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Protothecosis in Dogs: A Narrative Review

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ABSTRACT

Protothecosis is a rare and unusual disease that affects both humans and animals, including dogs. The causative agents are unicellular, achlorophyllous, "yeast-like" microalgae of the genus *Prototheca* (Trebouxiophyceae, Chlorophyta). Although usually saprophytic, *Prototheca* may, under conditions of immunologic compromise, become pathogenic and even lethal to the host. We present a synthesis of the current literature on protothecosis, with special emphasis on disease features in the dog. Five open-access scientific journal repositories were searched two times by two independent reviewers for original studies (including case reports, standard articles, and conference abstracts) pertaining to cases of protothecosis in dogs. Findings about protothecosis cases in dogs (e.g., animal metrics, type of infection, implemented treatment, and treatment outcome) were synthesized in independent data tables. Eighty studies describing 125 cases of protothecosis in dogs qualified for final analysis. Based on this investigation, protothecosis in dogs can be defined as an emerging disease that poses a serious challenge to the veterinary profession in terms of both diagnosis and management. In general, clinical signs and physical findings most often are referable to the gastrointestinal tract ($n = 68$; 54.4%). Yet the most common clinical manifestation in dogs is disseminated systemic infection ($n = 84$; 67.2%), including clinical signs referable to inflammation affecting more than one organ. We emphasize the complexity of *Prototheca* infection in dogs by summarizing clinical and laboratory findings from 125 cases of *Prototheca* infection in dogs published over the last half-century.

Abbreviations: AMB, amphotericin B; AMC, amoxicillin-clavulanic acid, amoxicillin/clavulanate; AMP, ampicillin; AMX, amoxicillin; AT, atropine; b.i.w., twice weekly; Bi, bismuth; c.t.d., cumulative total dose; CAM, chloramphenicol; CAR, carprofen; CEX, cephalexin; CEZ, cefazoline; CIM, cimetidine; CLI, clindamycin; CLT, clotrimazole; CM, capromorelin; CNS, central nervous system; CRO, ceftriaxone; CSF, cerebrospinal fluid; CUL., culture; DA, dopamine; DB, dobutamine; DCX, deracoxib; DEC, diethylcarbamazine; DOR, dorzolamide; DOX, doxycycline; DRM, doramectin; DSP, disophenol; DXM, dexamethasone; e., enema; ENP, enalapril; ENR, enrofloxacin; F, female; FBZ, fenbendazole; FEB, febantel; FIR, firocoxib; FLC, 5-flucytosine; FLU, fluconazole; FM, flunixin meglumine; FME, flumethasone; FMT, famotidine; FRS, furosemide; GEN, gentamicin; GPT, gabapentin; H.-P., histopathology; HCT, hydrocortisone; HTC, hetacillin; i.d., intradermal administration; i.m., intramuscular administration; i.t., intrathecal administration; i.v., intravenous administration; ITZ, itraconazole; KTZ, ketoconazole; LCM, lincomycin; M, male; MALDI-TOF MS, matrix-assisted laser desorption ionization-time of flight mass spectrometry; MAR, maropitant; MEC, meclizine; MES, mesalazine; MOL., molecular; MTZ, metronidazole; ND, no data; NEO, neomycin; NOR, norfloxacin; NOV, novobiocin; NST, nystatin; OFX, ofloxacin; p.o. (*per os*), oral administration; PAP, propionibacterium acnes preparation; PCZ, posaconazole; PDN, prednisolone/prednisone; PIC, pilocarpine; PIMO, pimobendan; PMB, polymixin-B; PNC, penicillin; PON, ponazuril; PRA, pradofloxacin; PRT, pyrantel; PST, phthalylsulfathiazole; PTX, pentoxifylline; PZQ, praziquantel; q.wk., once weekly; qXh, every X hours; RES, resorcinol; RMP, rifampicin; s.c., subcutaneous administration; s.i., subcutaneous infusion; SAM, S-adenosylmethionine; SCF, sucralfate; SDZ, sulfadiazine sodium; SM, simazine; SSZ, sulfasalazine; STR, streptomycin; t., topical administration; TAC, triamcinolone acetonide; TBZ, thiabendazole; TER, terbinafine; TET, tetracycline; THS, thiostrepton; TM, timolol maleate/timolol; TMC, tobramycin; TMP, trimethoprim; TPZ, trimeprazine; TRD, tramadol; TTC, tetracycline hydrochloride; u.o. (*uterque oculus*), ophthalmic application; VRZ, voriconazole; xw, x times weekly; ZNO, zinc oxide.

1 | Introduction

Protothecosis is a sporadic disease of vertebrates. It affects both humans and animals and is caused by saprophytic, achlorophyllous, unicellular algae of the genus *Prototheca* (Trebouxiophyceae, Chlorophyta), phylogenetically closely related to the genus *Chlorella* [1]. These two genera represent the only plants (Viridiplantae) capable of infecting humans and other mammals. Whereas *Chlorella* infections are extremely rare, protothecosis appears to be an emerging disease, the incidence of which has increased steadily over the past two decades [2–4].

The history of the *Prototheca* microorganisms dates back to the end of 19th century [5]. They were originally described as yeast-like fungi because of their morphological features and absence of chlorophyll [1, 2]. The taxonomy of the *Prototheca* genus has been revised repeatedly as more phenotypic and molecular data became available [3–9]. Recently, a new classification system of the *Prototheca* algae has been established based on the partial *cytb* gene fragment as a marker [6]. In this system, 18 *Prototheca* species have been described [10, 11].

Protothecosis affects a wide range of mammalian hosts, affecting both wild and domestic animals, with important differences in clinical signs, clinical course, and outcome among host species [2, 12–17]. *Prototheca* are found ubiquitously in the environment, with a strong predilection for areas of high humidity and high organic matter content. They have been isolated from soil [18, 19], aquatic environments, sewage [11, 20], and dairy farms [15, 16]. Importantly, the risk of environmental persistence of the algae, and thus transmission to animals, is promoted by their ability to survive a wide range of temperatures, including those used for pasteurization and chemical treatments, including chlorination employed in standard water treatment practices [21, 22].

