Received: 4 September 2023 | Revised: 29 September 2023 | Accepted: 9 October 2023

DOI: 10.1002/vrc2.757

Vet Record CaseReports

CASE REPORT

Companion or pet animals

A dog with protothecosis in the Netherlands

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Abstract

Prototheca spp. are parasitic algae that can infect humans, cattle, cats and dogs. Although it is rarely seen, if protothecosis occurs in dogs, it is often fatal due to dissemination of the algae and a poor response to treatment. A 3-year-old, Scottish Collie dog, female, neutered, was presented with large bowel diarrhoea, weight loss and lethargy of 2 months. The dog had also experienced a short transient episode of acute horizontal nystagmus, left-sided head tilt and cervical pain 2 weeks before presentation. Histology of the colon demonstrated an erosive neutrophilic colitis with periodic acid–Schiff stain-positive structures compatible with *Prototheca* spp. confirmed using polymerase chain reaction. Treatment with nystatin (100,000 IU orally every 8 hours) was unsuccessful, and euthanasia was performed soon after diagnosis. This case report documents the first reported case of canine protothecosis in the Netherlands, and demonstrates that nystatin treatment is not successful in every canine protothecosis case.

BACKGROUND

The genus *Prototheca* spp. consists of achlorophyllic algae that are ubiquitous in the environment, particularly in organic matter with a high moisture content, such as rivers, ponds, mud, sewage and animal waste. Although very few environmental studies have been performed, *Prototheca* spp. have been found in and around cattle farms, certain deciduous trees and food such as beef, raw milk, pork, clams and crabs.[1] *Prototheca* algae have lost their photosynthetic capabilities and have evolved to parasitism, infecting both humans and animals.[1–4] As protothecal infections typically develop over months, the algae are believed to survive or evade their host's immune system. They can survive digestion by macrophages, and they can form a biofilm, resulting in an increased resistance to both the host's immune system and drugs.[4,5] Of the acknowledged 15 *Prototheca* species, six species are currently recognised as pathogens for both humans and animals: *P. wickerhamii, P. bovis, P. cutis, P. miyajii, P. ciferrii* and *P. blaschkeae*. *P. bovis*, formerly known as *P. zopfii* genotype 2 and *P. wickerhamii* are most associated with infections in both humans and animals.[1,3,4,6,7] In cattle, *P. bovis* is a well-known causative agent of mastitis, with a prevalence of 11.2% in Italian herds and an estimated 8.3% in Polish cattle.[3,8] Bovine mastitis, caused by an ascending infection through the teat orifice, is often asymptomatic but leads to a significant reduction in milk production.[9] As there is no treatment for *Prototheca*-induced mastitis and affected animals need to be eradicated, this disease has significant economic consequences.[8–10] In humans, protothecosis is quite rare, with 211 cases reported worldwide since 1964.[11] Presentation and prognosis of human protothecosis is highly dependent on the site of infection and the host's immune system. Infection is often caused by environmental contamination of a wound, leading to localised skin lesions with a favourable prognosis. However, disseminated disease is also reported in predominantly immunocompromised patients, with a mortality rate of 56%.[2,4,11] In cats, protothecosis is a very rare (sub)cutaneous skin disease affecting forehead, nose, pinnae, distal limbs or the tail base.[12] In dogs, about 75 cases have been reported in literature so far.[6,7,13–17] In contrast to the relatively mild forms in cattle, immunocompetent humans and cats, protothecosis in dogs is often fatal due to dissemination of disease and a poor response to therapy. In dogs, protothecosis is thought to arise from the ingestion of large amounts of algae, although cutaneous infections resulting from penetrating injury have been reported as well.[13,18,19] After inoculation, infection can remain localised to the gastro-intestinal tract or skin, but often a haematogenous and/or lymphatic spread occurs to well-vascularised organs, leading to vision loss, neurological symptoms, polyurea/polydipsia, osteomyelitis or diskospondylitis.[1,7,15,19–23] Diagnosis can be made by means of cytology, histology, polymerase chain reaction (PCR) or culture of representative tissue or fluid specimens.[19,24] *P. wickerhamii* and *P. bovis* are most often isolated in canine cases, with *P. bovis* being the most common isolate and associated with more aggressive disease.[6,7,18,19] Most reported cases in dogs originate from areas with a warm, humid climate, where

the algae are especially prevalent (such as for southern and south-eastern United States, north-eastern Australia, southern continental Europe, Japan). Cases in colder countries are, to the best of our knowledge, rare. Here, we present a case of canine protothecosis in a young Collie dog originating from the Netherlands.

