

***Prototheca* spp. – an emerging udder health problem on dairy farms**

Ing. Kristína Tvarožková, PhD.

Slovak University of Agriculture in Nitra

Introduction

Mastitis, an inflammation of the mammary gland, is the most prevalent disease in dairy cattle worldwide. Economic losses are incurred through negative impact on milk production and its quality, animal welfare, and culling of affected animals or indirectly from treatment (Jagielski et al., 2019b; Libisch et al., 2022; Tvarožková et al., 2025). The etiological spectrum of mastitis in cows is broad and includes viruses; bacteria (streptococci, staphylococci, and enterobacteria); fungi, particularly yeasts; and non-photosynthetic, yeast-like algae of the genus *Prototheca* (De Vliegher et al., 2012; Holko et al., 2019).

Prototheca has recently been causing concern among dairy farmers. *Prototheca* is a colorless single-celled algae that is commonly found in the environment, especially in damp places—such as soil, barn floors, and animal bedding (Beinhauerova et al., 2023). *Prototheca* is a type of colorless algae that lacks chlorophyll and can cause mastitis. The first case of mastitis caused by this type of algae was recorded in 1952 (Lerche, 1952). Infections caused by *Prototheca* are usually subclinical—detectable based on high somatic cell counts and the presence of *Prototheca* in milk. In cases of clinical mastitis, it manifests in both acute and chronic forms (Shave et al., 2021). The first occurrence of *Prototheca* spp. in Hungary was recorded between 1997 and 1999 in a study by János et al. (2000). János et al. (2001) identified *Prototheca zopfii* in 1.6% of milk samples from cows with mastitis in Hungary. According to studies by Jagielski et al. (2019a) and Krukowski et al. (2020), *Prototheca* is responsible for 1.2% to 1.3% of mastitis cases in cows in Poland. Mastitis caused by *Prototheca* has been reported worldwide, in countries such as the United States (Anderson and Walker, 1988), Canada (Pieper et al., 2012), Belgium (Lagneau, 1996), Denmark (Aalbaek et al., 1998), Germany (Möller et al., 2007), Italy (Buzzini et al., 2004), Brazil (Bueno et al., 2006), and Japan (Osumi et al., 2008).

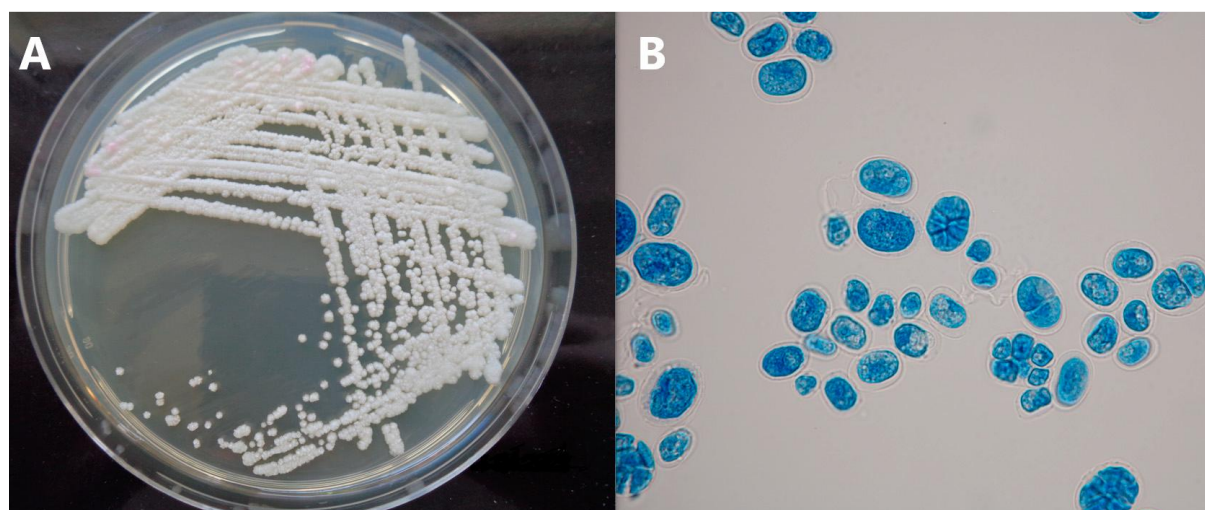


Figure1: Colonies of *Prototheca* spp. on Sabouraud dextrose agar (A), lactophenol cotton blue stained *Prototheca* spp. cells in 1000×magnification (B) (Beinhauerova et al., 2023).

National legislation

There are no legal regulations in Slovakia regarding the monitoring of *Prototheca* in the milk of dairy cows or small ruminants.

