

## Identification of *Prototheca* from the Cerebrospinal Fluid of a Cat with Neurological Signs

Lanave G<sup>1</sup>, Pellegrini F<sup>1</sup>, Palermo G<sup>2</sup>, Zini E<sup>3,4,5</sup>, Mercuriali E<sup>2,3</sup>, Zagarella P<sup>2</sup>, Bányai K<sup>6,7</sup>, Camero M<sup>1,\*</sup> and Martella V<sup>1</sup>

<sup>1</sup>Department of Veterinary Medicine, University of Bari Aldo Moro, Valenzano, Italy

<sup>2</sup>Veterinary Orthopaedic Traumatologic Centre of Arenzano, Arenzano, Italy

<sup>3</sup>Veterinary Institute of Novara, Granozzo con Monticello, Italy

<sup>4</sup>Department of Animal Medicine, Production and Health, University of Padova, Italy

<sup>5</sup>Clinic for Small Animal Internal Medicine, Vetsuisse Faculty, University of Zurich, Switzerland

<sup>6</sup>Veterinary Medical Research Institute, Hungarian Academy of Sciences, Hungary

<sup>7</sup>Department of Pharmacology and Toxicology, University of Veterinary Medicine, Hungary

### \*Corresponding author:

Michele Camero,  
Department of Veterinary Medicine, University of  
Bari Aldo Moro, Valenzano, 70010 Bari, Italy

Received: 02 Jan 2024

Accepted: 14 Feb 2024

Published: 19 Feb 2024

J Short Name: ACMCR

### Copyright:

©2024 Camero M. This is an open access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and build upon your work non-commercially

### Keywords:

Feline; Prototheca; Neurological symptoms; Infection; Alga; Diagnosis

### Citation:

Camero M, Identification of Prototheca from the Cerebrospinal Fluid of a Cat with Neurological Signs. Ann Clin Med Case Rep. 2024; V13(2): 1-6

## 1. Simple Summary

The genus *Prototheca* encompasses unicellular algae that are achlorophyllous and widespread in the environment. The genus is now included in the family Chlorellaceae, belonging to the order Chlorellales, which is included in the class, Trebouxiophyceae. *Prototheca* have repeatedly been reported to infect vertebrates. Cattle, dogs, and cats are the unique domestic animals in which *Prototheca* spp. have been reported, despite sporadic detection in goats, horses, and non-domesticated animals. *Prototheca* spp. have been reported to colonize different districts of the human body. Cats with protothecosis usually display a cutaneous disease, whereas dogs may develop both cutaneous and systemic forms. In this report, we identified molecularly *Prototheca* spp. in a cat with neurological signs. The animal presented a suspected diagnosis of multifocal lymphoma, and eventual immunological disorders/suppression likely triggered systemic diffusion of the achlorophyllous alga. Despite protothecosis not being regarded as a zoonosis, algal infections of animals should be recognized as indicators or sentinels of environmental risks for humans.

## 2. Abstract

*Prototheca* infections are rare in cats, and they are usually associated with cutaneous or subcutaneous infections by *P. wickerhamii*, with no evidence of neurological signs or systemic disease. In this study, we report the identification of *prototheca* in the cerebrospinal fluid (CSF) of a cat with neurological symptoms. Fourteen CSF samples were gathered from cats presented with neurological disease between 2012 and 2014. The inclusion criteria for the samples were an increase in CSF protein and cell number (pleocytosis), suggestive of an infectious inflammatory status of the central nervous system (CNS). Nine samples fulfilled the inclusion criteria (inflammatory samples), while five samples, used as control, did not (non-inflammatory samples). All the samples were screened molecularly for different pathogens associated with CNS disease in cats, including *prototheca*. Out of 14 CSF samples, only one inflammatory sample tested positive for *prototheca*. Upon sequence and phylogenetic analysis of the amplicon, the strain was characterized as *P. bovis*. This report is the first documented evidence of *prototheca* in the cerebrospinal fluid of a cat with neurological signs. *Prototheca* should be considered in the diagnostics

procedures on the CNS of cats presented with infectious diseases.