CASE PRESENTATION

A 3-year-old Scottish Collie dog, female, neutered, was referred to the first and last author at the Evidensia Referral Hospital Arnhem with clinical signs of large bowel diarrhoea, weight loss and lethargy of 2 months. Two weeks before referral, the dog had also suffered from neurological signs; a brief period of several hours of horizontal nystagmus and left-sided head tilt. Also, the owner had observed signs of neck pain for which the dog had received non-steroidal anti-inflammatory drugs. At the time of the referral, the neurological signs and neck pain were no longer present. At referral, the diarrhoea was characterised by an increased defecation frequency, tenesmus and haematochezia. Faecal flotation and a SNAP ELISA-antigen test (IDEXX) on *Giardia* spp. were both negative. A lightly digestible diet, diarrhoea inhibitors and treatment with metronidazole (20 mg/kg every 12 hours) for 7 days prescribed by the referring veterinarian had not resulted in improvement of the diarrhoea. The dog had no relevant medical history and had not been treated with any other medication other than the one mentioned above. Vaccination and deworming were not up to date. The dog was fed a raw meat diet. The dog was walked daily in open fields, which were visited by foxes, deer and hares as well. In summertime, the dog would often paddle in a large open water pool. As the symptoms of this dog started in wintertime, it had not visited this water pool for several months when presented.

INVESTIGATIONS

Physical examination of the dog was unremarkable. Biochemistry and complete blood count were unremarkable, besides a slightly elevated C-reactive protein (CRP) of 14.8 mg/L (ref: 0–10 mg/L). Abdominal ultrasound demonstrated a mesenterial lymphadenopathy (Figure 1). Cytology revealed a nonspecific reactive lymphadenitis, at which time the dog was referred. Based on the prior work-up, the owner was advised an endoscopic evaluation of both the upper and lower gastro-intestinal tract. The dog was premedicated with dexmedetomidine (2 μg/kg; Sedadex 0.5 mg/mL) and butorphanol (0.3 mg/kg; Butomidor 10 mg/mL) intravenously (IV). Anaesthesia was induced with propofol (3 mg/kg IV; Propovet 10 mg/mL) and maintained after intubation with inhaled isoflurane. Endoscopy was performed with a Karl Storz flexible video gastroscope ('Silver Scope') 7.9 mm × 140 cm. The macroscopic appearance of both the oesophagus and stomach was normal. The duodenum showed an irregular surface with fragile villi. Endoscopic evaluation of the colon demonstrated a thickened, pale surface with multiple small bleedings. Endoscopic biopsies of stomach, duodenum and colon were collected for histology. Samples were fixed in 10% neutral buffered formalin, embedded in paraffin, and

LEARNING POINTS/TAKE-HOME MESSAGES

- This is the first published case of canine protothecosis from the Netherlands, and therefore demonstrates that this disease also occurs in Northern European countries, even in wintertime.
- In any dog with refractory colitis or multisystemic disease, especially in case of a female Collie or Boxer dog, protothecosis should be included in the differential list and representative tissue or fluid samples should be tested by means of cytology, histology, culture or polymerase chain reaction.
- Protothecosis in dogs is often fatal due to dissemination of disease and a poor response to therapy. An early diagnosis and treatment might prevent dissemination and therefore improve prognosis, as indicated by the clinical improvement of some cases of more localised disease.
- Although treatment guidelines are lacking, parenteral amphotericin B seems to be the most effective treatment in dogs, even though some dogs have benefitted from oral azoles or nystatin as well. It is important to continue reporting on this (often) fatal disease in order to establish optimal treatment protocols.

