liver showed discrete heterogeneous echotexture. Other organs of the abdominal cavity had no ultrasonographic abnormalities. A blood sample was then collected from the saphenous vein for hematology and biochemical profile. A urine sample was obtained by gentle external pressure of the bladder. Initial therapy consisted of subcutaneous application of fluids (0.9% NaCl solution) and buprenorphine (0.02 mg/kg).

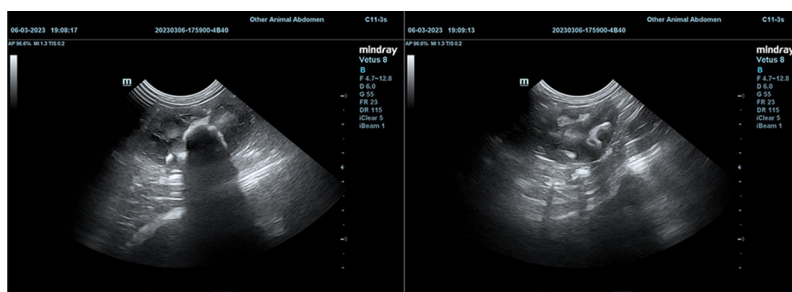


Figure 1. Bilateral kidney enlargement with indistinct cortex and medulla demarcation and a hyperechoic zone observed in the renal pelvis as revealed by ultrasonographic examination.

On the day following the initial examination, the rabbit was brought into the clinic with hypothermia (33 °C), bradycardia (90 beats/min) and bradypnea (22 breaths/min). The patient was provided with emergency treatment, that involved placing it in a forced-air warming unit (HoverHeat Veterinary Forced Air Warming System, Infinium Veterinary Health, US), and bolus application of warmed fluid (0.9% NaCl solution, IV) to address hypothermia and inadequate tissue perfusion, oxygen supplementation *via* face-mask (0.5 L/min), and glycopyrrolate (0.02 mg/kg, IV). When the patient experienced cardiac arrest, cardiac massage was initiated, followed by intubation, ventilation, and application of doxapram (7 mg/kg, IV). However, even with provided emergency care, the patient remained unresponsive, and died. With the owner's written consent, the carcass was sent for necropsy, and the obtained results were used for publication. The Ethical Committee at the Faculty of Veterinary Medicine, University

of Belgrade, Serbia, approved the usage of residual samples for scientific purposes (permission number: 01-07/2024).

Laboratory analyses

The blood sample was analyzed microscopically (Melillo, 2007), in duplicate, using Romanowsky-stained blood smears. Total white blood cell count was $5 \times 10^3/\mu\text{L}$, and in the reference range (Brandão et al., 2021). Differential formula was in accordance with the species profile (Brandão et al., 2021), and included 67% heterophils (neutrophils), 21% lymphocytes, 9% monocytes, 3% eosinophils, with basophils failing to be detected. Red blood cells presented with mild anisocytosis and poikilocytosis, and adequate hemoglobin distribution. Biochemistry analysis (Mindray BS-240, Shenzhen, China) showed increased levels of glucose, blood urea nitrogen (BUN), creatinine, creatine kinase (CK), alkaline phosphatase (ALP) and gamma-glutamyl transferase (γ -GT). Total bilirubin concentration and albumin/globulin (A/G) ratio were decreased (Table 1).

Table 1: Biochemistry profile with the obtained results and reference intervals (Brandão et al., 2021).

Analyte	Unit	Result	Reference interval
Total protein	g/dL	7.02	6.1 – 7.7
Albumin	g/dL	3.41	2.8 – 4.0
Globulins*	g/dL	3.61	2.1 – 3.7
A/G ratio*		0.94 ↓	>1
Glucose	mg/dL	232.07 ↑	109 – 161
Total cholesterol	mg/dL	51.14	6 – 65
Triglycerides	mg/dL	128.32	22 – 188
Alkaline phosphatase	U/L	19.9 ↑	6 – 14
Alanine transferase	U/L	71.9	52 – 80
Aspartate aminotransferase	U/L	53.8	48 – 96
Gamma-glutamyl transferase	U/L	10.2 ↑	0 – 7
Creatine kinase	U/L	516.7 ↑	23 – 247
Creatinine	mg/dL	2.89 ↑	1.0 – 2.2
Blood urea nitrogen	mg/dL	36.47 ↑	9 – 29
Phosphorous	mg/dL	3.96	3.0 – 6.2
Calcium	mg/dL	10.9	7.6 – 12.2
Total bilirubin	mg/dL	0.06 ↓	0.1 – 0.5