### 3. Introduction

The *Prototheca* spp. consist of microscopical and unicellular organisms that are obligatory heterotrophs because they lack chloroplasts capable of photosynthesis [1-3]. Despite their yeast-like morphology, based on genetic features, *Prototheca* spp. have been classified as algae and included in the *Prototheca* genus closely related to *Chlorella* genus in the family, *Chlorellaceae* [4]. *Prototheca* spp. are ubiquitous, may also colonize animal and human gastrointestinal tracts, and have been occasionally reported in the skin and nail beds of asymptomatic human patients [1,3,5]. *Prototheca* spp. Are also able to infect animals, but their specific pathogenic mechanisms of infection are yet to be elucidated. Several *Prototheca* spp., i.e., *P. cutis*, *P. miyajii*, *P. ciferrii*, *P. wickerhamii*, *P. bovis*, and *P. blaschkeae*, are able to infect both humans and animals [6-8].

Protothecosis is a rare and occasional disease reported in humans and domesticated as well as wild animals. Human and canine infections have been described worldwide [9]. Mucosal contact, ingestion, or traumatic introduction from contaminated fonts are regarded as the most common sources of transmission of *Prototheca* spp. The algae penetrate the body via the respiratory or gastrointestinal tract and may then diffuse via ocular, cerebral, and renal routes [10,11]. Over 95% of infections in human patients are due to *P. wickerhamii*, with a small number of cases by *P. bovis*, *P. miyajii*, *P. blaschkeae*, *P. ciferrii*, or *P. cutis* [12-14]. In dogs, most *prototheca* infections are caused by *P. zopfii*, with a few cases due to *P. wickerhamii* [11,15].

Feline protothecosis is quite infrequent, either due to natural resistance to infection or circumvention of environmental niches where algae commonly establish. The exiguous recorded cases have all been reported in clinically healthy adult cats with solid, non-ulcerated, cutaneous or subcutaneous masses located on the forehead, distal limbs, base of the tail, nose, or pinnae [16-19], and when the isolates have been speciated, they have all been characterized as *P. wickerhamii* [20]. Nasal localization of *prototheca* has also been reported in cats [15,21]. The lack of regional lymphadenomegaly and clinical signs associated with systemic infection/disease suggests that in cats, *prototheca* infection tends to be localized [10], although a unique cat displayed new distant nodules several months after excisional biopsy of an original solitary lesion [19]. Accordingly, unlike dogs, there is no evidence in the literature for neurological signs or systemic symptoms associated with *prototheca* infection in cats [10].

## 4. Materials and Methods

### 4.1. Collection of Samples

Fourteen cerebrospinal fluid (CSF) samples were gathered from cats with neurological disease at the veterinary clinics of Novara and Arma di Taggia, Imperia, Italy, between 2012 and 2014. The inclusion criteria for the samples were raised CSF protein and an increase in the CSF cell number (pleocytosis), parameters suggestive of an infectious inflammatory status of the central nervous system (CNS). Pleocytosis in a CFS sample (lumbar punctate) was categorized as positive with a protein fraction > 30 mg/dL or number of cells > 3 cells/ $\mu$ L, with a predominance of mononuclear cells. Nine samples fulfilled the inclusion criteria (inflammatory samples), while 5 samples did not and they were used as control.

### 4.2. Nucleic Acid Extraction

The nucleic acids were subjected to extraction from CSF samples employing the IndiSpin® Pathogen Kit (Indical Bioscience GmbH, Leipzig, Germany), following the manufacturer's instructions. Nucleic acid templates were stored at  $-70^{\circ}\text{C}$  until use.

### 4.3. Screening for *Prototheca* spp

Nucleic acid extracts were subjected to a PCR assay specific for the 18SrDNA of *prototheca*, using the forward primer Proto 18S-4F (5'-GACATGGCGAGGATTGACAGA-3') and the reverse primer Proto 18S-4R-1 (5'-ATCACAGACCTGTTATC-3') [22,23], which amplify a PCR product of approximately 250 bp (Table 1).

Amplification was conducted using the Accuprime PCR Kit (Invitrogen™ Thermo Fisher Scientific, Shanghai, China) and *P. blaschkeae* as a positive control. The bands were subjected to excision and purification by a QiaQuick Gel Extraction Kit (Qiagen GmbH, Hilden, Germany), and the sequence was determined. Sequencing was performed at Eurofins Genomics (Vimodrone, Milano) laboratories. As an internal control, primers targeting the 28S rRNA gene of the feline genome were used [33].

