Case Report

Algaemia in a dairy cow by *Prototheca blaschkeae*

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We describe the first known case of an algaemia by *Prototheca blaschkeae* in a dairy cow, which occurred after a chronic episode of mastitis caused by this pathogen. The organism was isolated from milk, joint fluid and blood samples, and microbiologic and molecular methods were performed to obtain a definitive identification of the algae. The affected cow was culled only after confirmation of a systemic infection by *Prototheca*.

**Keywords**  algaemia, bovine mastitis, *Prototheca blaschkeae*

**Introduction**

The only known plant-like organisms that cause infectious diseases in humans and animals are green algae of the genus *Prototheca* and *Chlorella* [1-4]. Members of the genus *Prototheca* are ubiquitous saprophytes, and can be isolated from a variety of environmental sources such as plants, soil, sludge, drinking and marine water, swimming pools, feces of domestic and wild animals, barn floors and meat products [5]. Some species may be opportunistic pathogens, causing pathology in immunocompromised hosts [6-8]. Similarly, they act as pathogens when there are predisposing factors, such as, in the case of dairy cows, poor animal care and poor milking hygiene are present [3,7,9]. Protothecal mastitis has been reported sporadically in dairy herds of Europe, Brazil and the United States [10-13]. These algae do not respond to routine mastitis therapy and the only control method to date has been the elimination of the infected animals [14]. Bovine mastitis caused by members of this genus has been almost exclusively associated with *Prototheca zopfii* genotype 2 [2]. Until recently the only species known to cause infectious diseases in humans and animals were *P. wickerhamii* and *P. zopfii*, respectively [15-17]. The new species *P. blaschkeae*, was identified from a human case of onychomycosis [18], was defined as biotype III of *P. zopfii* that together with biotype I was reported to be non-pathogenic [15]. Biotype classification was based on phenotypic characteristics, as well as auxonographical and biochemical analyses [15,19]. Recently, mastitis caused by *P. blaschkeae* has been reported in cattle [20] but never as an agent of systemic infections. We report the first known case of algaemia by *P. blaschkeae* in a dairy cow, after a chronic episode of mastitis.

**Case report**

A 4½-year-old Holstein cow was diagnosed with chronic mastitis in the udder's front right quarter. The culturing of milk samples from all udder quarters resulted in the isolation of *Prototheca* spp. in pure culture and was identified based on its growth and morphological features. Because mastitis by *Prototheca* is a chronic infection with no successful treatment, the owner was recommended to remove the cow from the herd. However, the owner decided to keep it in the farm because the cow was 3 months pregnant. Two months...
after the first milk analysis, the infection had extended to the right back quarter as determined by testing milk from all quarters. The cow was dried off and delivered a viable calf six months after our first diagnosis of mastitis. The animal was lethargic and anorectic for about 8 weeks after parturition.

A generalized mastitis by *Prototheca* spp. was later diagnosed (6 months after calving) in all quarters of the mammary gland. Somatic cell counts showed a steady increase since parturition up to the time the animal was culled (248 × 10⁵, 577 × 10⁵, 446 × 10⁵, 778 × 10⁵, 1.843 × 10⁶ and 1.248 × 10⁶ measured on a monthly basis). Meanwhile, the cow developed abscesses, first in both back leg joints (Fig. 1) then in the perinea region and some of these abscesses ruptured over time, and the animal was subsequently treated for the piodermatitis. At the time of our last visit to the farm, the cow presented an accelerated respiratory frequency rate but with no fever and fully recovered from her previous lethargic and anorectic condition. The following samples were collected; milk, whole blood (with and without EDTA) and joint fluids which appeared bloody and purulent.

The laboratory hematology findings revealed a monochromic and monocytic anemia represented by values below normal in red blood cells, hemoglobin and hematocrit. Cytology of all samples, stained by Gram and Diff-Quick, showed yeast like organisms in variable amounts under light microscopy. In order to increase the white blood cell fraction from the blood samples, mononuclear cells were separated by Lymphoprep (Axis-Shield PoC AS, Norway) gradient and washed. Stained smears of this preparation permitted an easier visualization of extracellular and intracellular algae bodies, with the evidence of protothecal bodies in wet-mount preparations (Fig. 2). The laboratory analysis consisted of inoculating samples on Columbia agar plates supplemented with 5% sheep blood (bioMérieux, Marcy l’Etoile, France) and Sabouraud dextrose agar plates (Merck Laboratories, Darmstadt, Germany) and incubation at 37°C for up to 96 h. After 96 h, the plates were kept at room temperature for up to 15 days. Following the incubation period, pinpoint colonies were observed in both Columbia and Sabouraud dextrose agar cultures, first from milk samples and later from specimens of other fluids. *Prototheca* spp. colonies recovered from milk were visualized in pure culture after 72 h of incubation and their abundance was related to the larger number of algae cells seen in the milk cytology. Colonies of *Prototheca* spp. isolated from the joint fluids presented a much slower growth and were only visualized 5 to 6 days post-inoculation. A mixed flora was retrieved from cultures of the

Fig. 1 Rear part of the cow with algaemia caused by *Prototheca blaschkeae*. An abscess in the left back leg joint can be observed. Atrophy of the udder is also noteworthy.