The predominant form of protothecosis in animals is mastitis in cattle, which causes heavy economic losses to the dairy industry worldwide [14, 15, 23–25]. The disease also has been reported in dogs [2, 17, 26, 27], cats [12, 13, 28–31], goats [32], horses [33], a beaver [34], and a carp [35]. Among dogs and cats, *Prototheca* infection leads to distinct clinical syndromes. In cats, disease generally is limited to cutaneous lesions after penetrating trauma, and the lesions tend to subside with appropriate topical and systemic treatment [7, 11]. In contrast, protothecosis in dogs is characterized by multiorgan involvement, refractoriness to treatment, and often a fatal outcome [2, 36].

Since the first report in 1969 [37], 125 cases of protothecal infection in dogs have been documented over the period under review. We have collated these cases, with the aim of achieving a comprehensive and accurate picture of protothecosis in dogs and exploring the potential risk factors for the disease. This comprehensive review summarizes available literature in a global context, with emphasis on current gaps in our knowledge and suggested areas for further research.

2 | Materials and Methods

2.1 | Literature Search

Five open-access scientific journal repositories, namely (i) PubMed (https://pubmed.ncbi.nlm.nih.gov), (ii) Google Scholar (https://scholar.google.com), (iii) Medline (https://www.nlm.nih.gov/bsd/pmresources.html), (iv) Web of Science (https://clarivate.com), and (v) Scopus (https://www.scopus.com) were searched two times—first in November 2019, and then again in March 2023, for any studies (e.g., peer-reviewed articles, books, and conference communications) pertaining to protothecosis in dogs. A case of protothecosis was defined as any case description with *Prototheca* identification using either histopathology, microbiology, or molecular testing. The form of the disease was determined based on the site of infection (e.g., skin infection, enteric infection, nervous system infection). The reference lists of all papers were further searched to detect additional papers on protothecosis in dogs.

2.2 | Study Selection

Two authors independently screened and extracted the eligible literature for inclusion, using the following keywords, their combinations, and their French, German, Italian, Polish, and Spanish equivalents: "*Prototheca*," "protothecosis," "canine," and "dog." Studies initially were assessed for their relevance. Every original study reporting cases of protothecosis in dogs was included. Only "literature review" studies were excluded, unless they also provided new, previously unreported cases of the disease in dogs. Finally, careful attention was directed so that cases were not duplicated. If two articles reported the same case, such was noted by assigning more than one reference (Table S1). Those articles were identified as follows: (i) when the author clearly indicated the inclusion of a case from the previous article, or (ii) when the same author described a case the details of which were identical to the case reported previously.

2.3 | Data Extraction

The extracted data were entered into MS Excel by two independent authors, crosschecked, and checked by the third author. Any discrepancies were resolved after data extraction. The spreadsheet was designed to record relevant information under appropriate (sub-) categories and to facilitate the exportation of the data to software for numerical analysis (Table S1).

Information collected for dogs with protothecosis included breed, sex, age, country of origin, and medical history, including details of *Prototheca* infection (e.g., clinical features, diagnostic methods, imaging, treatment), and any underlying conditions that could have influenced the immunologic status of the dog. Definitions and explanations of all data (sub-) categories were provided as a legend in Tables 1 and S1.

The compounds used in the treatment of protothecosis were categorized in terms of their chemical structure (alkaloid,

TABLE 1 | General characteristics of dogs under the study.

Characteristic		Type of protothecosis[i]				
		Cutaneous (*n* = 9)	**Neuroinfection (*n* = 6)**	**Systemic (*n* = 84)**	**Other (*n* = 26)**	**Total (*n* = 125)**
Breed[a]	Boxer	1 (11.1%)	4 (66.6%)	7 (8.3%)	3 (11.5%)	15 (12%)
	Cocker Spaniel	0 (0%)	0 (0%)	5 (6%)	1 (3.9%)	6 (4.8%)
	German Shepherd	0 (0%)	0 (0%)	4 (4.8%)	4 (15.4%)	8 (6.4%)
	Giant Schnauzer	0 (0%)	0 (0%)	3 (3.6%)	1 (3.9%)	4 (3.2%)
	Labrador Retriever	0 (0%)	1 (16.7%)	6 (7.1%)	5 (19.2%)	12 (9.6%)
	Mixed Breed	1 (11.1%)	1 (16.7%)	23 (27.4%)	3 (11.5%)	28 (22.4%)
	Rough Collie	1 (11.1%)	0 (0%)	7 (8.3%)	2 (7.7%)	10 (8%)
	Other*	6 (66.7%)	0 (0%)	29 (34.5%)	7 (26.9%)	42 (33.6%)
Sex[b]	F	2 (22.2%)	4 (66.6%)	52 (61.9%)	6 (23%)	64 (51.2%)
	M	3 (33.3%)	2 (33%)	21 (25%)	19 (73.1%)	45 (36%)
	ND	4 (44.5%)	0 (0%)	11 (13.1%)	1 (3.9%)	16 (12.8%)
Age[c]	Puppy (0 to < 1 years)	0 (0%)	0 (0%)	2 (2.4%)	2 (7.7%)	4 (3.2%)
	Adult (≥ 1 to < 7 years)	6 (66.7%)	6 (100%)	56 (66.6%)	12 (46.1%)	80 (64%)
	Senior (≥ 7 to < 12 years)	1 (11.1%)	0 (0%)	22 (26.2%)	7 (26.9%)	30 (24%)
	Geriatric (≥ 12 years)	1 (11.1%)	0 (0%)	2 (2.4%)	1 (3.9%)	4 (3.2%)
	ND	1 (11.1%)	0 (0%)	2 (2.4%)	4 (15.4%)	7 (5.6%)
	Range	1.5–13	2–6	3 months to 14	3 months to 12	3 months to 14
	Mean	5.8	4.6	5.3	5.1	5.1
	Median	4.5	4.8	4	5	4
Continent	Africa	0 (0%)	0 (0%)	1 (1.2%)	0 (0%)	1 (0.8%)
	Asia	1 (11.1%)	1 (16.7%)	4 (4.8%)	4 (15.4%)	10 (8%)
	Australia	1 (11.1%)	1 (16.7%)	18 (21.4%)	2 (7.7%)	22 (17.6%)
	Europe	6 (66.7%)	2 (33.3%)	23 (27.4%)	6 (23%)	37 (29.6%)
	North America	0 (0%)	2 (33.3%)	34 (40.4%)	12 (46.1%)	48 (38.4%)
	South America	1 (11.1%)	0 (0%)	4 (4.8%)	1 (3.9%)	6 (4.8%)
	ND	0 (0%)	0 (0%)	0 (0%)	1 (3.9%)	1 (0.8%)
Treatment outcome[d]	Success	5 (55.6%)	0 (0%)	1 (1.2%)	1 (3.9%)	7 (5.6%)
	Failure	1 (11.1%)	3 (50%)	47 (56%)	9 (34.6%)	60 (48%)
	No treatment	0 (0%)	2 (33.3%)	6 (7.1%)	1 (3.9%)	9 (7.2%)
	ND	3 (33.3%)	1 (16.7%)	30 (35.7%)	15 (57.6%)	49 (39.2%)