### 4.4. Quantitative Real Time PCR (qPCR), Specifically for *P. bovis*

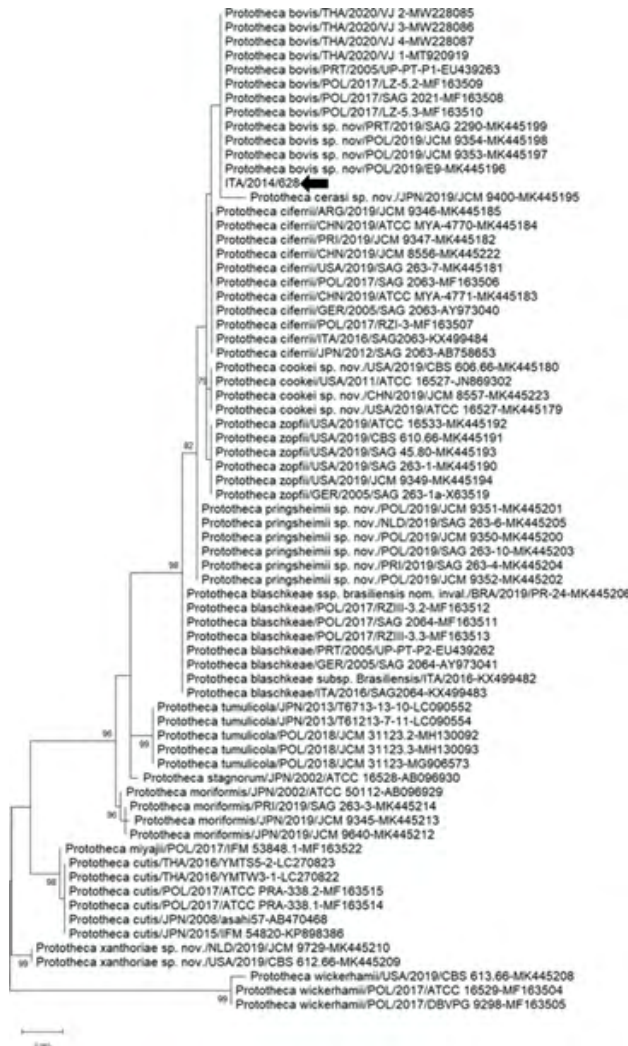
A qPCR specific for *P. bovis* was performed on samples testing positive for *Prototheca* spp. Ten  $\mu$ L of sample DNA was combined with the 15- $\mu$ L reaction master mix (IQ Supermix; Bio-Rad Laboratories SRL, Segrate, Italy), comprising 0.6  $\mu$ mol/L of each primer and 0.2  $\mu$ mol/L of the probe (Table 1). Thermal cycling was performed according to a previously described study [24].

### 4.5. Screening for Other Pathogens

Nucleic acid extracts were also screened for other feline pathogens, including feline infectious peritonitis, feline leukemia virus (FeLV), feline immunodeficiency virus (FIV), feline panleukopenia virus, rickettsia, neospora, toxoplasma, mycobacterium, and bacterial 16S rDNA (Table 1).

**Table 1:** Detailed list of protocols (pathogens, assays, primers, probes, and references) used for the molecular screening of samples included in the study.

