Protothecosis is an emerging environmental algal disease of humans, and animals caused by Prototheca species which are unicellular, achlorophyllous saprophytic algae. Human infections are primarily caused by P. wickerhamii, whereas animal disease is mainly due to P. zopfii. The disease can occur in sporadic as well as in epidemic form, and is reported from many countries of the world including India. The source of infection is exogenous as the organism exists in the environment as saprobe. The exact route of infection is not clearly established. However, accidental inoculation of organism into the skin through wound or traumatic injury may result in cutaneous infection. In dairy animals, trauma to teats may predispose to mastitis. In humans, Prototheca mostly cause cutaneous infections, and olecranon bursitis with repeated traumatic inoculation. However, in immunosuppressed individuals, it can disseminate to viscera causing life threatening sepsis. Cholestatic jaundice and hepatitis are the typical clinical presentation of systemic protothecosis. Direct detection of pathogen in clinical specimens, and its isolation in pure and luxuriant form still remains the mainstay of confirming the diagnosis of disease. There are evidences to believe that saprobic environment may serve as source of infection to man and animal. Immediate attention to skin injury, prompt chemotherapy in immunocompromised patient, avoiding contact with stagnant water, good animal husbandry practices, and hygienic methods of milking will certainly reduce the prevalence of disease in humans and animals. It is recommended that ‘Narayan” stain should be widely employed in Public Health and Microbiology laboratories to study the morphology of Prototheca which are attributed in various clinical disorders of humans and animals. Further studies on the chemotherapy and epidemiology of protothecosis seem imperative to control this emerging algal disease.

**Keywords:** Algae, Animal, Emerging disease, Environment, Human, Narayan stain, Protothecosis

**INTRODUCTION**

Protothecosis is an infectious algal disease of humans and animals reported from North America, Europe, Asia and Africa. The disease affects both male and female without any discrimination to age group (Pal, 2007).
disease is caused by *Prototheca*, which is a unicellular, saprobic, heterotrophic, achorophyllous algae (Pal, 2007; Pal *et al.*, 2011). When *Prototheca* species were first isolated from tree slime in 1894 by Kruger, they were thought to be fungi because of their microscopic morphology. Based on cell wall characteristics, the genus has been placed in the Kingdom Plantae, Phylum Chlorophyta, and Family Chlorophyceae. These ubiquitous saprophytes have been recovered from a wide variety of environmental sources, including water, mud, animal manure, sewage, salad, ice cream, banana, potato, and vegetation (Songer and Post, 2005, Pal, 2007). They are obligatory saprophytes because they lack chloroplasts capable of photosynthesis. All protothecae are morphologically similar, with individual cells being ovoid to oblong in tissue sections or spherical in laboratory cultures, and body fluids, and having granular, basophilic cytoplasm and a thick, hyaline cell wall. Organism diameter may range from 1.5 μm to 30 μm, with variations in size influenced by species, time since sporulation, and environmental or culture conditions. Historically, only three species of *Prototheca* were recognized: *Prototheca stagnora* (*P*.stagnora), *Prototheca wickerhamii* (*P*. wickerhamii) and *Prototheca zopfii* (*P*. zopfii) (Pal *et al.*, 2011). However, results of sequencing ribosomal RNA from several human and animal isolates indicate that previously described biotypes, or “variants” of *Prototheca zopfii* should perhaps be considered distinct species. *Prototheca zopfii* biotype 1 is naturally found in bovine liquid manure, whereas most cases of bovine mastitis are due to *Prototheca zopfii* biotype 2. *Prototheca zopfii* biotype 3, which is typically isolated from swine manure rather than bovine manure, has been renamed *Protothe cablaschkeae* (*P*.blaschkeae). Finally, provisional strain differences in *Prototheca wickerhamii* have been detected, and a novel *Prototheca* (*P*. cutis sp. nov.) has been tentatively identified based on ribosomal RNA sequencing of a single human isolate (Table 1).