Arrows: ↓ decreased values; ↑ increased values; Asterisk: * calculated values

The urine specimen was yellow without any visible sludge. The dipstick test (Dirui Industrial, Changchun, China) was positive for proteins, with pH being 6.5 and the specific gravity 1.012 (refractometer). The rest of the relevant analytes were found in traces (ketones) or were completely absent (glucose). Sediment evaluation confirmed crystalluria, predominantly of calcium oxalate mono and dihydrate, and a smaller amount of calcium carbonate and struvite crystals (Sink and Weinstein, 2012). Hyperplastic transitional cells were found in clusters, with mild to moderate anisocytosis, mild anisokaryosis and a variable nuclear-to-cytoplasmic ratio. Dipstick-detected proteinuria was further analyzed by the means of urine protein-creatinine (UPC) ratio and sodium dodecyl-sulfate polyacrylamide gel electrophoresis (SDS-PAGE), comparing these results with the ones of two healthy female rabbits of the same age. Protein concentration in the patient's urine was 129.28 mg/dL, with UPC ratio of 1.66, while UPC of the controls was 0.18 and 0.15, respectively. Following the procedure by Laemmli (1970), SDS-PAGE was performed. Detected protein bands had strong signals at the molecular weight (MW) lower than albumin, when compared with urine pattern of the controls, confirming proteinuria of tubular origin. However, strong albumin and mild signals above its MW also indicated towards selective glomerular proteinuria.

Upon urine sediment evaluation, spore-like formations were visualized, and having in mind patient's reported inappetence, the serum was further tested for IgM and IgG antibodies against *E. cuniculi*. Using indirect immunofluorescence antibody (IFA) assay, the IgM titer was determined to be at 1:160, while IgG at 1:1280. Both immunoglobulin titers are considered positive at 1:80, or higher (ProVet Laboratories, Center for animal research and diagnostics, Athens, Greece).

Macroscopic and microscopic findings

Necropsy revealed the good carcass condition of the animal, as well as petechial hemorrhages on the lungs, congestion of the spleen, and degenerative changes in the liver and kidneys. The liver had a normal shape but was slightly enlarged, dark brown in color and of friable consistency. The kidneys were enlarged, had a rough surface, and showed petechial and macular hemorrhages. The demarcation between the cortex and medulla was unclear, as also observed by ultrasonography, and parenchymal hemorrhages were also present on the cut surface of the kidney. A significant amount of sludgy, gritty, yellow material was found in the renal pelvis of both kidneys (Figure 2).

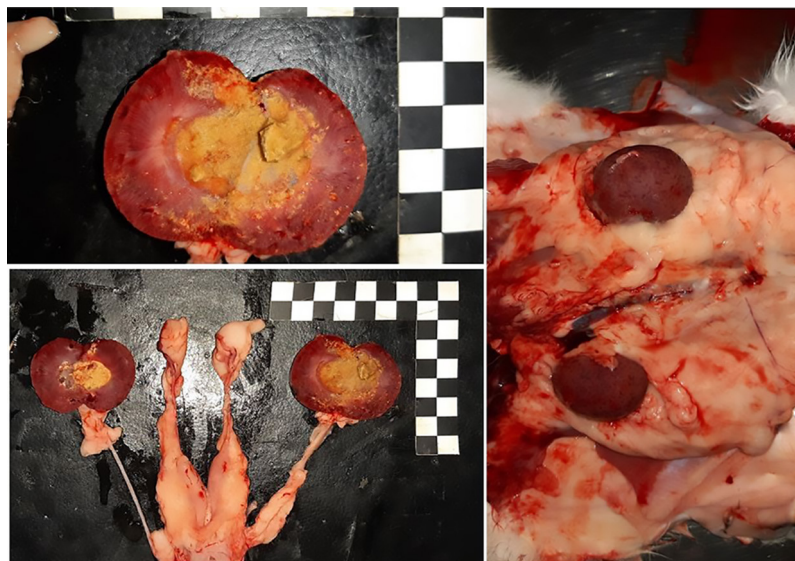


Figure 2. Representative image showing indistinct cortex and medulla demarcation, parenchymal hemorrhages, and sludgy, gritty, yellow material in the renal pelvis of bilaterally enlarged kidneys.