Pathogen	Assay	Primers and Probes	Oligonucleotide Sequence	Reference(s)
<i>Prototheca</i> spp.	PCR	Proto 18S-4F Proto 18S-4R-1	5'-GACATGGCGAGGATTGACAGA-3' 5'-ATCACAGACCTGTTATC-3'	[22] [23]
<i>Prototheca bovis</i>	qPCR	PZg2F SPZg2 PZg2R	5'-GACGATGATCCTAGTTATGGTGTAC-3' 5' <i>Fam</i> - TGGTAGAAGACAAATAATGTACCAAAACCA- BHQ13' 5'-TATAAAAGCAAGTCCAGTTACAGCAC-3'	[24]
feline infectious peritonitis	qPCR	FCoV1128f FCoV1200p FCoV1229r	5'-GATTTGATTTGGCAATGCTAGATTT-3' 5'-AACAATCACTAGATCCAGACGTTAGCT-3' 5' <i>Fam</i> -TCCATTGTTGGCTCGTCATAGCGGA- Tamra3'	[25]
feline leukemia virus	PCR	118for 119rev	5'-TTACTCAAGTATGTTCCCATG-3' 5'-CTGGGGAGCCTG GAGACTGCT-3'	[26]
feline immunodeficiency virus	PCR nPCR	158for 159rev 160for 161rev	5'-GAGTAGATACWTGGTTRCAAG-3' 5'-CATCCTAATTCTTGCATAGC-3' 5'-CAAAATGTGGATGGTGGAAAY-3' 5'-ACCATTCWATAGCAGTRGC-3'	[27]
feline panleukopenia virus	qPCR	FPV/CPV-For FPV-Pb CPV-Pb FPV/CPV-Rev	5'-ACAAGATAAAAGACGTGGTGTAAC TCAA-3' 5' <i>Vic</i> -ATGGGAAATACAGACTATAT-MGB3' 5' <i>Fam</i> -ATGGGAAATACAAACTATAT-MGB3' 5'-CAACCTCAGCTGGTCTCATAATAGT-3'	[28]
ricketsia	PCR	RSFG 877 RSFG1258	5'-GGGGCCTGCTCACGGCGG-3' 5'-ATTGCAAAAAGTACAGTGAACA-3'	[29]
neospora	qPCR	Neo For Neo Probe Neo Rev	5'-GCATCGGAGGACACTGCT-3' 5' <i>Fam</i> -CTGACTCTGAACACCGGAGGCACG- Tamra3' 5'-ATGTCGTAAATCGGAGTTGCTTC-3'	[30]
toxoplasma	qPCR	Tox For Tox Probe Tox Rev	5'-GTCCTATCGCAACGGAGTTCTT-3' 5' <i>Fam</i> -CCAGACGTGGATTTCGGTTGGTTCC- Tamra3' 5'-TTCGTCCGTCGTAATATCAGGC-3'	
mycobacterium	PCR nPCR	Myc For Myc Rev Myc NFor Myc Nrev	5'-CATGCAAGTCGAACGGAAAG-3' 5'-CGGTGCTTCTTCTCCACCTA-3' 5'-TACTCGAGTGGCGAACGGGT-3' 5'-CGGACCTTCGTGATGGTGA-3'	[31]
bacterial 16S rDNA	PCR	B-V5 A-V6	5'-ATTAGATACCCYGGTAGTCC-3' 5'-ACGAGCTGACGACARCCATG-3'	[32]
Internal control	PCR	feline 28S rDNA Fw feline 28S rDNA Rv	5'-AGCAGGAGG TGTTGGAAGAG-3' 5'-AGGGAGAGCCTAAATCAAAGG-3'	[33]



**Figure 1:** Partial 18SrDNA sequence (250 nt)-based phylogenetic tree of *Prototheca* spp. strains reported in this study and reference strains retrieved from the NCBI database. The Maximum Likelihood method and Kimura model (two parameters), with a discrete gamma distribution, were applied for the phylogenetic analysis. One thousand bootstrap replicates were employed to assess the robustness of individual nodes on the phylogenetic tree. Bootstrap values higher than 75% were displayed. Black arrows denote strains reported in this study. Numbers of nucleotide substitutions are connoted by the scale bar.

**4.6. Sequence and Phylogenetic Analyses**

The online tool BLAST (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>, accessed on 15 September 2023) was used to find the highest nt identity in the NCBI database. Sequence editing was performed by the software package Geneious Prime v. 2021.2 (Biomatters Ltd., Auckland, New Zealand). Sequence alignments were performed by the MAFFT [34] plugin implemented in Geneious Prime. The best-fitting substitution model settings for the phylogeny were explored by the tool “Find the best protein DNA/Protein Models” of the MEGA X v. 10.0.5 software [35]. The evolutionary history was deduced by using the maximum-likelihood method, the Kimura 2-parameter model, a discrete gamma distribution to model

evolutionary rate differences among sites (6 categories), and supplying statistical support with 1000 replicates. Bayesian inference and neighbor-joining phylogenetic analyses were also performed to explore the phylogeny of *Prototheca* spp.