### Table 1: Pathogenic *Prototheca* spp. and Associated Disease Conditions

<table>
<thead>
<tr>
<th>Prototheca spp.</th>
<th>Hosts and Disease conditions</th>
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<tbody>
<tr>
<td><em>P</em>.blaschkeae</td>
<td>Cows (M, C) and people (C)</td>
</tr>
<tr>
<td><em>P</em>. cutis sp. nov.</td>
<td>People (C)</td>
</tr>
<tr>
<td><em>P</em>. wickerhamii</td>
<td>Cat (C), dog (CS) and people (C,O,S)</td>
</tr>
<tr>
<td><em>P</em>. zopfii non biotyped</td>
<td>Cow (M, S), dog (CS) and people (C,O,S)</td>
</tr>
<tr>
<td><em>P</em>. zopfii biotype 2</td>
<td>Cow (M) and dog (S)</td>
</tr>
</tbody>
</table>

Note: C – Cutaneous; M – Mastitis; O - Olecranon bursitis; S - Systemic.

Source: Craig, 2012

The present communication describes the growing importance of protothecosis as an emerging environmental borne algal disease in medical and veterinary medicine.

**Epidemiology**

*Prototheca* species are ubiquitous, and can be routinely isolated from plant surfaces, soil, or water, may occur as transient flora within animal and human gastrointestinal tracts, and are occasional asymptomatic colonizers of human skin and nail beds. However, because of their saprophytic nature, these algae preferentially occupy ecologic niches that offer a continuous source of partially digested or decomposing organic matter in wet environments, such as raw or treated sewage, cattle or swine manure and tree slime flux (Pal *et al.*, 2011; Craig, 2012).
Protothecosis is an uncommon, sporadic disease in people, dogs, cats, and non-domesticated mammals, with human and canine infections reported worldwide (Pal, 2007). Prototheca spp. infections in dogs occur more frequently in warm, humid regions. Although protothecosis is recognized with sufficient frequency that individual reports of disease are not routinely published, the relative prevalence of disease is still considered low: by the year 2000, only 108 human infections had been reported in the medical literature; by 2006, only 44 canine infections had been reported; and by 2009, only 5 feline infections had been reported. In dairy cows, however, outbreaks and endemic herd infections are regularly reported. Prototheca spp. infection most commonly causes mastitis in cows (Pal and Lee, 1997) with the difference in epidemiology likely because these algae thrive in their manure, resulting in indirect, and continued exposure of animals to high concentrations of organisms. Milking machines have been suggested as a possible fomite, however, the association between protothecal mastitis, and the lactation cycle is disputed (Craig, 2012).

In dogs, 75% to 90% Prototheca infections are due to non-biotyped P. zopfii, with one report of P. zopfii biotype 2; the remaining reported infections were due to P. wickerhamii. When they have been speciated, all organisms infecting cats have been P. wickerhamii. Non-biotyped P. zopfiior P. zopfiigenotype 2 have been isolated from the majority of affected cows, with only occasional case reports of P. blaschkeae infection. However, P. blaschkeae was only recognized as a novel species in 2006, and therefore, reported cases of human or canine P. zopfii infections before this time must be questioned. More than 95% of infections in people are due to P. wickerhamii, with only single report of infection with P. blaschkeae or P. cutis sp. nov. associated illness, and the remaining ones with non-biotyped P. zopfii. Because Prototheca spp. infections are acquired from the environment, and are not transmissible through contact, the risk of animals infecting people is minimal. Infections in animals are a sentinel for a risk of environmental exposure for humans (Craig, 2012). It is believed that both human and animals acquire infection from saprobic environment (Pal, 2007).

In Brazil ptotothecosis was reported in goat due to P. wickerhamii, and with probable source of infection by the contact of the nostrils mucosa or skin with contaminated water. It was speculated due to the observation of Prototheca in the farm pond during dry season, in that, most ponds have a progressive loss of water, and the water quality decreases, with high concentration of organic matter (Camboim et al., 2010).

**PATHOGENESIS**

Traumatic inoculation or contamination of open wounds has been associated with cutaneous infections. In people, some infections occurred with infection of pre-existing wounds, such as olecranonbursitis, presumptively due to repeated trauma to this region. Infection of previously recognized open wounds has not been reported in dogs or cats; however, trauma and contamination may be associated with protothecal mastitis in cows (Craig, 2012).