Authorities involved in legislation and investigation, national controlling bodies (who they are and what they do)

Main legislative and regulatory authorities:

- Ministry of Agriculture and Rural Development of the Slovak Republic (MARD SR): Drafts legislation (decrees, food code) and sets policy on milk hygiene.
- State Veterinary and Food Administration of the Slovak Republic (SVFA SR): The main authority for official control of food of animal origin.
- Regional Veterinary and Food Administrations (RVFA): Conduct direct inspections of primary production (small farms, milking parlors) and milk processing.
- National Reference Laboratory for Milk (NRLM) – Institute of Veterinary Medicine and Food Science (IVMFS) Bratislava/Dolný Kubín: Provides supervisory and reference laboratory analyses.
- Public Health Authority of the Slovak Republic (PHA SR): Monitors milk hygiene in food service establishments (restaurants, school cafeterias).

Activities of the authorities:

Primary production inspections (RVFA): They inspect the health of dairy cows, milking hygiene, and the cooling and storage of raw milk on farms.

Sampling and laboratory testing (NRLM/ RVFA): They analyze milk for the presence of pathogens (e.g., *Listeria monocytogenes*), antibiotic residues, somatic cells, and total microbial count.

Approval of facilities (SVFA SR): Dairies and milk collection centers must have an approval number and meet strict hygiene requirements.

Rapid Alert System (RASFF): If unsafe milk is detected, authorities ensure its withdrawal from the market.

Sales inspection: They monitor compliance with standards during the direct sale of raw milk to consumers.

Legislative Framework:

Slovak Food Code: Regulates food safety, hygiene, and composition.

Act No. 39/2007 Coll. on Veterinary Care: The basis for the control of products of animal origin.

Slovak Government Regulation No. 312/2003 Coll.: Health requirements for the production and marketing of raw milk.

European Regulations (EC) 852/2004 and 853/2004: General and specific hygiene rules.

The inspection of milk hygiene and safety is a comprehensive process that encompasses hygiene in primary production, collection, storage, and processing. The inspection system complies with EU food safety regulations, which ensure food safety from farm to consumer.

Since there is no legislation in Slovakia regulating the presence of the *Prototheca* in milk, there are no national controlling bodies here involved in drafting such legislation or conducting inspections.

Country statistics (occurrence, historical data if available to see trends, etc.)

We do not have data on the presence of *Prototheca* in milk in Slovakia, as this is not part of the standard testing protocol for identifying the causes of mastitis.

Any former research and research results (list of relevant research activities or publications, main findings)

Since the presence of *Prototheca* is not regulated by law, testing for *Prototheca* in milk is conducted only at the request of farmers and only on a minimal scale. Based on data obtained from the veterinary laboratory, we found that only two milk samples from two farms were tested for the presence of *Prototheca* during 2024, with one sample testing positive and the other negative. During 2025, the laboratory tested 14 samples for the presence of *Prototheca* in milk, and all samples tested negative.

Gancárová et al. (2025) detected the genus *Prototheca* spp. in 0.8% of bacteriologically positive goat milk samples.

Recommendations on country (and V4) level for legislation and/or control, research areas

Since the presence of *Prototheca* in milk is not monitored, I would recommend conducting a survey to assess the current situation regarding the prevalence of *Prototheca* in dairy cows. Given that *Prototheca* is resistant to standard mastitis treatments, prevention and regular laboratory testing of raw milk quality are crucial. Only after assessing the current prevalence of *Prototheca* should attention be turned to potential legislative standards for ensuring that milk is safe for consumption.

References:

- Aalbaek B, Jensen HE, Huda A. 1998. Identification of *Prototheca* from bovine mastitis in Denmark. APMIS 106:483–488.
- Anderson KL, and Walker RL. 1988. Sources of *Prototheca* spp. in a dairy-herd environment. J. Am. Vet. Med. Assoc. 193:553–556.
- Beinhauerova M, Moravkova M, Seydlova R, Crhanova M. 2023. Eradication of Bovine Mastitis Caused by the Pathogenic Microalga *Prototheca bovis* on a Dairy Cattle Farm: A Case Report. Microbiolog. Res. 14(3):1343-1352.
<https://doi.org/10.3390/microbiolres14030091>
- Bueno VF, de Mesquita AJ, Neves RB, de Souza MA, Ribeiro AR, Nicolau ES, and de Oliveira AN. 2006. Epidemiological and clinical aspects of the first outbreak of bovine mastitis caused by *Prototheca zopfii* in Goiás State, Brazil. Mycopathologia 161:141–145.
- Buzzini, P, Turchetti B, Facelli R, Baudino R, Cavarero F, Mattalia L, Mosso P, Martini A. 2004. First large-scale isolation of *Prototheca zopfii* from milk produced by dairy herds in Italy. Mycopathologia 158:427–430.
- De Vlieghe S, Fox LK, Piepers S, McDougall S, Barkema HW. 2012. Invited review: Mastitis in dairy heifers: nature of the disease, potential impact, prevention, and control. J Dairy Sci. 95(3):1025-1040. doi: 10.3168/jds.2010-4074.
- Gancárová B, Tvarožková K, Oravcová M, Uhrinčat' M, Mačuhová L, Vašíček D, Černek Ľ, Tančin V. 2025. Somatic cell count and presence of microbial pathogens in milk of goats in Slovakia. J. Dairy Res. 92(1):78-80. doi:10.1017/S0022029925000354
- Holko I, Tančin V, Vršková M, Tvarožková K. 2019. Prevalence and antimicrobial susceptibility of udder pathogens isolated from dairy cows in Slovakia. J. Dairy Res. 86: 436–439. doi:10.1017/S0022029919000694
- Jagielski T, Krukowski H, Bochniarz M, Piech T, Roeske K, Bakula Z, Wlazło Ł, Woch P. 2019. Prevalence of *Prototheca* spp. on dairy farms in Poland - a cross-country study. Microb Biotechnol. 12(3):556-566. doi: 10.1111/1751-7915.13394.
- Jagielski T, Roeske K, Bakula Z, Piech T, Wlazło Ł, Bochniarz M, Woch P, Krukowski H. 2019. A survey on the incidence of *Prototheca* mastitis in dairy herds in Lublin province, Poland. J Dairy Sci. 102(1):619-628. doi: 10.3168/jds.2018-15495.
- Jánosi S, Ratz F, Lauke T, Szigeti G, Kerenyi J, Tenk M, Katona F, Kulscár M, Huszenica G. 2000. *Prototheca zopfii* mastitis in cattle: first diagnosis in Hungary. Magyar-Allatorvosok-Lapja. 122:7–14.
- Jánosi S, Szigeti G, Rátz F, Laukó T, Kerényi J, Tenk M, Katona F, Huszenicza A, Kulcsár M, Huszenicza G. 2001. *Prototheca zopfii* mastitis in dairy herds under continental climatic conditions. Vet Q. 23(2):80-83. doi: 10.1080/01652176.2001.9695087.
- Krukowski H, Lassa H, Zastempowska E, Smulski S, Bis-Wencel H. 2020. Etiological agents of bovine mastitis in Poland. Med Weter. 76(04):221–225.

- Lagneau, P. E. 1996. First isolation of *Prototheca zopfii* in bovine mastitis in Belgium. J. Mycol. Med. 6:145–148.
- Lerche M. 1952. Eine durch Algen (*Prototheca*) hervorgerufene Mastitis der Kuh. Berl Munch Tierarztl Wochenschr. 4:64–69.
- Libisch B, Picot C, Ceballos-Garzon A, Moravkova M, Klimesová M, Telkes G, Chuang S-T, Le Pape P. 2022. *Prototheca* Infections and Ecology from a One Health Perspective. *Microorganisms*. 10(5):938. <https://doi.org/10.3390/microorganisms10050938>
- Möller, A., U. Truyen, and U. Roesler. 2007. *Prototheca zopfii* genotype 2—The causative agent of bovine protothecal mastitis? Vet. Microbiol. 120:370–374.
- Osumi, T., Y. Kishimoto, R. Kano, H. Maruyama, M. Onozaki, K. Makimura, T. Ito, K. Matsubara, and A. Hasegawa. 2008. *Prototheca zopfii* genotypes isolated from cow barns and bovine mastitis in Japan. Vet. Microbiol. 131:419–423.
- Pieper, L., A. Godkin, U. Roesler, A. Polleichtner, D. Slavic, K. E. Leslie, and D. F. Kelton. 2012. Herd characteristics and cow-level factors associated with *Prototheca* mastitis on dairy farms in Ontario, Canada. J. Dairy Sci. 95:5635–5644.
- Shave CD, Millyard L, May RC 2021. Now for something completely different: *Prototheca*, pathogenic algae. PLoS Pathog 17(4): e1009362. <https://doi.org/10.1371/journal.ppat.1009362>
- Tvarožková K, Gancárová B, Černek Ľ, Tančin V. 2025. Occurrence of mastitis pathogens and their antibiotic resistance in dairy cows in Slovakia. J. Dairy Res. 92(2):204-207. doi:10.1017/S0022029925101258