Histopathological examination of the kidneys revealed congestion and tubulonecrosis, characterized by the loss of cellular structure of the tubulocytes, as well as interstitial nephritis with multifocal infiltration of inflammatory cells (lymphocytes, plasma cells, and histiocytes) in the interstitial space, associated with marked hemorrhage in this space (Figure 3A). Multifocal proliferation of fibrous tissue in the form of fibrotic changes was also observed (Figure 3B).

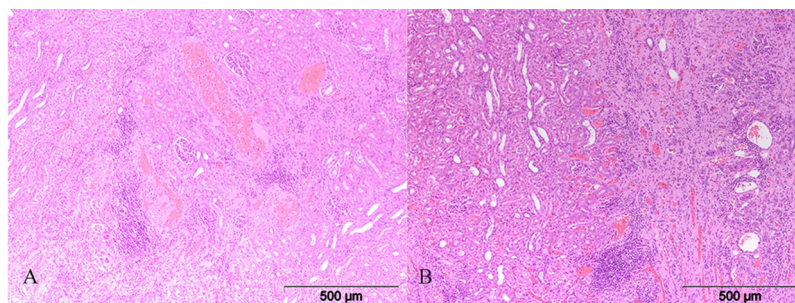


Figure 3. **A)** Histopathological examination of the kidneys showing kidney congestion, tubulonecrosis with loss of tubulocyte cellular structure, interstitial nephritis with multifocal infiltration of inflammatory cells (lymphocytes, plasma cells, and histiocytes) in the interstitial space, and pronounced hemorrhage in the interstitial space. HE×100; **B)** Histopathological examination of the kidneys revealing multifocal proliferation of fibrous tissue characterized by fibrotic changes. HE×100.

Microscopic examination of the liver showed degenerative changes in the form of acute cellular swelling and multifocal lymphocytic pericholangitis. The heart also showed

degenerative and inflammatory changes in the form of myocardial degeneration and multifocal lymphocytic myocarditis. Non-purulent encephalitis characterized by perivascular infiltration of lymphocytes and histiocytes, and focal gliosis were noted.

Differential diagnoses

As *E. cuniculi* infection is followed by cell rupture and inflammation in target tissues – central nervous system, kidneys, liver, heart, and lungs (Latney et al., 2014), the list of differential diagnoses includes infectious and noninfectious causes, able to affect these organs in the same manner.

DISCUSSION

While rabbits are increasingly popular as pets (DeMello, 2016), there is still little knowledge about the effects of plant toxins in this species. The patient described in this report, known to have consumed *D. marginata*, a saponin-containing plant, presented with non-specific clinical signs and laboratory findings, and sudden death, due to *E. cuniculi* infection.

D. marginata is a common foliage ornamental houseplant. The leaves and stems of this plant contain steroidal saponins (furostanol), with a proven toxic effect on the gastrointestinal system, liver, and kidneys, following excessive intake (Bertero et al., 2020; Johnston, 2008). The exact mechanism of saponin toxicity is still unknown, but it has been shown to have cytotoxic and genotoxic effects, causing kidney damage by oxalate crystals, and liver damage by saponins and saponin metabolites (Sobolewska et al., 2020). As this case presents findings in a pet rabbit, known to have species-specific calcium metabolism, it is difficult to identify the exact limit between physiological and pathological amounts of calcium oxalate and carbonate crystals in the urine. However, in most species, an intense calcium-oxalate monohydrate crystalluria is related to toxin-induced renal failure (Sink and Weinstein, 2012). Ultrasonographic evaluation revealed bilaterally enlarged kidneys with indistinct corticomedullary differentiation and a hyperechoic area in the renal pelvis, supporting the presence of significant renal parenchymal damage.

