

Intelligent prevention and control of Prototheca in dairy cattle farming

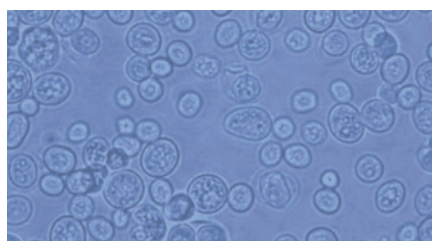
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One of the current issues in the area of dairy cattle health is the presence of the micro-alga Prototheca. This alga is significant because it causes mastitis problems that can lead to large economic losses due to reduction in the quality and quantity of milk produced and the slaughter of infected animals, amongst other losses.

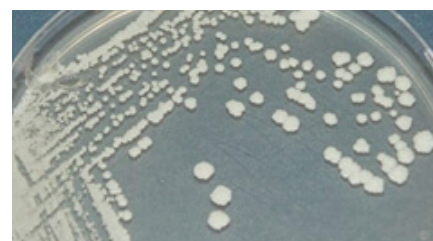
Prototheca is a heterotrophic unicellular alga, phylogenetically correlated with the Chlorella genus. It is a stationary micro-organism, oval or spherical in shape, and 7-10 microns in diameter. This alga reproduces asexually, does not photosynthesise and is widely distributed in the environment.

A saprotrophic agent, Prototheca metabolises simple sugars and amino acids. It is ubiquitous in nature and has been isolated from soil, waterways, and faeces of cattle, pigs, dogs, rodents, and even humans affected by gastro-enteric disease. It is usually present in tap water at levels below 10 CFU/ml, while in the biofilm and sediments deposited in the different parts of the water distribution system, it can reach values greater than 10,000 CFU/ml or g.

On dairy farms, the most favourable areas for the growth of Prototheca are cracks in the pavement, the water system biofilm, small puddles of water existing in the transit areas of the milking parlour, microscopic pores of the rubber components in the milking system, and other similar places.



Identification of suspect colonies by microscopic observation preceded by staining with Lactophenol Blue.



Given that Prototheca has proven highly resistant to disinfection with chlorinated products, the cleaning and disinfection programs of the installations, especially milking equipment, should be carried out using specific products and work protocols.

It should be noted that the commonly used chlorinated detergents are not effective against this alga. Therefore, the only effective action is to disinfect using products based on hydrogen peroxide and peracetic acid, whose algaecide action has been certified.

Clinical aspects

Normally, mastitis caused by Prototheca manifests as a chronic disease of the mammary gland. The occurrence of mastitis is usually asymptomatic or subclinical, with no obvious associated clinical signs, although the increase in somatic cell count in milk can be observed, with values exceeding 10^6 cells/ml.

As the infection progresses and the parenchyma consequently deteriorates, some clinical signs may manifest, such as blood clots in the foremilk, watery appearance of milk, increased thickness of the parenchyma and progressive decrease in milk production, perhaps associated with atrophy of the affected quarter.

Under normal conditions, the majority of animals affected by chronic mastitis caused by Prototheca excrete a significant amount of the alga, although this is often intermittent.

The appearance of acute mastitis caused by Prototheca is usually sporadic. When this occurs, there is an obvious alteration of the mammary secretions (purulent serum) and a considerable increase in the volume of the affected quarter. The most consistent feature of this type of mastitis is the permanent increase in somatic cell count. However, there are cases, mainly in recent infections, in which the somatic cell count stays within the normal range.

In chronic and acute cases, clinical signs of systemic disease are usually not observed. However, in the presence of predisposing factors, after a time, mammary infection can cause systemic complications related to Prototheca spreading through the bloodstream.

Since, as discussed above, mastitis associated with Prototheca does not generally display obvious clinical signs, there is a serious risk of not detecting these sub-clinical mastitis infections, thus facilitating their dissemination to other animals.

The greatest risk of mastitis infection by Prototheca exists during the first weeks of lactation because of the state of immunosuppression that accompanies calving and

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Table 1. Positive samples for Prototheca on dairy cattle farms (Cammi et al., 2008).

Sample	Prototheca samples		
	No. samples	No. positive	Positive (%)
Drinking water	56	10	19
Milking equipment rinse water	6	2	30
Swab of teat cup inner rubber liner	31	4	13
Swab of milking parlour surface	21	4	19
Feed	3	0	0
Bedding material	26	5	19
Faeces negative to mammary excretion	5	0	0

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the onset of milk production. Spontaneous cure in dairy cows is extremely rare. In addition, mammary infection by *Prototheca* persists during the drying-off phase, and reappears again after calving.

Epidemiology

Prototheca is ubiquitous in the environment, preferring moist habitats rich in organic matter. Thus, favourable conditions for the survival and growth of this alga are widely present in dairy farms. In this type of facilities, *Prototheca* can be found in the troughs (in water, in the biofilm attached to

the inner walls, and surrounding damp areas), in forage and silage, in faeces, in bedding material, in wet surfaces (rubber floor of the waiting room and hallways), in teat liners, in milking equipment, etc.

Prototheca can be ingested by animals, for example, by drinking the water from the trough, ingesting contaminated food or licking surfaces where the alga is present. *Prototheca* is able to reproduce in the rumen, resulting in a healthy carrier animal.

This animal will excrete the alga in its faeces, contaminating the bedding, the floor, and other parts of the facilities. Risk of contagion in other animals from environmental exposure increases considerably when *Prototheca* is present in the faeces of



healthy carrier animals. Up to this point, the alga can be considered an 'environmental pathogen', with an unusually high risk of contagion. Given the proliferation of the alga in the rumen, it is necessary to stress the high risk of horizontal transmission through water troughs.