Fig. 2 Photomicrograph obtained by light microscopy of wet mount preparations of *Prototheca* spp. from the white blood cell fraction separated by Lymphoprep. Extracellular *Prototheca* spp. sporangia (arrow). Magnification ×1000.

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collected joint fluids on the Columbia agar plates, consisting of *Corynebacterium* spp. and *Streptococcus* spp. that were visualized after an incubation period of 24 h at 37°C. Recovery of *Prototheca* spp. from blood samples was the slowest, with visible colonies detected only after 10 days post-inoculation. Pregnancy and parturition may have contributed to lower the cow’s immunocompetence and allow the dissemination of the algae from the mammary gland to the blood stream. Following the evidence of systemic involvement of *Prototheca* spp. in this cow, the owner agreed to remove the animal from the herd.

The identification of *Prototheca* spp. in this study was attempted using the API 20C system (BioMerieux, Portugal Lda). However, the database of the API 20C system only includes *Prototheca wickerhamii*, and the biochemical reaction of trehalose was inconclusive. Therefore, the species identification of the isolates recovered from all indicated samples was accomplished through the use of PCR amplification of the 18S rDNA, amplified rDNA restriction analysis, and by direct sequencing of the 18S rDNA sequences. The results indicated that the isolates were all *P. blaschkeae* (GenBank accession no. EU439262). This isolate is also deposited in the Sammlung von Algenkulturen Goettingen (SAG) culture collection with the strain number SAG2291. PCR amplification of the 18S rDNA region was carried out as previously described [20], presenting a fragment of about 1800 bp. Amplified rDNA restriction analysis was performed using HaeIII (NEB, Inc., Frankfurt, Germany), which presented a restriction pattern similar to the one determined for *P. blaschkeae* in a recent study [20] (Fig. 3). The amplified rDNA restriction analysis of the isolate (SAG 2291), presented differences when compared to the restriction patterns of the type strains of *P. zopfii* genotype 1 (SAG 2063) and 2 (SAG 2021). However, its rDNA restriction pattern (SAG 2291) was similar to that of *P. blaschkeae* type strain (SAG 2064) which lack the 400 bp fragment (Fig. 3). The sequence had a length of 1815 bp and showed 99.8% identity within the 18S rDNA sequence available for *P. blaschkeae* SAG2064 (AY973041).

**Discussion**

*Prototheca* are unicellular organisms closely related to the green alga of the genus *Chlorella* but lacking chlorophyll [5]. Members of the genus *Prototheca* are ubiquitous saprophytes, and can be isolated from a variety of environmental sources [5]. The genus comprises several species, but until recently the only species known to cause infectious diseases in humans and animals were *P. wickerhamii* and *P. zopfii*, respectively [15–17]. Among domestic animals, dogs have been affected with severe disseminated forms of the disease involving several organs. In cattle, this pathogen is in
general associated with chronic mastitis. The first report associating *P. zopfii* with mastitis in cattle was published in 1952 [12]. Taniyama *et al.* [21], described the first case of disseminated protothecosis caused by *P. zopfii* in several organs of a cow through the use of immunohistochemical and histopathological techniques. While *P. wickerhamii* is in general associated with systemic infections in humans [22–24], *P. blaschkeae* was first identified from a human case of onychomycosis [18]. There have been reports of algaeemia [20] with *P. wickerhamii* in culture after its visualization in peripheral blood smears but this has only been demonstrated in a few human cases [22,24]. In addition, *Prototheca* in this case study could be seen in cytology of all collected samples, but its recovery in culture from blood specimens was somehow difficult. To our knowledge this is the first report of an algaeemia in a cow involving this infectious pathogen. The stress of pregnancy, aggravated by the lethargic and anorectic condition that followed the parturition, may have induced a debilitating status reducing the cow’s defenses and resistance mechanisms. Although generally saprophytic, some *Prototheca* species may cause pathology when the host immunological defenses are impaired [5–7], or when predisposing factors such as deficient animal care and poor milking hygiene occur in dairy management [3,7,9]. The literature [13,20,25,26] suggests that the incidence of bovine mastitis by *Prototheca* spp. is increasing all over the world. This fact probably reflects the inclusion by laboratories of explicit tests in their diagnostic routines.

Recently, Roesler *et al.* [15] suggested that isolates belonging to biotype II of *P. zopfii* represented by strain SAG 2021, are the causative agents of bovine mastitis [2]. Moreover, *P. zopfii* was divided into three biotypes on the basis of phenotypic characteristics and auxonographical and biochemical analyses. In this scheme, *P. blaschkeae* belongs to biotype III [15, 19,25]. More recently, mastitis by *P. blaschkeae* has been reported in cattle [20] but never involved in a systemic type of condition. With the emergence of infectious diseases in the world, the potential consequences of bovine protothecosis on public health remains to be determined through studies of the resistance of the new taxonomic groups to milk pasteurization. This study reports the first known case of algaeemia by *P. blaschkeae* in a dairy cow, after a chronic episode of mastitis.

**Acknowledgements**

The authors thank the suggestions of Volker Huss for the molecular characterization of the isolate, and Dr Uwe Roesler for providing the type strains (SAG 2021, SAG 2063, and SAG 2064).

**Declaration of interest:** The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

**References**