(Continues)

TABLE 1 | (Continued)

Characteristic			Type of protothecosis[i]				
			Cutaneous ($n=9$)	Neuroinfection ($n=6$)	Systemic ($n=84$)	Other ($n=26$)	Total ($n=125$)
Etiology[e]	*Prototheca* sp.		1 (11.1%)	2 (33.3%)	32 (38.1%)	21 (80.7%)	56 (44.8%)
	*P. bovis**		1 (11.1%)	0 (0%)	12 (14.3%)	1 (3.9%)	14 (11.2%)
	*P. ciferrii***		0 (0%)	0 (0%)	2 (2.4%)	0 (0%)	2 (1.6%)
	P. wickerhamii		7 (77.8%)	0 (0%)	9 (10.7%)	0 (0%)	16 (12.8%)
	*P. zopfii****		0 (0%)	4 (66.7%)	28 (33.3%)	4 (15.4%)	36 (28.8%)
	P. zopfii and *P. wickerhamii*****		0 (0%)	0 (0%)	1 (1.2%)	0 (0%)	1 (0.8%)
Diagnostic method[f]	Histopathology	POS.	8 (88.9%)	5 (83.3%)	75 (89.3%)	26 (100%)	114 (91.2%)
		NEG.	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
		ND	1 (11.1%)	1 (16.7%)	9 (10.7%)	0 (0%)	11 (8.8%)
	Culture	POS.	8 (88.9%)	3 (50%)	48 (57.1%)	2 (7.7%)	61 (48.8%)
		NEG.	0 (0%)	1 (16.7%)	4 (4.8%)	1 (3.9%)	6 (4.8%)
		ND	1 (11.1%)	2 (33.3%)	32 (38.1%)	23 (88.4%)	58 (46.4%)
	Molecular	POS.	6 (66.7%)	2 (33.3%)	25 (29.8%)	3 (11.5%)	36 (28.8%)
		NEG.	0 (0%)	0 (0%)	2 (2.4%)	0 (0%)	2 (1.6%)
		ND	3 (33.3%)	4 (66.7%)	57 (67.8%)	23 (88.5%)	87 (69.6%)
Underlying disease[g]	Yes		0 (0%)	2 (33.3%)	11 (13.1%)	1 (3.9%)	14 (11.2%)
	ND		9 (100%)	4 (66.7%)	73 (86.9%)	25 (96.1%)	111 (88.8%)
Source of infection[h]	Specified		2 (22.2%)	3 (50%)	10 (11.9%)	3 (11.5%)	18 (14.4%)
	ND		7 (77.8%)	3 (50%)	74 (88.1%)	23 (88.5%)	107 (85.6%)

[a]Other: American Staffordshire Terrier ($n=1$); Australian Cattle Dog ($n=1$); Bernese Mountain ($n=1$); Border Terrier ($n=1$); Bull Arab ($n=1$); Corgi ($n=1$); Dachshund ($n=2$); Dalmatian ($n=1$); Dobermann ($n=3$); English Setter ($n=1$); French Bulldog ($n=2$); German Shorthaired Pointer ($n=1$); Golden Retriever ($n=1$); Gordon Setter ($n=1$); Greyhound ($n=1$); Hungarian Vizsla ($n=2$); Maltese ($n=3$); Maremma Sheepdog ($n=2$); Miniature Schnauzer ($n=1$); Poodle ($n=1$); Rhodesian Ridgeback ($n=3$); Samoyed ($n=1$); Scottish Shepherd ($n=1$); Setter Llewellin ($n=1$); Siberian Husky ($n=2$); Springer Spaniel ($n=2$); Staffordshire Bull Terrier ($n=1$); Yorkshire Terrier ($n=1$); ND = no data ($n=2$).
[b]F = female; M = male; ND = no data.
[c]ND = no data.
[d]Success = cure/improvement; Failure = death/euthanasia/treatment failure; ND = no data about treatment or treatment outcome.
[e]*Included four cases diagnosed as *P. zopfii* gen. 2 and one case diagnosed as *P. zopfii* var. *hydrocarbonea* = now *P. bovis*; **included one case diagnosed as *P. zopfii* gen. 1; ***Species designation which most probably includes species that currently would be identified as *P. ciferrii* or *P. bovis*; ****case diagnosed with two different *Prototheca* species (i.e., *P. zopfii* and *P. wickerhamii*).
[f]POS = positive outcome of diagnostic method for *Prototheca* sp.; NEG. = negative outcome of diagnostic method for *Prototheca* sp.; ND = no data of performed assay.
[g]Yes = underlying disease was indicated in the article; ND = underlying disease was not diagnosed/no data.
[h]ND = no data (unspecified).
[i]Other = enteric infection ($n=13$), eye infection ($n=11$), heart infection ($n=2$); neuroinfection ($n=4$); neuroinfection + eye infection ($n=2$).