Udder infection occurs through entry of *Prototheca* when the teat sphincter opens during milking. In this way, the alga passes from quarter to quarter through the traditional milking contagion vectors (water, hands, inner walls of the teat cup liner, etc) leading to the appearance of contagious mastitis (*Prototheca* acts as 'contagious pathogen').

Elimination of the alga through the milk occurs intermittently and can lead to false negatives if it is not present in sufficient quantity to be detected in the laboratory. Calves feed with milk from infected cows can act as an important source of environmental contamination due to the excretion of *Prototheca* in faeces.

When no new animals have recently been brought to the farm, the infection usually has an environmental origin. The subsequent spread from one animal to another occurs mainly through milking.

Diagnosis

Diagnosing mastitis caused by *Prototheca* is impossible from a clinical point of view because of the absence of pathognomonic symptoms. Laboratory testing is therefore essential. Likewise, to assess the degree of environmental contamination, the presence of *Prototheca* in different sample types (animal drinking water, milking equipment washing water, teat cup swabs, food, bedding material, faeces, etc) can be identified in the laboratory.

Laboratorios OX performs diagnostic tests for the presence of *Prototheca* using a unique procedure that follows the guidelines of the National Mastitis Council and includes cultivation in PIM (*Prototheca* Isolation Medium) (37°C 72 hours) and the subsequent identification of suspect colonies by

microscopic observation preceded by staining with Lactophenol Blue.

Prototheca sensitivity

Prototheca species have been shown to be resistant to antibiotic therapy both in vitro and in vivo. Sensitivity studies performed in vitro have shown limited sensitivity of Prototheca zopfii to some antibiotics and fungicides, such as Polymyxin B, Gentamicin, Nystatin, Amphotericin B, Fluconazole, Itraconazole and Posaconazole. In vivo trials with these and other active ingredients have shown only a temporary reduction of symptoms and excretion of algae, at best. Moreover, Prototheca has proved to be highly resistant to chlorinated disinfection products. Of particular interest is the resistance of Prototheca to water treatment using traditional chlorination methods.

Prevention and control

Considering the great resistance to antibiotic and fungicidal therapy shown by Prototheca, the application of preventive measures is of paramount importance. However, it is necessary to note that the alga's resistance to chlorinated products requires the implementation of intelligent work protocols that include the use of biocidal products of proven efficacy against Prototheca. As part of its Intelligent Biosecurity Operations Management Program for Dairy Cattle, Grupo OX has developed a specific protocol for preventing and controlling Prototheca. This work program includes the implementation of the following measures:

● Identification of infected animals:

It is necessary to periodically monitor the milk of all the animals on the farm in order to identify any cows with subclinical infections. Prototheca-positive animals should be separated from healthy ones and milked last, in order to contain the spread of infection. Once the Prototheca-positive animals have been removed or separated, it is advisable to monitor the presence of Prototheca in the negative group of animals by testing the milk in the laboratory.

● Identification and control of sources of environmental contamination:

Prototheca's resistance to chlorinated products requires the introduction of alternative water treatment methods:

- Treatment of water used on the farm (animal drinking water, milking parlour cleaning water, milking equipment cleaning water, and so on) with OX-AGUA 2nd Generation. This product, which is formulated with hydrogen peroxide stabilised by the inclusion of the exclusive OX-RPLUS core, has shown its effectiveness against Prototheca, and guarantees the absence of the alga in the water, thus avoiding potential horizontal transmission through the watering troughs.

Pens and beds must be kept clean and dry. Any contact between the udders and dirty rest areas increases the likelihood of micro-organisms invading the udder. In dairy cattle farms, the 'all in-all out' system cannot be applied since the occupation of the stables is continuous. For this reason, extreme hygiene measures must be permanently applied. Also, after cleaning the milking equipment with the corresponding detergent, it must be disinfected using a specific product.

The following actions are recommended:

- Disinfection of pens, equipment and milking parlour, teat cups, calve pens, and so on with OX-VIRIN. This unique, 100% biodegradable disinfectant, based on hydrogen peroxide and peracetic acid, has proven its efficacy against Prototheca even at low doses, and can be used within the existing legal framework for all required disinfection tasks (including inside the milking circuit).
- Application of the powdered disinfectant product OX-S4 to the bedding material (especially in critical areas such as delivery room and veterinary treatment area). Its specific formulation guarantees absolute humidity control and destruction of Prototheca due to its exclusive peroxyacetic compounds content. The characteristics of these products make its application easy and convenient, guaranteeing the control of Prototheca in facilities in which the alga has been detected, and its prevention in those installations that test negative for the presence of this pathogen.

● Controlling risk factors for mammary infection:

Those risk factors that affect the integrity and functionality of the teat canal, facilitating the entry of the alga, must be monitored. Ensure that the milking system and operations carried out by the staff during milking process are correct. Pre- and post-milking teat disinfection should be carried out using effective products approved for this application. For pre-milking disinfection, Grupo OX recommends using PRE-OX Mousse, which ensures maximum skin care without sacrificing total biocidal efficacy. For post-dipping process, BLUE-OX is recommended. Both products contain compounds based on active oxygen to ensure their effectiveness against micro-organisms of interest, including Prototheca.

This work protocol will help prevent entry of Prototheca in dairy cattle farms and, in places where the presence of the alga has been detected, will prevent Prototheca spreading. Considering the significant economic losses that Prototheca may cause, it is not surprising that intelligent biosecurity management has proven to offer an economically profitable opportunity for improvement in the short, medium and long term. ■

References are available from the author on request