Because of the ubiquitous nature of Prototheca spp. and common exposure of animals to these organisms, disseminated disease is attributed to immunosuppression. The most frequently identified causes of
immunosuppression in people with disseminated protothecosis are local or systemic glucocorticoid administration, neoplasia (particularly hematologic cancers), diabetes mellitus, and acquired immunodeficiency syndrome. Despite these associated comorbid disorders in people, similar associations have not been made in dogs. Rather than exploiting host immunosuppression, Prototheca spp. may directly alter neutrophil function, thus promoting establishment, persistence, or dissemination of infection. P. zopfii increases antioxidant enzyme and hydrogen peroxide production by bovine milk neutrophils, although these changes in function do not alter opsonization or killing of organisms (Craig, 2012).

CLINICAL AND PATHOLOGICAL FINDINGS

Animals

Dogs

Dogs with disseminated Prototheca spp. infection most commonly have clinical signs referable to infection of the distal gastrointestinal tract, Central Nervous System (CNS), and eyes. As a result of colitis, severe, intermittently persistent large bowel diarrhoea with mucus, hematochezia, with or without melena are most commonly reported, and occasional signs include voiding of sloughed mucosa, weight loss, or vomiting. Approximately, 50% of dogs may have clinical signs attributable to CNS infection with or without concurrent colitis. Neurologic abnormalities, associated with meningoencephalomyelitis, may include seizures, central vestibular disease, altered mentation, blindness, deafness, ataxia, or lateralizing or generalized lower motor neuron deficits (Pal et al., 2011). In most cases, owner perception is that neurologic signs were of acute onset. Acute blindness may be the result of CNS infection or due to severe ocular inflammation. Ocular disease includes uveitis-associated glaucoma, miosis, and aqueous fluid are due to leukokoria, buphthalmos, posterior synechiae, chorioretinitis with raised, white, retinal granulomas, and retinal or vitreous haemorrhage (Figure 1). Less commonly noted clinical signs may reflect infection of other organs, including peripheral lymphadenomegaly, clinical signs of uraemia, lameness due to osteomyelitis, and sudden death due to presumptive protothecal myocarditis. The appearance and occurrence of cutaneous infections are variable in affected dogs (Figure 2A and B). Nonulcerated, multifocal/military to nodular skin lesions are usually observed first. More severely affected dogs may have ulcerated lesions on the trunk, pinnae, scrotum, foot pads, or planum nasale with associated nasal discharge. The skin lesions either may occur as the sole overt clinical sign or may accompany the previously described GI, neurologic, ophthalmic abnormalities (Craig, 2012). Prototheca zopfii genotype 2 causes severe...
neurologic signs such as seizures, progressive hind limb ataxia, polyuria, and polydipsia with no overt enteric signs (Lane et al., 2012).

The colon and ileum in dogs with systemic protothecosis are usually erythematous, thickened, and friable, with increased mucosal granularity, erosions, nodules, and large amounts of mucus and frank blood often times seen on endoscopic examination. Gross necropsy findings in dogs with disseminated *Prototheca* spp. infection may be confined to the gastrointestinal tract, but more typically include pinpoint to several millimetre-diameter white to yellow plaques or nodules on the serosal surfaces of numerous organs, including the heart, liver, spleen, mesenteric lymph nodes, diaphragm, and thyroid glands (Figure 3). Larger green to white plaques that extend into underlying tissues, particularly the lungs, may also be present. *Prototheca* spp. associated microscopic changes within the distal gastrointestinal tract include oedema with mild to severe mononuclear inflammation and numerous organisms in aggregates and cords throughout all intestinal layers, but predominantly within the lamina propria (Figure 4A and B) (Craig, 2012).

**Cats**

Protothecosis is very rare in cats, because of either natural resistance to infection or avoidance of environmental niches where algae are typically found. The few published cases have all described clinically healthy adult cats with firm,

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**Figure 2: Cutaneous lesions due to protothecosis (A) Heavy crusts on the footpadsof a dog with the rarer, cutaneous form of protothecosis. All four footpads wereinvolved, as were (B) other areas of the skin**

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**Figure 3: Heart and lungs from a dog with disseminated *P. zopfi*i infection. Small, granular, white to tan 2- to 4-mm nodule-like plaques are visible on the surface of the myocardium**

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Source: Craig, 2012
This article can be downloaded from http://www.ijlbpr.com/currentissue.php

non-ulcerated, cutaneous or subcutaneous masses on the forehead, distal limbs, tail base, nose, or pinnae (Figure 5). The absence of regional lymphadonomegaly, and lack of any clinical signs of systemic disease in these cats suggest that these infections were localized. However, one cat developed new distant nodules several months after excisional biopsy of an original solitary lesion (Craig, 2012).