Unlike gastrointestinal irritations caused by saponin consumption, reported in animals and humans (Bertero et al., 2020), the patient in this case had normal stools, without abdominal pain and clinical signs of intestinal tract disorders. Furthermore, the necropsy confirmed the good carcass condition, but the owners stated that the rabbit had lost weight during the previous month. Primarily a marker of liver dysfunction, detected higher ALP level could also be a consequence of the increase of the intestinal isoenzyme, known to be abundant in pet rabbits (Melillo, 2007).

Urine analysis showed an acidic pH, which is rather uncommon in rabbits (Brandão et al., 2021). This adds up to a possible metabolic imbalance due to inadequate feeding during the previous month. As the body tends to adapt to lower energy input,

ketoacidosis occurs. Due to a negative energy balance, the liver increases β -oxidation of free fatty acids, followed by ketone production and metabolic ketoacidosis. Rabbits are known to be affected by ketoacidosis, as they lack key metabolic pathways, that include activity of carbonic anhydrase in the ascending limb of renal tubules and ammonia production, in comparison to other animal species (Harcourt-Brown, 2010). The patient's urine was proven to contain ketones in traces. However, as the dipstick only detects acetoacetate, and not β -hydroxybutyrate or acetone, it is possible that the intensity of ketonuria was even higher (Sink and Weinstein, 2012). An additional explanation for acidic urine is the supersaturation by calcium oxalate monohydrate crystals. Although it is known that carbonate crystals are found in healthy rabbits' urine, the presence of large amounts of oxalate monohydrates should not be excluded as indicative of a pathological state, due to possible poisoning and/or tubular damage (Sink and Weinstein, 2012).

Azotemia found in the blood points towards renal dysfunction, primarily on the glomerular level, and is known to be present in *E. cuniculi* infection (Melillo, 2007). Proteinuria advocates for the presence of both albumin and globulins in the urine. Low MW proteins, found in the urine, indicate tubular damage, which was confirmed by histopathology. The isosthenuria found in the patient could be a result of compromised ability of the kidneys to concentrate urine (Melillo, 2007). These laboratory findings, combined with ultrasonography and histopathology reports, corroborate renal dysfunction.

As an obligate intracellular parasite, *E. cuniculi* is known to evade host immune response, leading not only to an acute infection, but possible outcomes of reactivation, reinfection, as well as a subclinical form of the disease, in apparently healthy animals (Cray et al., 2009). It is known that IgM levels increase from day 0 to day 35 post-exposure, while the IgG levels increase after 2-3 weeks (Magalhães et al., 2022). Our patient was positive for both IgM and IgG against *E. cuniculi*, which suggests that acute infection, reactivation or reinfection occurred during the last month of its life. In this case, the most probable explanation was that a previously acquired infection, rather dormant in the patient, was activated upon its evident metabolic stress. It is interesting to mention that, even though immunoglobulins and proteinuria were found, sera analysis showed total protein and albumin levels within the reference range. This is not a common protein distribution in clinically-manifested encephalitozoonosis followed by the presence of hypoproteinemia, hypoalbuminemia and (γ) hyperglobulinemia (Cray et al., 2009). However, the calculated A/G ratio confirmed slightly higher globulin levels in comparison to albumin, indicating possible changes were underway, likely at the beginning of the clinical form.

As a widespread disease, encephalitozoonosis manifests as a neurological, renal, ocular and/or gastrointestinal form (Latney et al., 2014). However, it was noted that roughly 25% of pet rabbits, seropositive with both IgM and IgG antibodies against *E. cuniculi*, were categorized as (apparently) healthy, due to lack of clinical manifestation of the disease (Škrbec et al., 2023; Cray et al., 2009). The rabbit presented in this case did not

show the most common signs of the disease, but changes were found on both brain and kidney tissue, in the form of non-purulent encephalitis and tubular necrosis and interstitial nephritis, respectively. It was previously reported that there are correlation discrepancies in the severity of inflammatory lesions found in the brain, and the severity of clinical signals related to the neurological form of the disease (Latney et al., 2014). As a species, rabbits are prone to stress, and high glucose levels in blood of the patient may be stress-related. However, chronic stress in the patient could have been provoked due to prolonged nibbling of a plant known to be toxic in other animals (Caloni et al., 2013). Any changes in rabbits' diet, as well as ingestion of poisonous plants, could have caused gastrointestinal disorders (Škrbec et al., 2023). In the frame of the proven *E. cuniculi* infection, it is possible that it started affecting the gastrointestinal tissue, as it was weakened during prolonged exposure to *D. marginata* and its compounds. As the endgame included death of the patient, it is possible to speculate that the host's cell-mediated immune response against *E. cuniculi* was compromised and that the chronic ingestion of *D. marginata* added to the immunosuppression.