**5. Results**

Fourteen CSF samples collected in this study were subjected to molecular screening for *Prototheca* spp. and a panel of feline pathogens. Out of 14 CSF samples, 1 sample (#628/14) tested positive for *Prototheca* spp. by PCR, and the sequence was determined. By BlastN analysis performed on a 250 bp sequence of 18SrDNA of *Prototheca* spp., strain #628/14 shared the highest nucleotide (nt) identity (100%) with *P. zopfii* var. *hydrocarbonea* strain UP-PT-P1 (EU439263). All the samples tested negative for feline infectious peritonitis, feline leukemia virus, feline immunodeficiency virus, feline panleukopenia virus, rickettsia, neospora, toxoplasma, mycobacterium, and bacterial 16S rDNA.

Partial 18SrDNA sequence (250 nt)-based phylogenetic analysis was performed using the sequence of *Prototheca* spp. generated in the study and the cognate sequences of the closest relatives retrieved from the NCBI database. Different phylogenetic approaches were explored for *Prototheca* spp., and similar topologies with slight differences in bootstrap values at the nodes of the tree were noticed. Accordingly, the maximum-likelihood (ML) tree was used. Upon ML analysis, strain ITA/2014/628 segregated with strains belonging to the *P. bovis* clade (Figure 1). Upon qPCR specific for *P. bovis*, sample #628/14 yielded 27 Ct.

The animal that tested positive for *Prototheca* spp. was a 9-year-old male domestic European cat, presented at the veterinary clinic with a 24 h history of seizures, incoordination, circling, and disorientation. Clinical pathological evaluation included a complete blood count and clinical chemistry panel. Blood analysis showed a marked increase in creatine kinase (9827 U/L, reference interval [ref.]: 91–326 U/L), alanine aminotransferase (517 U/L, ref.: 22–45 U/L), and aspartate aminotransferase (98 U/L, ref.: 21–41 U/L). The other parameters were not altered. Complete blood count parameters were within the reference interval. In addition, at the time of clinical examination, the animal tested negative for FIV and FeLV, using a quick test (SNAP FIV/FeLV Combo Test—IDEXX Laboratories). Abdominal ultrasound examination revealed multiple spleen and liver nodules. Fine needle biopsy specimens taken from the spleen and liver nodules revealed many lymphoid cells, and a suspect of lymphoma was included in the differential diagnosis.

Within 24 h, the clinical condition of the animal worsened, and the owner opted for the gentle suppression of the animal. Extreme care was employed to guarantee that death had happened prior to discarding the animal remains [36]. Before euthanasia, a CSF sample (#628/14) was collected with the permission of the owner, exclusively for research purposes.

Examination of the CSF displayed a distinct increase in total protein (2432 mg/L, range <300 mg/L) and cytological features consistent with marked mixed-type pleocytosis (2448 cells/ $\mu$ L, range <3 cells/ $\mu$ L), composed mainly of small and medium-sized lymphocytes with no red blood cells. The owner did not give permission for further investigations (i.e., necropsy) in the animal.