5 μm sections were stained with haematoxylin and eosin and periodic acid–Schiff stain (PAS). Histology of the stomach was unremarkable. The duodenum demonstrated a moderate increase of eosinophilic granulocytes in the lamina propria of the intestinal villi, compatible with a moderate eosinophilic enteritis. Biopsies of the colon showed multifocal erosions of the surface, along with a moderate, mixed infiltrate in the lamina propria composed of macrophages, neutrophils and lesser lymphocytes and macrophages (Figure 2). In between, and more in the superficial lamina propria, a limited number of intra- and extracellular, 4–9 μm diameter, non-budding oval spheres with a PAS-positive capsule were found (Figure 3). The occasional presence of two to four endospores inside a sporangium was considered mostly consistent with *Prototheca* spp. granulomatous to mixed colitis.[25] A PCR test on *Prototheca* spp. (Genefast SRL) confirmed this diagnosis. DNA was extracted by using a commercial kit (Maxwell RSC Blood DNA kit, Promega) with an automatic extractor (Maxwell RSC Instrument, Promega). DNA was amplified and *Prototheca* species were demonstrated according to Ricchi et al.[26]

DIFFERENTIAL DIAGNOSIS

The dog in our case was presented with chronic large bowel diarrhoea, weight loss, lethargy and transient vestibular symptoms and neck pain. Differential diagnoses for the neurological signs and neck pain were infectious, idiopathic, toxic, traumatic or neoplastic causes. Despite of the relatively high dosage, metronidazole toxicity was considered unlikely as the neurological signs had started before administration of metronidazole. As the nystagmus and neck pain were no

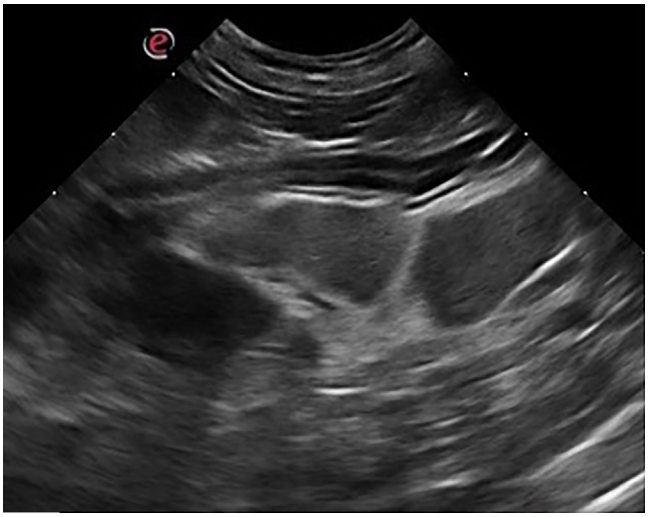

FIGURE 1 Ultrasonic presentation of the mesenterial lymphadenopathy in this dog.

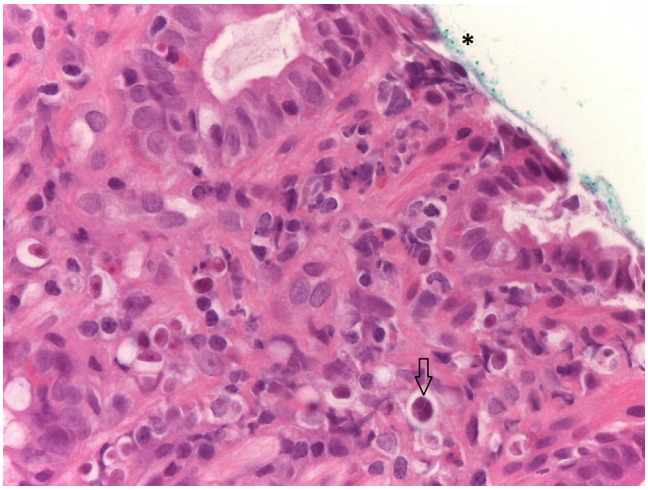

FIGURE 2 Colon, 630×, haematoxylin and eosin stain. Histiocytic and neutrophilic to mixed colitis with erosion (asterisk), and presence of multiple oval to irregular *Prototheca* spheres with a refractile capsule. There is focal endosporulation (arrow).