allylamine, aminocoumarin, aminoglycoside, polyene, ansamycin, azole, beta-lactam, catecholamine, chloramphenicol, corticosteroid, fluoroquinolone, lincosamide, oligopeptide antibiotic, polymyxins, sulfonamide, tetracycline, and other), type of drug (antibiotic, azole, herbicide, steroidal and non-steroidal anti-inflammatory drug, sulfonamide, and other), and biological activity (antibacterial, antifungal, anti-inflammatory, antiparasitic, antiprotozoal, and other). The drug categorization was based on data excerpted from the PubChem database (https://pubchem.ncbi.nlm.nih.gov). Based on the extracted data, summary statistics were performed (percentage, mean, median, and range) and presented in Table 1.

3 | Results

The first case of *Prototheca* sp. infection in a dog was observed in 1963 in the United States (USA), but not published until 1969 [37] (no. 1; Table S1). Since then, up until 2023, 79 reports have appeared, describing a total of 124 cases of protothecosis in dogs. It is noteworthy that the number of new cases has been steadily increasing over the decades, with 10 cases in the 1990s, 29 cases in the 2000s, 43 in the 2010s, and 23 between 2020 and 2023 (Figure 1). We summarize those 125 described cases of protothecal infections in dogs. All reports with complete data sets are listed in Table S1.

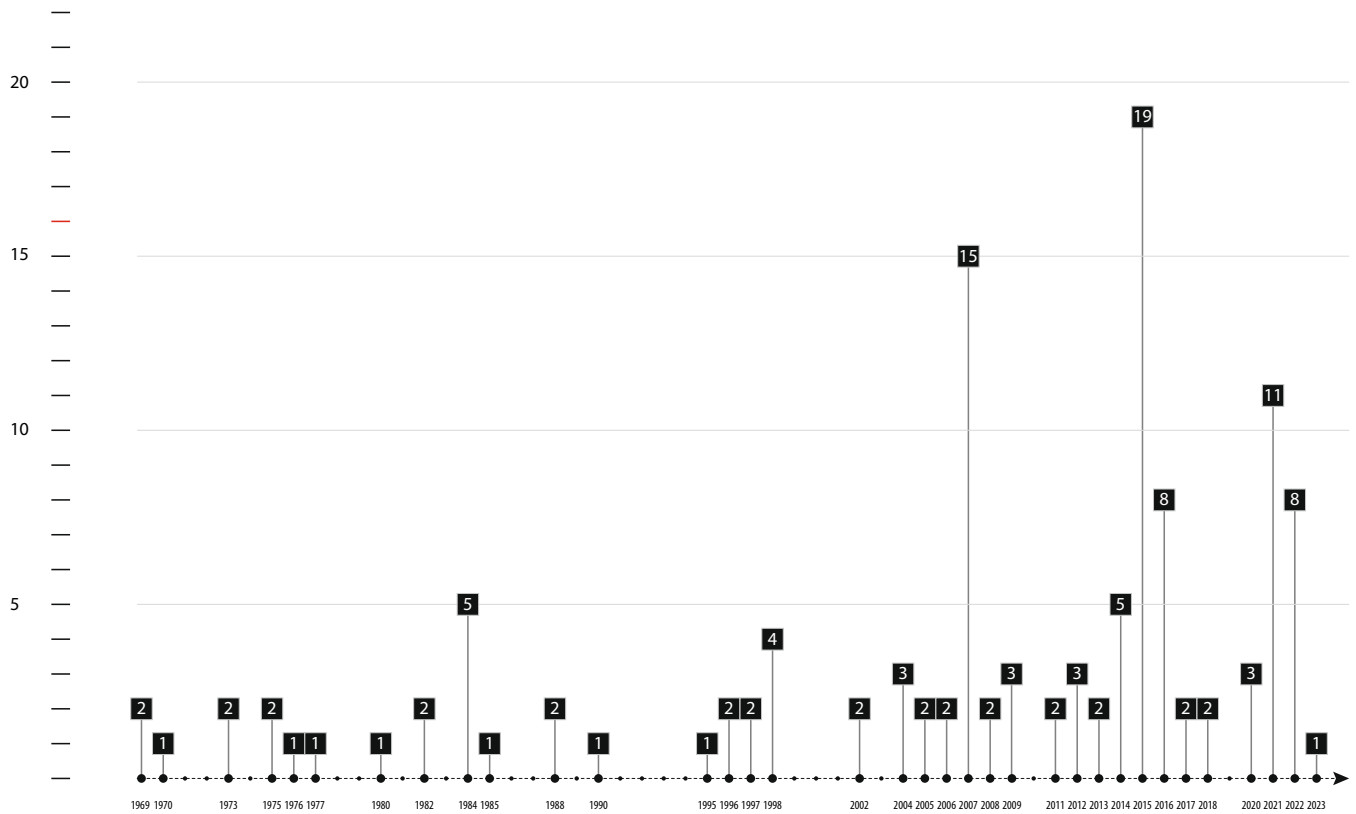

FIGURE 1 | Number of cases of protothecosis in dogs published in the literature over time (1969–2023).

Geographically, the cases covered 21 countries located on all continents except Antarctica. The largest proportion ($n = 48$; 38.4%) of the cases originated from North America. A third of the cases came from Europe ($n = 37$; 29.6%), and almost as many were scattered across Australia ($n = 22$; 17.6%), Asia ($n = 10$; 8%), South America ($n = 6$; 4.8%), and Africa ($n = 1$; 0.8%). In one case, it was impossible to identify the provenance ($n = 1$; 0.8%; Figure 2).