**Cattle**

*Prototheca zopfii* can cause chronic progressive pyogranulomatous lesions in bovine mammary glands and associated lymph nodes. Indurative mastitis may affect a number of quarters. Because of their intracellular location, protothecal cells may be difficult to eliminate from the glands. Although the organisms are excreted intermittently in milk, they may not be demonstrable in samples, and some cases of the disease may be overlooked. *Prototheca zopfii* can persist in the tissues throughout a dry period and may be excreted during the next lactation. Treatment is unsuccessful. Affected cows should be culled because they are potential sources of infection and their milk yields are permanently reduced. Disseminated protothecosis has been recorded in cattle on rare occasions (Quinn et al., 2011).

**Goat**

In goat protothecosis due to *P. wickerhamii* is responsible for mucopurulent nasal discharge, inspiratory dyspnoea, sneezing with inflation of the cheeks during expiration and proliferative nodules, with 0.3-0.5 in diameter in the nasal mucosa of both nostrils. Thickening of mucocutaneous junction with ulcerative nodules in the mucocutaneous junctions and the upper lip were also reported. It was suggested that in goats protothecosis caused by *P. wickerhamii* is a chronic, slowly progressive infection, which affects immunologically competent goats, causing multifocal ulcerative pyogranulomatous, and necrotising lesions of the nasal vestibule,
mucocutaneous junctions of the nostrils and subcutaneous tissues and skin of face and head (Camboim et al., 2010).

**HUMANS**

Slightly more than 30 cases of protothecosis have been described, 60% of them in men. With the exception of one case due to *P. zopfii*, the causal agent in all other cases in which the species was identified was *P. wickerhamii*. Recently, an infection caused by green algae was described. Humans acquire the infection, possibly through skin lesions, when they come into contact with contaminated water or other habitats of these agents. The profusion of these agents in the environment, as well as the few cases described in humans, indicate that they are not very virulent and that lowered host resistance is required for them to act as pathogens. In fact, five of nine patients with cutaneous or subcutaneous protothecosis had a pre-existing or intercurrent disease. Similarly, seven of eight patients with the olecranon bursitis form had previously sustained a trauma to the elbow. The incubation period is unknown. Protothecosis manifests itself in two principal clinical forms. One is progressive ulcerative or verrucous lesions of the cutaneous (Figure 6A), and subcutaneous tissue on exposed skin. The other is chronic olecranon bursitis, with pain and swelling. In one case of dissemination, intraperitoneal and facial nodules were observed (Acha and Szyfres, 2001). Kwong et al. (2013) reported clinical case of protothecosis in the context of isolated hypogammaglobulinemia, though it cannot be completely established whether the patient’s infection was directly attributable to this, or whether the two conditions were coincidental by chance. Seok et al. (2013) described a case of human cutaneous protothecosis (Figure 6B) caused by *Prototheca zopfii*. In addition, post-transplant protothecosis is a rare but significant infection and is associated with a grave prognosis (Narita et al., 2008).

Figure 6: Cutaneous protothecosis. (A): Mixed *Aspergillus* and *Prototheca* cellulitis in a neutropenic patient (Source: Barrak, 2010) (B): Erythematous nodular lesion with crust is seen on the left wrist (Source: Seok et al., 2013)
**DIAGNOSIS**