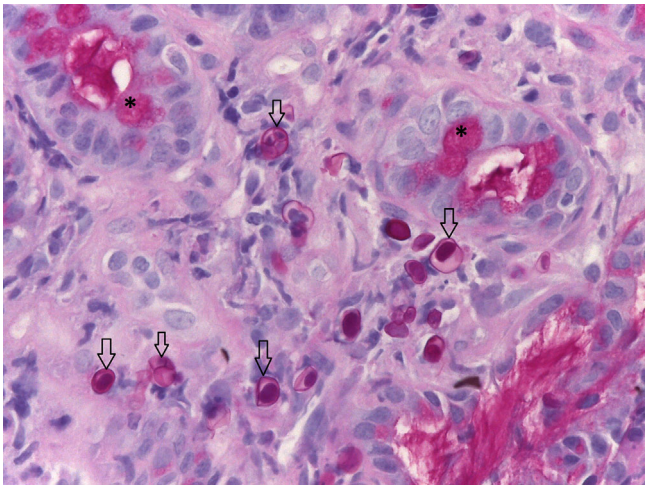

FIGURE 3 Colon, 630×, periodic acid–Schiff (PAS) stain. The capsule of sporangia is highlighted (arrows). Intestinal crypts also show PAS positivity (asterisk).

longer present at the referral, the owner asked us to focus on the large bowel diarrhoea. This was considered the main problem for work-up. As no endoparasites were found with faecal analysis and there had been no clinical response to both the dietary and metronidazole trial, an endoscopy of the proximal and distal gastro-intestinal tract was performed to differentiate between idiopathic inflammatory bowel disease, neoplastic colitis or, less likely, granulomatous colitis related to intracellular *Escherichia coli* infection (as seen with Boxers and French bull dogs).

TREATMENT

The dog was treated with nystatin (100,000 IU orally [PO] every 8 hours), as described by Ribeiro et al.[27]

OUTCOME AND FOLLOW-UP

The dog was euthanased shortly after initiation of therapy due to persisting colitis and lethargy, weakness, nausea and recurrence of mild ataxia.

DISCUSSION

We were surprised by the diagnosis of a *Prototheca* spp. colitis in this dog. Although, retrospectively, the weight loss and neurological symptoms in this dog were not typical for a primary colitis, protothecosis is very rare in dogs and infections have not been reported from the Netherlands so far. Up until now, canine cases originated from Australia, New Zealand, Japan, Canada, the United States, Brazil, Uruguay, Israel and European countries, such as Greece, Spain, Italy and England.[6,7,13–17] As *Prototheca* spp. are ubiquitous in organic matter with a high moisture content, their occurrence in the cold Dutch climate, especially in wintertime, was unexpected. In our dog, protothecosis due to ingestion of algae was considered the most likely infectious route, as the primary signs were gastro-intestinal. The outdoor lifestyle of the dog, leading to an increased exposure to environmental algae, or the raw meat diet, might have facilitated the ingestion of algae. There are several reports of dogs with chronic large bowel diarrhoea and weight loss during several months before dissemination occurs.[19,24,27,28] The clinical signs of our dog were, in retrospect, in line with these earlier studies. The transient neurological signs and the re-occurrence of mild ataxia soon after diagnosis might have been indicative of disseminated disease, which was unfortunately not confirmed with neuroimaging, liquor analysis or postmortem examination. The owner had made the request to focus on the intestinal problems, but in retrospect it would have been ideal if we could have performed additional examinations, such as a cerebrospinal fluid tap.

Besides the outdoor lifestyle and raw meat diet, this dog seemed to be predisposed to infection by both sex and breed. In one Australian study reviewing 17 *Prototheca* spp. cases, 70% were female dogs and 41% were Boxers. The same authors reviewed an additional 27 cases from outside Australia and found an increased prevalence of female dogs (63%) and

Collies (22%).[19] Collies and Boxers therefore might have an underlying genetic defect in innate immunity predisposing them to infection. In addition, as there is a higher incidence of granulomatous colitis in Boxers, colonic colonisation and invasion of *Prototheca* might be facilitated in the already diseased gut.[29] Our female Collie dog had no history of gastro-intestinal symptoms, but considering these statistics, she seemed to be predisposed to infection by both sex and breed.