The disease mostly affected females (females: $n = 64$; 51.2% vs. males: $n = 45$; 36% and no data: $n = 16$; 12.8%) and pedigreed dogs ($n = 95$; 76%). However, purebred dogs may have been tested more frequently. Most cases were medium-sized to large breeds, perhaps reflecting the propensity of these breeds to interface more with the environment. Boxers were most commonly observed ($n = 15$; 12%), followed by Labrador Retrievers ($n = 12$; 9.6%), Rough Collies ($n = 10$; 8%), German Shepherds ($n = 8$; 6.4%), Cocker Spaniels ($n = 6$; 4.8%), and Giant Schnauzers ($n = 4$; 3.2%). Mixed breeds accounted for approximately a fifth of the cases ($n = 28$; 22.4%; Table 1).

Most of the dogs ($n = 80$; 64%) were adults (≥ 1 to < 7 years), whereas a quarter ($n = 30$; 24%) were older (≥ 7 to < 12 years). Both puppies (0 to < 1 year) and geriatric dogs (≥ 12 years) were equally represented by four animals each ($n = 4$; 3.2%). The age of seven ($n = 7$; 5.6%) dogs could not be determined. The overall median age at the time of diagnosis was 4 years (range, 3 months to 14 years; mean, 5.3 ± 3.1; Table 1).

The disease was manifested in one of six forms: (i) systemic ($n = 84$; 67.2%), defined by involvement of more than one organ; (ii) enteric ($n = 13$; 10.4%); (iii) ocular infection ($n = 11$; 8.8%); (iv) cutaneous ($n = 9$; 7.2%), (v) nervous system infection ($n = 6$; 4.8%) or (vi) myocardial infection ($n = 2$; 1.6%).

Most ($n = 111$; 88.8%) of the dogs had no mention of underlying or comorbid diseases in their clinical histories. Less than one-third (28.6%) of the animals were affected with co-existing conditions and presented with more severe health conditions (e.g., adenocarcinoma and lower respiratory tract disease). The most common comorbid disorder was a parasitic infection caused by either nematode species or other (unspecified) endoparasites (Tables 1 and S1).

Possible sources of infection were surmised for 18 cases (14.4%). The transmission was speculated to have occurred through either direct contact with the environment or other animals ($n = 15$; 83.3%) or during surgery ($n = 3$; 16.7%). In general, clinical signs and physical findings were most often referable to the gastrointestinal tract ($n = 68$; 54.4%), yet the exact anatomical site sampled was not always recorded. For 33 cases (48.5%), alimentary involvement was confirmed based on more than a single specimen, collected from different gastrointestinal tissues at the same time-point. Of 111 gastrointestinal samples positive for *Prototheca* spp., 33 (29.7%) were collected from colon, 31 (27.9%) from rectum, 24 (21.7%) from liver, 9 (8.1%) from pancreas, 8 (7.2%) were fecal samples, 3 (2.7%) from small intestine, and 3 (2.7%) from stomach.

The most prevalent form of protothecosis was a systemic disease, which accounted for 67.2% ($n = 84$) of the cases. In this kind of infection, among the organs and fluids most commonly involved were eyes ($n = 37$; 44%), kidneys ($n = 35$; 41.7%), heart ($n = 25$; 29.8%), colon ($n = 25$; 29.8%), liver ($n = 24$;