Suitable specimens for laboratory examination include milk samples and biopsy or post-mortem tissues. An indirect ELISA has been described for the detection of antibodies in serum, and whey. Gomerimethenamine silver (GMS) or Periodic Acid Schiff (PAS) techniques can be used to demonstrate algal cells and sporangia in histological sections of granulomatous lesions (Pal, 2007). Immunofluorescent techniques are used to identify *P. zopfi* and *P. wickerhamii* in tissues. The organisms grow on blood agar and Sabouraud dextrose agar without cyclohexamide. The pathogen may be isolated from contaminated specimens on Prototheca isolation medium with added phthalate and 5-fluorocytosine. Culture plates are incubated aerobically at 35°C to 37°C for 2 to 5 days. Carbohydrate assimilation test kits for differentiating *Prototheca* species are available commercially. *Prototheca wickerhamii* assimilates trehalose but not 1-propanol, whereas *P. zopfi* assimilates 1-propanol but not trehalose. Identification criteria for isolates includes, colonial morphology, microscopic appearance of sporangio spores and carbohydrate assimilation tests. Pal (2004) has demonstrated that “Narayan” stain can be successfully employed to study the morphology of *Prototheca* isolated from humans and animals. Molecular methods are available for the identification of *P. zopfi*. Antibodies in dairy cows can be detected by enzyme immunoassay. A strong serologic response may be present in infected cows (Quinn et al., 2011).

**Isolation**

Definitive diagnosis of *Prototheca* spp. infection requires culture or genetic identification methods. All *Prototheca* species grow readily on most laboratory media, including blood agar; therefore, even in cases when protothecosis is not suspected, culture of biologic samples such as urine, CSF, or vitreous humour often results in abundant algal growth. *Prototheca* spp. form white to light tan colonies on blood agar and Sabouraud's cycloheximide-free dextrose agar at 25°C to 37°C; differences in sugar (Table 2) and alcohol assimilation or antibacterial susceptibility are required for routine species differentiation, whereas ribosomal RNA sequencing is needed to differentiate *P. zopfi* biotypes. Differentiation of *Candida* spp. from *Prototheca* spp. (both of which may be found in urine) using ribostamycin-

<table>
<thead>
<tr>
<th>Table 2: Some Characteristic Features of <em>Prototheca</em> spp.</th>
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<tbody>
<tr>
<td><strong>P. wickerhamii</strong></td>
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<tr>
<td>Growth at 37 °C</td>
</tr>
<tr>
<td>Glucose</td>
</tr>
<tr>
<td>Trehalose</td>
</tr>
<tr>
<td>L-propanol</td>
</tr>
<tr>
<td>Acetate</td>
</tr>
<tr>
<td>Galactose</td>
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<tr>
<td>Capsule</td>
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Source: David et al., 2007
impregnated disks has been described, but cytologic examination of colonies is sufficient and more rapid (Craig, 2012).

Cultivation is readily accomplished within 72 h on routine fungal media without cycloheximide and also on blood agar (Figure 7). Isolates grow between 25° and 37°C as white to cream, dull, moist to mucoid yeast-like colonies. Following isolation, yeast-like colonies should be examined in lactophenol cotton blue or Narayan (Pal, 2004) wet mounts. The rounds to oval sporangiospores are characterized by hyaline cell walls and prominent nuclei. Sporangia, varying from 7 to 25 µm in diameter, depending on their stage of development, and containing 2 to 20 sporangiospores are observed (Figure 8). Hyphal formation and budding do not occur. The cells of *P. zopfii* tend to be larger (14-25 µm in diameter) than those of *P. wickerhamii* (7-13 µm in diameter). *Prototheca* spp. can be identified through carbon assimilation tests in several commercial yeast identification systems (Songer and Post, 2005).

**Clinical Laboratory Findings**

Hematologic and serum biochemical abnormalities in dogs with disseminated protothecosis generally reflect nonspecific inflammation (i.e., neutrophilic leukocytosis and hyperglobulinemia). In animals with kidney or liver involvement, abnormalities may be those typically associated with renal failure or hepato cellular damage. Urinalysis abnormalities may include minimally concentrated urine or active urine sediment. These findings may be due to renal dysfunction and the frequent renal shedding of *Prototheca* spp. Cerebrospinal Fluid (CSF) from dogs with CNS signs, and vitreous fluid from dogs with uveitis may be grossly discolored, and have marked increases in both polymorpho nuclear, and mononuclear leukocytes and protein concentration (Craig, 2012).