Negative effects of *D. marginata* steroid compounds on the host's immune response could be the accelerator of death in a pet rabbit with *E. cuniculi* infection. The described case also raises awareness regarding the presence of *E. cuniculi* in the pet rabbit population in Serbia.

Ethical statement

The owner provided written consent that the obtained results could be used for publication. The Ethical Committee at the Faculty of Veterinary Medicine, University of Belgrade, Serbia, approved the usage of residual samples for scientific purposes (permission number: 01-07/2024).

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Authors' contributions


AP was the leading clinician and wrote the article. KS performed and interpreted the laboratory analyses and reviewed the article. MM interpreted the intoxication effects and wrote the article. DM performed the necropsy and histopathological examination, interpreted the results and reviewed the article. MV supervised the case and wrote and reviewed the article.

Competing interests


The authors declare that they have no competing interests.


ORCID iDs

Ana Pešić  <https://orcid.org/0009-0003-5423-534X>

Kristina Spariosu  <https://orcid.org/0000-0003-4943-2907>

Mirjana Milovanović  <https://orcid.org/0000-0001-6721-7771>

Darko Marinković  <https://orcid.org/0000-0002-5456-1343>

Miloš Vučićević  <https://orcid.org/0000-0002-3208-147X>

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DA LI BILJNI TOKSINI MOGU DA UBRZAJU SMRT KUNIĆA (*ORYCTOLAGUS CUNICULUS*) INFCIRANOG PARAZITOM *ENCEPHALITOOZON CUNICULI*?

Ana PEŠIĆ, Kristina SPARIOSU, Mirjana MILOVANOVIĆ,
Darko MARINKOVIĆ, Miloš VUČIĆEVIĆ

Kratak sadržaj

Kao biljojedi, kunići kućni ljubimci imaju sklonost da jedu sobne biljke, čak i one za koje je poznato da mogu da izazovu trovanje. Prethodno zdrava dvogodišnja ženka kunića dovedena je na pregled zbog inapetence tokom poslednjih 12 sati. Primećeno je da je kunić, koji se slobodno kretao po stanu, prethodnih mesec dana grickao listove nove sobne biljke – dracene (*Dracaena marginata*), koja sadrži saponine i toksična je za (druge) kućne ljubimce. Prateći klinički pregled, analize krvi i urina potvrdile su azotemiju i tubularnu proteinuriju. Pored toga, u serumu kunića su utvrđena IgM i IgG antitela protiv *Encephalitozoon cuniculi*. Ultrazvučni pregled je pokazao da su oba bubrega uvećana, nejasne demarkacije kore i srži, uz prisustvo hiperehogene zone u bubrežnoj karlici. Dan kasnije, konstatovana je smrt pacijenta. Tačkasta krvarenja na plućima, kongestija slezine i degenerativne promene na bubrežima, jetri i srcu, bile su uočene na obdukciji. Patohistološki nalaz bubrega potvrdio je nekrozu tubula i intersticijalni nefritis. Takođe, utvrđen je negnojni encefalitis sa perivaskularnom infiltracijom limfocita i histiocita. Visok titar IgM i IgG otvara mogućnost da je aktivna *E. cuniculi* infekcija doprinela pogoršanju zdravstvenog stanja kunića, pri čemu je smrt nastupila kao posledica prolongirane „ishrane“ toksičnom biljkom, ili – obrnuto. Laboratorijski – potvrđena *E. cuniculi* infekcija kod kunića u Srbiji podiže svest o prisustvu ovog zoonotskog patogena u populaciji kunića kućnih ljubimaca.

Ključne reči: *Dracaena marginata*, encefalitozoonoza, nekroza tubula, saponini