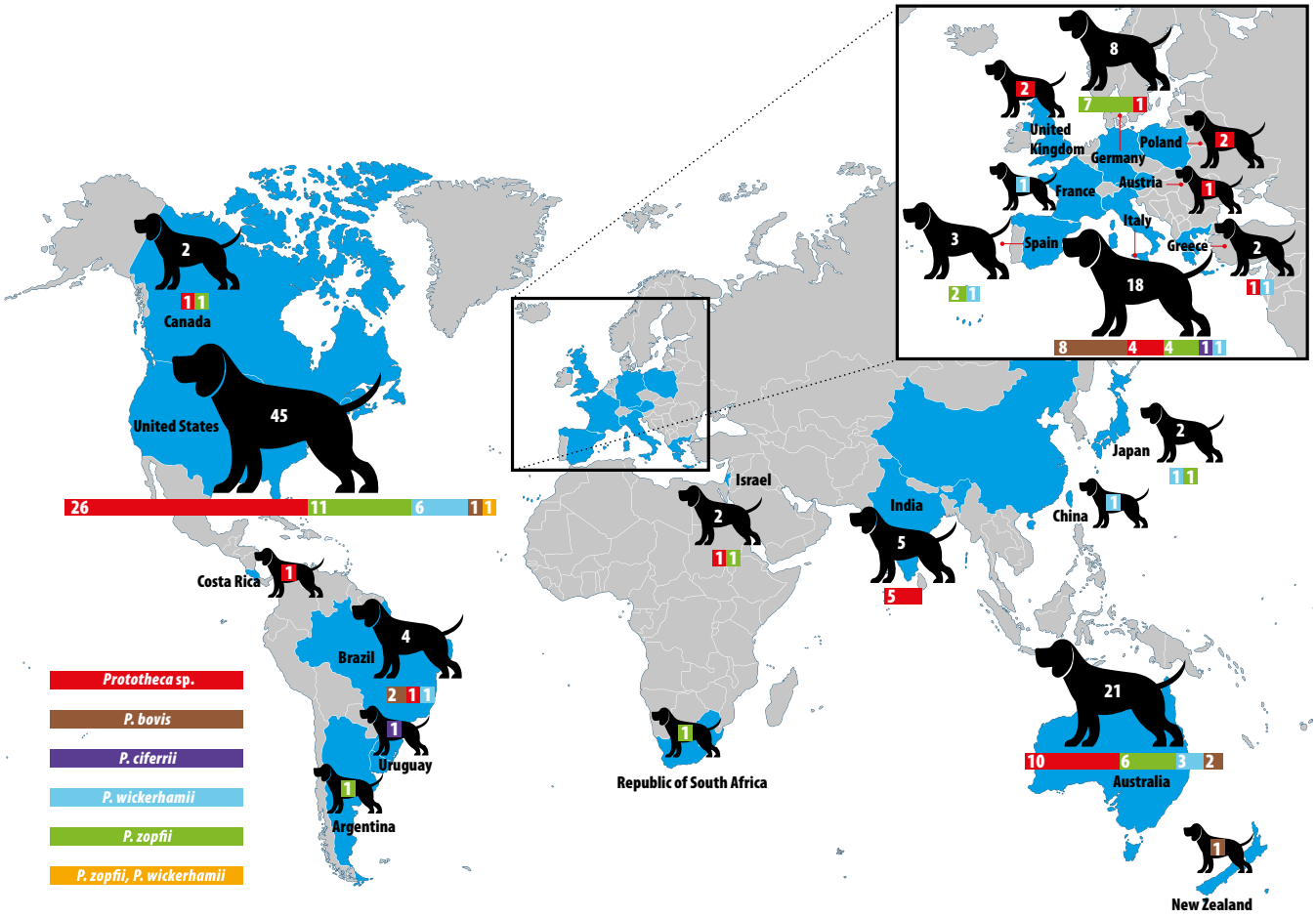

FIGURE 2 | Geographical distribution of all published cases of protothecosis in dogs from 1969 to 2023.

28.6%), rectum ($n = 23$; 27.4%), brain ($n = 22$; 26.2%), cerebrospinal fluid (CSF; $n = 10$; 11.9%), and lungs ($n = 9$; 10.7%). Interestingly, of 25 cases of systemic protothecosis involving colonic infection, 15 (60%) initially were presented with large bowel signs that progressed over time (from 1 month to 2 years).

Other forms of *Prototheca* disease were less common. Enteric infections were confirmed in 13 (10.4%) dogs by examination of the following samples: colon ($n = 8$; 61.5%), rectal scrapings ($n = 5$; 38.5%), small intestine ($n = 2$; 15.4%), or feces ($n = 1$; 7.7%). Ocular involvement alone was observed in 11 (8.8%) dogs. Among these, bilateral and unilateral involvement was equally common (both eyes: $n = 5$ or 45.4%; right eye: $n = 4$ or 36.4%; left eye: $n = 1$ or 9.1%; and no data [ND]: $n = 1$ or 9.1%).

In nine dogs (7.2%), the disease was manifested as cutaneous lesions, with biopsy samples taken from affected skin ($n = 5$; 55.6%), cutaneous nodules ($n = 3$; 33.3%), or nasal mucosa ($n = 1$; 11.1%). Only six (4.8%) cases were diagnosed as neural infections (i.e., infection of the central nervous system [CNS]), with clinical samples collected from CSF ($n = 4$; 66.7%), brain ($n = 3$; 50%), or spinal cord ($n = 2$; 33.3%). Finally, in two (1.6%) dogs, the disease was confined to the heart alone. We also found a possible association between the type of infection and *Prototheca* species. Whereas cutaneous infections were primarily linked to *P. wickerhamii* (80%), systemic disease was associated with species other than *P. wickerhamii*, namely *P. bovis*, *P. ciferrii*, or *P. zopfii* (48.8% combined).

Overall, the diagnosis was obtained by histopathological examination of representative biopsy tissue ($n = 114$; 91.2%), culture ($n = 61$; 48.8%), molecular testing ($n = 36$; 28.8%) or some combination of these. Speciation was guided by a variety of methods, including auxanographic assays (carbohydrate and alcohol assimilation profiling) and immunofluorescent antibody testing ($n = 23$; 18.4%). In nearly half of the cases ($n = 56$; 44.8%), identification of the etiologic agent was done at the genus level only. In 36 (28.8%) cases, the pathogen was described as *P. zopfii*, which most probably would now be identified as either *P. bovis* or *P. ciferrii* [10]. In six (4.8%) cases, the same species (*P. zopfii*) was found, but further genotyped as genotype 1 ($n = 1$; 0.8%) or 2 ($n = 5$; 4%), which now are referred to as *P. ciferrii* and *P. bovis*, respectively. The genotyping results for two of those cases were further confirmed (cases nos. 57 and 100; Table S1) [38]. In two case reports [27, 39], the current nomenclature was used, resulting in the identification of nine strains as *P. bovis* (7.2%) and one (0.8%) as *P. ciferrii*. The species identity of four of those strains was confirmed (cases nos. 108, 110, 111, and 113; Table S1) [38]. In 16 (12.8%) dogs, *P. wickerhamii* was the causative species, which later was confirmed in four cases (cases nos. 39, 109, 114, and 125; Table S1) [38]. Interestingly, in one dog, two different species, namely *P. zopfii* and *P. wickerhamii*, were identified upon

fluorescent antibody studies performed on the brainstem, cerebellum, and kidneys (no. 10; Table S1).

Treatment was attempted in 69 (55.2%) dogs, but only half ($n = 35$; 50.7%) of the cases were correctly diagnosed before or during initial treatment, with a subsequent change of drug regimen. For the remainder, the identification of the causative organism was made long after empirical and symptomatic medication had been instituted, typically at necropsy examination ($n = 21$; 30.4%). Most animals ($n = 55$; 79.7%) received different anti-infectives with either antibacterial ($n = 51$; 92.7%) or antifungal ($n = 15$; 27.3%) activity. Corticosteroids also were frequently prescribed ($n = 41$; 59.4%), usually in treatments integrating three or more different medications ($n = 37$; 90.2%). Typically, corticosteroids were combined with antibiotics ($n = 39$; 95.1%) and azoles ($n = 21$; 51.2%). Among dogs treated with antibiotics, enrofloxacin (ENR) was the most commonly administered drug ($n = 17$; 30.9%), followed by amphotericin B (AMB; $n = 11$; 20%), and doxycycline (DOX; $n = 9$; 16.4%). Treatments often included azoles ($n = 36$; 52.2%), with itraconazole (ITZ) being used most frequently ($n = 21$; 58.3% of all dogs treated with azoles), followed by ketoconazole (KTZ; $n = 11$; 30.6%), and metronidazole (MTZ; $n = 11$; 30.6%). Fenbendazole (FBZ) and clotrimazole (CLT) were used in four (11.1%) and three (8.3%) dogs, respectively. Other azoles (fluconazole [FLU], posaconazole [PCZ], thiabendazole [TBZ], and voriconazole [VRZ]) were used in single cases. Overall, treatment options usually consisted of combined regimens involving three or more different medications ($n = 49$; 71%).