## References

- Shave CD, Millyard L, May RC. Now for Something Completely Different: Prototheca, Pathogenic Algae. *PLoS Pathog.* 2021; 17, e1009362.
- Jagielski T, Gawor J, Bakula Z, Decewicz P, Maciszewski K, Karnkowska A. Cytb as a New Genetic Marker for Differentiation of Prototheca Species. *J. Clin. Microbiol.* 2018; 56: 10-1128.
- Kano R. Emergence of Fungal-Like Organisms: Prototheca. *Mycopathologia.* 2019; 185: 747-54.
- Irrgang A, Murugaiyan J, Weise C, Azab W, Roesler U. Well-Known Surface and Extracellular Antigens of Pathogenic Microorganisms among the Immunodominant Proteins of the Infectious Microalgae Prototheca Zopfii. *Front. Cell. Infect. Microbiol.* 2015; 5: 67.
- Lass-Flörl C, Mayr A. Human Protothecosis. *Clin. Microbiol. Rev.* 2007; 20: 230-42.
- Jagielski T, Dylag M, Roesler U, Murugaiyan J. Isolation of Infectious Microalga Prototheca wickerhamii from a Carp (Cyprinus carpio)—A First Confirmed Case Report of Protothecosis in a Fish. *J. Fish. Dis.* 2017; 40: 1417-21.
- Jagielski T, Roeske K, Bakula Z, Piech T, Wlazlo L, Bochniarz M, et al. A Survey on the Incidence of Prototheca Mastitis in Dairy Herds in Lublin Province, Poland. *J. Dairy Sci.* 2019; 102: 619-28.
- Masuda M, Jagielski T, Danesi P, Falcaro C, Bertola M, Krockenberger M, et al. Protothecosis in Dogs and Cats—New Research Directions. *Mycopathologia.* 2021; 186: 143-52.
- Jagielski T, Lagneau PE. Protothecosis. A Pseudofungal Infection. *J. Mycol. Med.* 2007; 17: 261-70.
- Pressler BM. Infectious Diseases of the Dog and Cat. In *Protothecosis and Chlorellosis*; Greene, C.E., Ed, Saunders: St. Louis, MO, USA. 2012; 1: 696-701.
- Stenner VJ, MacKay B, King T, Barrs VRD, Irwin P, Abraham L, et al. Protothecosis in 17 Australian Dogs and a Review of the Canine Literature. *Med. Mycol.* 2007; 45: 249-66.
- Hirose N, Hua Z, Kato Y, Zhang Q, Li R, Nishimura K, et al. Molecular Characterization of Prototheca Strains Isolated in China Revealed the First Cases of Protothecosis Associated with Prototheca Zopfii Genotype 1. *Med. Mycol.* 2018; 56: 279-87.
- Masuda M, Hirose N, Ishikawa T, Ikawa Y, Nishimura K. Prototheca miyajii sp. Nov., Isolated from a Patient with Systemic Protothecosis. *Int. J. Syst. Evol. Microbiol.* 2016; 66: 1510-20.
- Khan ID, Sahni AK, Sen S, Gupta RM, Basu A. Outbreak of Prototheca wickerhamii Algaemia and Sepsis in a Tertiary Care Chemotherapy Oncology Unit. *Med. J. Armed Forces India.* 2018; 74: 358-64.