Our canine patient was treated with nystatin (100,000 IU PO every 8 hours), as described by Ribeiro et al.[27] They successfully treated an 8-year-old dog with chronic colitis, hyporexia and weight loss of 2 months with nystatin prescription for a period of 90 days. Our dog unfortunately did not respond to nystatin treatment, which might have been due to the presence of more disseminated disease when compared to the case studied by Ribeiro et al. Our case therefore demonstrates that nystatin is not suitable for every canine *Protothecа* spp. infection. Nystatin is registered for dogs, and for this reason we were forced to use this medication first. If it is not effective, only at that time the so-called cascade arrangement allows us to make other choices. An early diagnosis and treatment might prevent dissemination of this disease and therefore improve prognosis. As canine protothecosis is rare, treatment is based on single case reports and general treatment recommendations are lacking. It is therefore important to continue reporting on the treatment of this remarkable disease. In humans, apart from surgical excision of localised skin lesions, oral itraconazole or fluconazole are often prescribed for mild cases. For more serious infections, or those unresponsive to oral azole treatment, intravenous amphotericin B (AMB) is considered most effective, sometimes combined with tetracyclines.[11] In dogs, variable results have been established with treatment with parenteral AMB, azoles and nystatin. Localised skin lesions were successfully treated with long-term pulse therapy with oral itraconazole in one dog, but only partially with oral fluconazole and AMB in a second dog.[13,30] Stenner et al. reported a survival of 12 and 17 months, respectively, with parenteral AMB treatment in two dogs with granulomatous colitis and a survival of at least 12 months with parenteral AMB and oral itraconazole treatment in another dog with disseminated protothecosis.[19] Oral ketoconazole seemed to prolong survival with 6 months after surgery in a dog with a spinal *Prototheca* granuloma, although this same treatment was not effective in two other dogs with disseminated disease.[15,19] Apart from the grave prognosis of protothecosis, treatment with azoles or AMB can be quite expensive, further discouraging the owner to attempt treatment. Stenner et al. established 12–17 months survival with a twice weekly subcutaneous AMB injection treatment protocol during a period of 5 weeks. This protocol is financially more feasible when compared to extensive IV treatment protocols, especially when the less expensive Fungizone formulation is available for use.[19] The addition of an azole to AMB therapy is recommended by several veterinary authors,[7,19] although this is not considered to be more effective in human medicine.[11]

AUTHOR CONTRIBUTIONS

Conceptualisation: Ilona Bontekoning and Paul Mandigers. Methodology: Ilona Bontekoning. Investigation: Ilona Bontekoning, Sofie Maes and Paul Mandigers. Data curation: Ilona Bontekoning and Sofie Maes. Writing—original draft preparation: Ilona Bontekoning. Writing—review and editing: Sofie Maes and Paul Mandigers. Visualisation: Sofie Maes. Supervision: Paul Mandigers. All authors have read and agreed to the published version of the manuscript.

ACKNOWLEDGEMENTS

We kindly thank the owner of the dog for cooperation and her help in finishing this study.

CONFLICT OF INTEREST STATEMENT

The authors declare they have no conflicts of interest.

ETHICS STATEMENT

This case report describes a spontaneous *Protothecа* spp. infection in a client-owned dog. Diagnostics were performed in consultation with the owner. This article was written with consent of the dog owner.

FUNDING INFORMATION

This research received no external funding.

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How to cite this article: Bontekoning I, Maes SMJ, Mandigers PJJ. A dog with protothecosis in the Netherlands. Vet Rec Case Rep. 2023;e757. https://doi.org/10.1002/vrc2.757

IMAGE QUIZ

Figure 2 What is visible at the asterisk and to what is the arrow pointing?

MULTIPLE-CHOICE QUESTION

This dog was suffering from clinical signs suggestive for a colitis/chronic large bowel diarrhoea. The history also revealed that there was weight loss and transient neurological signs. Is weight loss a typical clinical sign of a colitis?

POSSIBLE ANSWERS TO MULTIPLE-CHOICE QUESTION

a. Yes, this is a typical clinical sign.
b. No, it is not a typical clinical sign and is never seen.
c. It is not a typical clinical sign.

CORRECT ANSWER

c. It is not a typical clinical sign.

It is a typical clinical sign in a chronic small bowel diarrhoea, but not in a chronic large bowel diarrhoea. If it occurs in a chronic large bowel diarrhoea case, it does not exclude the often-seen ordinary colitis, but it is more suggestive for a neoplastic colitis or a severe chronic ulcerative histiocytic colitis. In this case, the final diagnosis was in line with all the clinical signs seen in this dog.