Of 67 (53.6%) dogs that received treatment and for which the outcome was reported, as many as 60 (89.6%) did not respond to any drugs and ultimately were euthanized ($n = 37$; 61.7%) or died ($n = 22$; 36.7%; Tables 1 and S1). One dog (1.7%) survived, but the outcome was described as a failure because there were no signs of improvement (no. 103; Table S1). The overall success rate was very low ($n = 7$; 10.4%): three dogs improved while on treatment and four were apparently cured. For two (2.9%) dogs, in which treatment was undertaken, the outcome was not reported (nos. 37 and 102; Table S1).

Among 82 dogs that died or were euthanized, 64 (78%) suffered from disseminated systemic infection. However, the highest mortality was recorded for cases with confirmed nervous system infection, where treatment often was abandoned, and euthanasia was undertaken for animal welfare reasons ($n = 5/6$; 83.3%; Table S1). In contrast, for cutaneous protothecosis, the mortality rate was low ($n = 1/9$; 11.1%). Also, the best treatment responses were observed in dogs with cutaneous protothecosis, and more than half ($n = 5/9$; 55.6%) of these cases achieved a favorable outcome (Tables 1 and S1).

4 | Discussion

In light of our review, protothecosis can be described as a sporadic but devastating pathological process, which typically manifests as primary colitis, progressing over time after vascular invasion to a disseminated systemic infection with multiple organ involvement, poor response to treatment, and high mortality.

Protothecosis affects dogs on every continent, particularly in North America and Europe, which together account for nearly 70% of the global caseload. The disease can be diagnosed in any dog, regardless of age, sex, or breed. Although protothecosis in dogs appears to be extremely rare, its true prevalence is likely to be underestimated because of underreporting and under- or mis-diagnosis, associated with generally poor clinical awareness of the condition. The low number of cases of protothecosis in dogs contrasts with an extensive environmental reservoir of the *Prototheca* algae, which is considered as the primary source of infection for other animals and humans [3, 15, 16, 23, 40–44]. Environmental transmission is particularly relevant for the spread of *Prototheca* mastitis among dairy herds. The housing and milking environments of cows serve as the main source from which infection originates [13, 14, 22]. Among 125 cases of protothecosis in dogs evaluated in our study, only 15 were clearly linked to the environment or other animals. Still, given that dogs often encounter contaminated objects and excretions, especially in rural areas, combined with the fact that the infection, at its early stage, is usually associated with mild signs of large bowel dysfunction, *Prototheca*-infested water and soil may be an important source of infection. Without doubt, the fecal-oral route is the most plausible mode of transmission.

Besides heavy environmental exposure, underlying diseases that potentially compromise the immune system of the host may be a factor conducive to the development of *Prototheca* infection. Alterations of immunologic status, because of neoplastic disease or exposure to immunosuppressive drug regimens have been identified in many human patients, particularly those with systemic disease [3, 44–48]. Such association has not been conclusively demonstrated in animals. In cattle, various farm management deficiencies, including poor hygiene of housing, feeding, and milking, as well as heavy and unaddressed environmental contamination, have been recognized as potential risk factors for protothecal mastitis rather than an underlying deterioration of the animals' health [15, 16, 23]. In cats, only one individual with immunological impairment, associated with feline immunodeficiency virus and papillomavirus type 2 infections, has been described [28]. In our analysis, we did not observe any important co-morbidities.

Interestingly, the disease primarily affected pedigreed dogs. It is commonly observed that purebred dogs are at a higher risk for some diseases, including infectious diseases compared with mixed-breed dogs, typically related to genetic or inherited disorders [49–51]. Some studies suggest that specific breeds or groups of breeds may be more prone to develop certain infections [52, 53]. Likewise, it has been shown that Boxers, French Bulldogs, and their hybrids (breeds most frequently diagnosed with protothecosis) have a higher predisposition for granulomatous colitis associated with mucosal colonization and invasion by adhesive *Escherichia coli* strains compared with other breeds [53]. Thus, we suspect that there might be a common underlying defect that makes these breeds vulnerable to colonization, particularly of the colon.

Protothecosis is an uncommon clinical entity that can pose a diagnostic challenge because of unfamiliarity. In it is early stages, it gives rise to signs of colitis such as straining,

excessive mucus, and sometimes fresh blood in the feces. Unfortunately, nonspecific colitis and large bowel diarrhea are commonly seen in dogs for a variety of reasons. Thus, in cases of protothecal colitis, much valuable time often is wasted treating clinical signs symptomatically, without obtaining a definitive diagnosis. This delay may give the organism the opportunity to invade the colonic venules and subsequently disseminate widely to give rise to systemic infection. A further problem is that *Prototheca* spp. organisms mimic yeasts in stained smears and needle aspirates, and this characteristic organism morphology is not invariably recognized as pathogenic by the primary clinician or diagnostic laboratory. Although conventional cytology (e.g., rapid stains such as DiffQuik and Gram stain) can easily detect *Prototheca* organisms in a variety of clinical specimens (e.g., rectal scrapings, skin lesions, fine needle aspirates, cytospin preparations of CSF, urine sediment), it may not always capture these distinctive features (i.e., pathognomonic morphology of moruloid sporangia), resulting in misidentification [54, 55]. The algae thus can be mistakenly identified as fungi that have yeast or yeast-like forms in mammalian tissues, such as *Blastomyces* spp., *Coccidioides* spp., *Candida* spp., or *Cryptococcus* spp. [3, 44, 54–56] These fungi can be easily differentiated from *Prototheca* algae both morphologically (in smears) and on culture, especially in laboratories equipped with matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS) technology. Thus far, MALDI-TOF MS has been successfully used for species identification in four dogs [57–59]. For dimorphic fungi, yeast-to-hyphal morphology transition, which can be induced by temperature, is useful diagnostically, and cryptococcal species usually have a prominent capsule. Moreover, infections caused by dimorphic fungi are typically airborne-transmitted and thus produce respiratory signs, which are rare in protothecosis [26, 60–65].

