Prototheca-Induced Infections in Dairy Cows: A Multiresistant Environmental Pathogen

Márcio Garcia Ribeiro*
School of Veterinary Medicine and Animal Sciences, São Paulo State University-UNESP, Botucatu, Brazil

Abstract

Prototheca spp is an achlorophyllous alga found ubiquitously in the environment. The organism is opportunistic in nature and has been considered an emerging disease that affects humans and some domestic animals, although bovine mastitis is a major clinical manifestation of algae-infections among livestock. The pathogen is commonly nonresponsive to conventional therapy, which has led to segregation and premature culling of chronic cases as main measures to control of protothecal mastitis. In this scenario, this mini-review focused on epidemiological aspects, in vitro resistance of algae, and measures applied to control and prevention of protothecal mammary infections in dairy cows.

Etiology

Members of the genus Prototheca consist of unicellular, colorless, achlorophyllous intracellular yeast-like micro-algae able to infect humans [1] and animals [2]. Based on a current taxonomic proposal using mitochondrial cytochrome b gene molecular markers, fourteen Prototheca species are assigned: typical dairy cattle-associated, i.e., Prototheca bovis (formerly Prototheca zopfii genotype 2), Prototheca blastheae, and Prototheca ciferrii (formerly Prototheca zopfii genotype 1), human-associated, i.e., Prototheca wickerhamii, Prototheca catus, and Prototheca miyajii, as well as Prototheca moriformis (Prototheca ulmns), Prototheca stagna, Prototheca tumulicola, Prototheca zopfii, Prototheca cookei, Prototheca pringhemii, Prototheca xanthorae, and Prototheca ceras, the last four ones, recently named [3]. Among these species, Prototheca ciferrii has been found in manure and environment of dairy farms, whereas Prototheca blastheae and Prototheca bovis, have been described globally causing mammary infections, besides predominance of Prototheca bovis as a most frequent cause of clinical protothecal mastitis in dairy herds [2].

Epidemiology

Prototheca species inhabit a wide variety of environmental niches and surfaces, particularly with high humidity and abundant organic matter contents [1,2]. On endemic-dairy farms, these saprophytic algae have been recovered in farm-sources where animals are driven or resting, including streams or stagnant ponds, mud and manure surround milking areas, feed, cattle drink water, feces of cattle and calves, the material of cow’s bed and compost barn, as well as milking machine [4,5]. Improper teat hygiene procedures and poor hygiene in the intramammary infusions approach, particularly the use of dry cow teat sealant and infusions with nonintramammary formulations, revealed strong herd-risk factors to protothecal mastitis [6]. Transmission between infected-to-healthy hosts may also occur due to deficiencies in milking hygiene procedures, improper pre-milking practices, and excess of organic material on the environment of dairy cows [4]. Variable prevalence (≤10%) of Prototheca species isolation has been seen among mastitis cases and bulk tank milk samples subjected to microbiological culture worldwide [2,6-8]. Nonetheless, increasing rates of clinical protothecal mastitis have been reported globally [2,3,5], with marked outbreaks recorded in South (Brazil) and North America (USA, Canada, and Mexico), Asia (Japan and China), Oceania (New Zealand), and some countries of Europe (Denmark, England, and Italy). A large-scale study involving molecular characterization of 342 Prototheca isolates obtained of bovine mastitis from different countries confirmed high prevalence of Prototheca bovis (90.6%), followed by minor frequency of Prototheca blastheae (8.8%), and Prototheca ciferrii (0.6%), reinforcing Prototheca bovis as a major cause of mammary infections in cattle-herds [2].

Pathogenicity and Clinical Aspects

The virulence and pathogenicity of Prototheca-induced infections remain poorly understood, although the rigid structure of cell wall, suppression of cell-mediated immune response, evasion of phagocytic cells [1,9], and biofilm formation [10,11] probably enables intracellular persistence that, in turn, results in pyogranulomatous reactions with the destruction of mammary tissues and epithelia, leading to limited immune response and tissue resolution [9]. Mostly cows develop clinical indurative mastitis, during lactation or dry period. Mammary protothecosis produces a decrease in milk yield, as opposed to increasing SCC. Sporadically the pathogen spread from mammary glands to infect other organs. Some animals may develop subclinical form or solve clinical signs spontaneously, although the pathogen tends long-term infections [4]. Mammary infections usually are refractory to conventional intramammary and/or systemic therapy [12], which results in poor prognosis or outcome, especially when two or more teats are affected. In this regard, on endemic-farms, economic losses have been related to the deep impact of decrease milk production and early culling of clinical cases [2,4,12].

Diagnostic Approach

Routine identification of the Prototheca species has been based on conventional microbiological culture, staining (Gram, Romanowsky variants, lactophenol cotton blue), micromorphology, and biochemical activity (carbohydrate and alcohol assimilation) [1,2,5,9]. However, the algae may be eliminated intermittently by milk, a fact that may limit microbiological diagnosis on farms [4]. In the coming years, the speciation, genotyping, taxonomic reclassification, and novel species characterization of algae has been allowed using various molecular methods, e.g., conventional and multiplex PCR, PCR-restriction enzyme analysis (PCR-REA) assay (partial cyto b gene), sequencing (18S rRNA gene), and MALDI-TOF MS [2,3,6,10].

Treatment

Since the treatment of mammary infections usually is unsuccessful, Prototheca sp isolated from milk of dairy cows have been...
subjected to a number of in vitro studies with a wide variety of pharmacological products with well-known microbiocide effect, and have shown susceptibility to some antifungal (amphotericin B, itraconazole, ketoconazole) [13], antimicrobials (gentamicin, netilmicin, colistin sulfate, kanamycin) [11,13] and, recently, dinotroanilamines, a group of herbicides [11]. Also, in vitro algicidal effect in low concentrations has been seen in antiseptic, disinfectants and/or sanitizing such as iodine, sodium hypochlorite [14], peracetic acid [10], guanidine [15], as well as other products that have been assessed, e.g., hydrogen peroxide, thimerosal, chlorhexidine, copper sulfate, and silver nitate. Nonetheless, there is no strong association between in vitro susceptibility and in vivo microbiological cure of mammary infections, in addition to none standard guidelines to in vitro susceptibility tests for Prototheca. Besides in vitro action of some of these pharmacological products against Prototheca species, commonly have been seen only temporary in vivo clinical regression, which no effectively alter the outcome of clinical mammary infections [12] and, then, in a great number of animals, if not all, usually is observed the return of clinical signs.

Control and Prevention

Measures recommended avoiding protothecal mastitis are similar to prevent and control other environmental pathogens, due to opportunistic behavior and environmental nature of the algae [4]. Therefore, these cares include a routine of clinical/subclinical and microbiological diagnosis of mastitis, adequate milking hygiene, teat dip practices, offering food after milking, water chlorination used in milking procedures, clean and dry pre- and postmilking area, and management of bedding and compost barn material. On endemic herds, early diagnosis, segregation of affected animals to end of milking, dry off teat (only one teat affected), or culling of multi-teat and/or chronic animals appears to be main measures to control protothecal mammary infections.

Public Health Issue

Ingestion of contaminated milk and derivatives from bovines has been seen as a source of the transmission of the Prototheca species from cows-to-people [16], and it poses as a public health concern because these algae may resist to industrial heat producing ability and efficiency of sanitizing agents against Prototheca zopfii isolates from bovine subclinical mastitis. Journal of Dairy Science 98(6): 3613-3362.

References