- Wolf AM. Fungal Diseases of the Nasal Cavity of the Dog and Cat. *Vet. Clin. N. Am. Small Anim. Pract.* 1992; 22: 1119-32.
- Kaplan W, Chandler FW, Holzinger EA, Plue RE, Dickinson RO. Protothecosis in a Cat: First Recorded Case. *Med. Mycol.* 1976; 14: 281-6.
- Finnie JW, Coloe PJ. Cutaneous Protothecosis in a Cat. *Aust. Vet. J.* 1981; 57: 307-8.
- Coloe PJ, Allison JF. Protothecosis in a Cat. *J. Am. Vet. Med. Assoc.* 1982; 180: 78-9.
- Dillberger JE, Homer B, Daubert D, Altman NH. Protothecosis in Two Cats. *J. Am. Vet. Med. Assoc.* 1988; 192: 1557-9.
- Endo S, Sekiguchi M, Kishimoto Y, Kano R, Aoki S, Sichinohe T, Hasegawa A. The First Case of Feline Prototheca wickerhamii Infection in Japan. *J. Vet. Med. Sci.* 2010; 72: 1351-3.
- Huth N, Wenkel RF, Roschanski N, Rösler U, Plagge L, Schöniger S. Prototheca Zopfii Genotype 2-Induced Nasal Dermatitis in a Cat. *J. Comp. Pathol.* 2015; 152: 287-90.
- Roesler U, Möller A, Hensel A, Baumann D, Truyen U. Diversity within the Current Algal Species Prototheca Zopfii: A Proposal for Two Prototheca Zopfii Genotypes and Description of a Novel Species, Prototheca blaschkeae sp. Nov. *Int. J. Syst. Evol. Microbiol.* 2006; 56: 1419-25.
- Sobukawa H, Yamaguchi S, Kano R, Ito T, Suzuki K, Onozaki M, et al. Short Communication: Molecular Typing of Prototheca Zopfii from Bovine Mastitis in Japan. *J. Dairy Sci.* 2012; 95, 4442-4446.
- Bacova R, Kralik P, Kucharovicova I, Seydlova R, Moravkova M. A novel TaqMan qPCR assay for rapid detection and quantification of pro-inflammatory microalgae Prototheca spp. in milk samples. *Med. Mycol.* 2021; 59: 784-92.
- Gut M, Leutenegger CM, Huder JB, Pedersen NC, Lutz H. One-Tube Fluorogenic Reverse Transcription-Polymerase Chain Reaction for the Quantitation of Feline Coronaviruses. *J. Virol. Methods.* 1999; 77: 37-46.
- Stiles J, Bienzle D, Render JA, Buyukmihci NC, Johnson EC. Use of Nested Polymerase Chain Reaction (PCR) for Detection of Retroviruses from Formalin-Fixed, Paraffin-Embedded Uveal Melanomas in Cats. *Vet. Ophthalmol.* 1999; 2: 113-6.
- Endo Y, Cho K.-W, Nishigaki K, Momoi Y, Nishimura Y, Mizuno T, et al. Molecular Characteristics of Malignant Lymphomas in Cats Naturally Infected with Feline Immunodeficiency Virus. *Vet. Immunol. Immunopathol.* 1997; 57: 153-67.
- Decaro N, Desario C, Lucente MS, Amorisco F, Campolo M, Elia G, et al. Specific Identification of Feline Panleukopenia Virus and Its Rapid Differentiation from Canine Parvoviruses Using Minor Groove Binder Probes. *J. Virol. Methods.* 2008; 147: 67-71.