Of the 18 currently recognized *Prototheca* species, only seven have been associated with infections in humans and animals. Six species (*P. wickerhamii*, *P. blaschkeae*, *P. ciferrii*, *P. cutis*, *P. miyajii*, and *P. bovis*) have been recognized as pathogens in humans, with *P. wickerhamii* being responsible for most cases. In animals, the spectrum of pathogenic species is equally wide, with six species identified. Different species appear to show some degree of host predilection. In cats, only three species have been shown to be the causative agents, with *P. wickerhamii* demonstrated in all but two cases [12, 28, 29, 40, 66, 67], with these two cases being caused by *P. bovis* and *P. cutis* [30, 31]. Whereas *P. bovis* has been responsible for the majority of mastitis cases in cows, *P. blaschkeae*, *P. ciferrii*, and *P. wickerhamii* also have been reported [14, 15, 24, 25]. Four species (*P. bovis*, *P. ciferrii*, *P. wickerhamii*, and *P. zopfii*) have been implicated in infections of dogs, with *P. zopfii* accounting for 29.6% of them. The species name *P. zopfii* however recently has been changed, and all strains that hitherto were referred to under this name would now most probably be assigned to either *P. bovis* or *P. ciferrii*, according to the *Prototheca* taxonomic revision [10]. Interestingly, different *Prototheca* species showed possible predilections for different types of infection. Cutaneous infections largely were caused by *P. wickerhamii*, whereas systemic disease was generally attributable to other species.

The potential host specificity of *Prototheca* algae might reflect local adaptation to the most abundant host species in each environmental niche. This specificity also may relate to behavioral differences among host species. For instance, cats drink much less water and are more fastidious about where they drink water than dogs. Given, however, the striking contrast between the environmental ubiquity of the algae and the rarity of *Prototheca* infections, the host-pathogen associations may be far more complex, and may include genetic make-up and stress exposure [68]. Intra-species differences in the expression of pathogenicity-related genes also have been demonstrated, suggesting that even greater inter-species variations exist, potentially influencing host preferences and interactions [69]. The genetic background of the canine host also might influence the ability of different *Prototheca* species to infect a specific anatomical location. This protothecal tropism towards specific organs and tissues may explain the possible association of some *Prototheca* species with certain clinical forms, as described above (e.g., *P. wickerhamii* and cutaneous infection).

The overall cure rate for protothecosis in dogs is very low, calculated at 10.4%, with marked variation depending on the clinical manifestations, exceeding 83% in dogs with cutaneous lesions and as low as 2% in dogs with systemic disease. This pattern replicates the situation in cases of protothecosis in humans, where skin infections had a relatively high success rate (73%), whereas disseminated infection had the worst (33%) [4].

Treatment regimens that produced a positive outcome mostly incorporated at least two drugs, usually of different classes. Out of five such cases, two received KTZ and three ITZ (in two cases in combination with AMB). Only in two instances, did a single drug (KTZ or NST) lead to complete recovery of the dog.

Surgery as a treatment for protothecosis in dogs was performed only in one case of skin infection, leading to a complete cure with no signs of relapse. In cats, in which the protothecal disease is usually limited to the skin and subcutis, the lesions resolved whenever surgery was employed [12, 29, 66]. In humans, surgical excision as a sole treatment was attempted, mostly in patients with olecranon bursitis or skin infections, yielding a high success rate (89%) [4].

In the search for more efficient treatments against *Prototheca* algae, new potential agents have been evaluated, including nanoparticles, iodinated carbamates, guanidine, or 3-bromopyruvate [70–74]. Although early, laboratory-based results are promising, the in vivo efficacy and safety of these new agents have yet to be established. The use of orally active cochleated amphotericin B (CAMB) formulations [75, 76] and renal-sparing polyene antifungals [77] also may prove very helpful in the management of invasive systemic protothecosis in the future.

To conclude, protothecosis in dogs is an important infectious disease that generally is characterized by an insidious onset, aggressive clinical course, and high mortality. Early clinical signs are vague and nonspecific, and the diagnosis often is delayed or confused with other causes of colitis during the initial diagnostic evaluation. Poor clinical awareness of protothecosis as a

differential diagnosis may further contribute to the rarity of this diagnosis.

Among the clinical signs that should particularly alert the clinician are acute or chronic, hemorrhagic large bowel diarrhea, progressive impairment of vision, cranial nerve palsies, and other neurologic signs. Even small and benign-appearing skin lesions (e.g., nodules, ulcers) must not be dismissed but closely inspected and preferably biopsied for histopathology and microbiological culture. Finally, one should not neglect taking a careful history, which can facilitate the diagnosis of protothecosis, and address not only the clinical history but also information about the dog's behavior and its outdoor environment.

A limitation of our study is the relatively small number of cases of dogs diagnosed with protothecosis. Additionally, not all studies provided detailed descriptions of the animals and the clinical course of their disease. These factors should be taken into consideration when making comparisons among studies. Because *Prototheca* spp. have long remained on the margin of clinical and scientific interest, knowledge gaps exist concerning their biology and pathogenicity, in particular. Our narrative review highlights the problem of *Prototheca* infections in canine medicine, urges special attention to the disease, and makes a strong case for more extensive research in this field.

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Disclosure

The authors declare no off-label use of antimicrobials.

Ethics Statement

The authors declare no Institutional Animal Care and Use Committee (IACUC) or other approval was needed. The authors declare human ethics approval was not needed.

Conflicts of Interest

The authors declare no conflicts of interest.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section.