**Cytologic Examination**

Initial presumptive diagnosis of cutaneous or disseminated *Prototheca* spp. infection most commonly occurs via cytologic identification of
morphologically consistent organisms in colonic biopsy impression smears, lymph node fine-needle aspirates, or skin or colonic biopsies (Figure 9A, B and C). Although definitive diagnosis requires culture, the concurrent presentation of large intestinal diarrhoea with hematochezia and uveitis, along with the unusual cytologic appearance of *Prototheca* spp., makes cytologic diagnosis relatively reliable. The organisms in cytologic samples stain well with modified Wright or Gram stain with surrounding nonstaining clear cell walls. Dogs with clinical signs of GI disease may have organisms noted in rectal or colonic scrapings, but this appears to be a less reliable method of diagnosis than endoscopic or full-thickness biopsies of the distal intestine. Examination of routine urine sediment or cytospin preparations identifies organisms in greater than 50% of infected dogs. The pathogens have also been noted in vitreous humor samples from dogs with uveitis. In dogs with atypical presentations (e.g., acute renal failure), organisms may be first noticed in biopsies from affected organs (Craig, 2012).

**Molecular Techniques**

Molecular techniques are currently developed for the diagnosis of both *P. blaschkeae* and *P. zopfii*. These includes genotype-specific PCR (Osumi et al. 2008), which targets 18S rDNA sequencing (Marques et al., 2008) and by Restriction Fragment Length Polymorphism (RFLP) analysis (Moller et al., 2007). In addition Ricchi et al. (2010) developed two-step Real Time PCR reaction
followed by DNA Resolution Melting Analysis (qPCRD RMA) for *P. zopfii* genotype 2, *P. wickerhamii* and *P. blaschkeae* targeting 18S rDNA which is helpful for the diagnosis of animal or human protothecosis.

**TREATMENT AND PREVENTION**

Cutaneous protothecosis has been treated successfully by surgical excision. Although isolates are susceptible to gentamicin, amphotericin B, nystatin, and polymyxin B *in vitro*, systemic administration of these agents does not produce a clinical response. In the dairy environment, disease may be controlled by improving management conditions, such as not using muddy lodging areas, culling infected animals that may shed organisms in their faeces and serve as a source of infection, and using teat dips after milking (Songer and Post, 2005; Pal, 2007).

Results of studies have examined the in vitro susceptibility of *Prototheca* spp. to various antifungal or antibacterial drugs. Most *P. zopfii* isolates from cows with mastitis are susceptible to amphotericin B (AMB), pimaricin, and posaconazole; resistant to caspofungin; and have variable susceptibility to nystatin, filipin, and voriconazole. Asingle *P. wickerhamii* mastitis isolate had a similar susceptibility pattern and was susceptible to voriconazole. Susceptibility to clotrimazole is regularly observed with isolates of *P. wickerhamii* but not those of *P. zopfii*. This feature is commonly used for speciation of *Prototheca* spp. cultures by microbiology laboratories. Some algal isolates may be susceptible to aminoglycosides, but the most promising agent in this class, ribostamycin, is not commercially available. All protothecal species are resistant to fluconazole, and 5-flucytosine. Interestingly, two essential plant oils, tea tree and bergamot oil, have also been studied based on previously reported antimicrobial effects and both demonstrated in vitro algicidal activity against nonspeciated *Prototheca*. Despite the demonstration of in vivo susceptibility to various drugs, the clinical response to treatment of cutaneous or disseminated protothecal infections in people and animals has been very poor (Pal, 2007; Craig, 2012).

**CONCLUSION**

Protothecosis is an infectious saprozoonic algal disease of world wide distribution. The organisms occur as saprophyte in the environment, and is isolated from a variety of natural substances including the water, soil, and plant. The route of transmission is not clearly established. Infection is introduced through trauma to the skin, mucous membrane, and subcutaneous tissue. Though disease is recorded in man and many species of animals, the source of infection is not well understood. More research is warranted to elucidate the reasons why the organisms, though widely distributed in environment, occasionally produce the disease, and also to determine a successful treatment regimen by developing a safe, effective, and low cost chemotherapeutic agent for better management of disease in humans and animals. The factors which predispose the subject to *Prototheca* infection should be investigated. Further studies are imperative to establish the source of infection to humans as well as animals. It is advised that veterinarian should be vigilant about protothecosis as animals serve as sentinel for a risk of environmental exposure to humans.
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