29. Claerebout E, Losson B, Cochez C, Casaert S, Dalemans AC, De Cat A, et al. Ticks and Associated Pathogens Collected from Dogs and Cats in Belgium. *Parasit. Vectors.* 2013; 6: 183.
30. Reitt K, Hilbe M, Voegtlin A, Corboz L, Haessig M, Pospischil A. Aetiology of Bovine Abortion in Switzerland from 1986 to 1995? A Retrospective Study with Emphasis on Detection of *Neospora Caninum* and *Toxoplasma Gondii* by PCR. *J. Vet. Med. Ser. A* 2007; 54: 15-22.
31. Hashimoto A, Koga H, Kohno S, Miyazaki Y, Kaku M, Hara K. Rapid Detection and Identification of Mycobacteria by Combined Method of Polymerase Chain Reaction and Hybridization Protection Assay. *J. Infect.* 1996; 33: 71-7.
32. Stecher B, Chaffron S, Käppeli R, Hapfelmeier S, Friedrich S, Weber TC, et al. Like Will to Like: Abundances of Closely Related Species Can Predict Susceptibility to Intestinal Colonization by Pathogenic and Commensal Bacteria. *PLoS Pathog.* 2010; 6: e1000711.
33. Helps C, Reeves N, Egan K, Howard P, Harbour D. Detection of *Chlamydomytila felis* and feline herpesvirus by multi-plex real-time PCR analysis. *J. Clin. Microbiol.* 2003; 41: 2734-6.
34. Katoh K. MAFFT: A Novel Method for Rapid Multiple Sequence Alignment Based on Fast Fourier Transform. *Nucleic Acids Res.* 2002; 30: 3059-66.
35. Kumar S, Stecher G, Li M, Knyaz C, Tamura K. MEGA X: Molecular Evolutionary Genetics Analysis across Computing Platforms. *Mol. Biol. Evol.* 2018; 35: 1547-9.
36. Meyer RE. Euthanasia and Humane Killing. In *Veterinary Anesthesia and Analgesia*, 5th ed, Grimm, K.A., Lamont, L.A., Tranquilli, W.J., Greene, S.A., Robertson, S.A., Eds, Wiley Blackwell: Chichester, UK, 2015; 130-43.
37. Pore RS, Barnett EA, Barnes WC, Walker JD. *Prototheca* Ecology. *Mycopathologia.* 1983; 81: 49-62.
38. Satoh K, Ooe K, Nagayama H, Makimura K. *Prototheca cutis* sp. Nov., a Newly Discovered Pathogen of Protothecosis Isolated from Inflamed Human Skin. *Int. J. Syst. Evol. Microbiol.* 2010; 60: 1236-40.
39. Severgnini M, Lazzari B, Capra E, Chessa S, Luini M, Bordoni R, et al. Genome Sequencing of *Prototheca Zopfii* Genotypes 1 and 2 Provides Evidence of a Severe Reduction in Organellar Genomes. *Sci. Rep.* 2018; 8: 14637.
40. Thomas J, Preston N. Generalised Protothecosis in a Collie Dog. *Aust. Vet. J.* 1990; 67: 25-7.
41. Schultze AE, Ring RD, Morgan RV, Patton CS. Clinical, Cytologic and Histopathologic Manifestations of Protothecosis in Two Dogs. *Vet. Ophthalmol.* 1998; 1: 239-43.
42. Lane LV, Meinkoth JH, Brunker J, Smith SK, Snider TA, Thomas J, et al. Disseminated Protothecosis Diagnosed by Evaluation of CSF in a Dog. *Vet. Clin. Pathol.* 2012; 41: 147-52.
43. Vince AR, Pinard C, Ogilvie AT, Tan EO, Abrams-Ogg ACG. Protothecosis in a Dog. *Can. Vet. J.* 2014; 55: 950-4.
44. Carfora V, Noris G, Caprioli A, Iurescia M, Stravino F, Franco A. Evidence of a *Prototheca Zopfii* Genotype 2 Disseminated Infection in a Dog with Cutaneous Lesions. *Mycopathologia.* 2017; 182: 603-8.
45. Sonne L, de Oliveira EC, Argenta FF, Monteggia RS, Ferreiro L, Rösler U, et al. *Prototheca Zopfii* Genotype 2 Disseminated Infection in a Dog with Neurological Signs. *Ciência Rural.* 2017; 47: e20160877.
46. Ribeiro MG, Rodrigues de Farias M, Roesler U, Roth K, Rodigheri SM, Ostrowsky MA, et al. Phenotypic and Genotypic Characterization of *Prototheca Zopfii* in a Dog with Enteric Signs. *Res. Vet. Sci.* 2009; 87: 479-81.
47. Walker A, MacEwan I, Fluen T, Hardcastle M. Disseminated Protothecosis with Central Nervous System Involvement in a Dog in New Zealand. *N. Z. Vet. J.* 2022; 70: 238-43.
48. Kessell AE, McNair D, Munday JS, Savory R, Halliday C, Malik R. Successful Treatment of Multifocal Pedal *Prototheca wickerhamii* Infection in a Feline Immunodeficiency Virus-Positive Cat with Multiple Bowenoid in Situ Carcinomas Containing Papillomaviral DNA Sequences. *J. Feline Med. Surg. Open Rep.* 2017; 3: 205511691668859.
49. Mayorga J, Barba-Gómez JF, Verduzco-Martínez AP, Muñoz-Estrada VF, Welsh O. Protothecosis. *Clin. Dermatol.* 2012; 3: 432-6.
50. Todd JR, Matsumoto T, Ueno R, Murugaiyan J, Britten A, King JW, et al. Medical Phycology. 2017. *Med. Mycol.* 2018; 56: S188-204.
51. Jinatham V, Cantoni DM, Brown IR, Vichaslip T, Suwannahitatorn P, Pophuechai S, et al. *Prototheca bovis*, a Unicellular Achlorophyllous Trebouxiophyte Green Alga in the Healthy Human Intestine. *J. Med. Microbiol.* 2021; 70: 001415.
52. Hillesheim PB, Bahrami S. Cutaneous Protothecosis. *Arch. Pathol. Lab. Med.* 2011; 135: 941-4.
53. Silveira CS, Cesar D, Keating MK, DeLeon-Carnes M, Armién AG, Luhers M, et al. A Case of *Prototheca zopfii* Genotype 1 Infection in a Dog (*Canis lupus Familiaris*). *Mycopathologia.* 2018; 183: 853-58.
54. Tsuji H, Kano R, Hirai A, Murakami M, Yanai T, Namihira Y, et al. An Isolate of *Prototheca wickerhamii* from Systemic Canine Protothecosis. *Vet. Microbiol.* 2006; 118: 305-11.