

Prevalence and distribution of *Prototheca* species among dairy herds in Poland between 2015 and 2017

Zofia Bakula¹, Łukasz Wlazło², Mariola Bochniarz³, Tomasz Piech³, Władysław Wawron³, Henryk Krukowski², Tomasz Jagielski¹

¹Department of Applied Microbiology, Institute of Microbiology, Faculty of Biology, University of Warsaw, I. Miecznikowa 1, 02-096 Warsaw, Poland

²Department of Animal and Environmental Hygiene, University of Life Sciences in Lublin, Akademicka 13, 20-950 Lublin, Poland

³Department and Clinic of Animal Reproduction, Faculty of Veterinary Medicine, University of Life Sciences in Lublin, Głęboka 30, 20-612 Lublin, Poland

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Objectives: *Prototheca* spp. are unicellular, colorless, saprophytic, yeast-like algae ubiquitously distributed in the environment, with a particular predilection for moist areas. Occasionally, the algae may cause opportunistic infections in animals and humans. Of the eight currently postulated species, five (*P. zopfii*, *P. wickerhamii*, *P. blaschkeae*, *P. cutis*, and *P. miyajii*) are described as the causative agents of protothecosis. *P. zopfii* genotype 2 and *P. blaschkeae* are among the key aetiological agents of bovine mastitis, which persists as the most common and economically important disease of dairy herds worldwide.

The aim of the study was to investigate the prevalence of *Prototheca* spp. among dairy herds in Poland.

Methods: The survey included milk samples from 108 dairy cows originating from 23 dairy herds in 13 voivodeships of Poland. Samples with a positive California mastitis test (CMT) result (152 samples, 38 cows) were plated on the *Prototheca* Isolation Medium (PIM). Furthermore, 280 control milk samples (CMT-negative) collected from 70 healthy animals were used for PIM inoculation. The plates were incubated at 37°C for 72 hours, under aerobic conditions. Each grown isolate was subjected to species identification with both phenotype-based and molecular methods. Conventional differentiation involved micromorphological characteristics and carbohydrate assimilation profiles (API 20C AUX system, Biomerieux®, France), while molecular speciation was done using genotype-specific PCR assays for *P. zopfii* genotype 1, *P. zopfii* genotype 2, and *P. blaschkeae*, as described previously (Int. J. Syst. Evol. Microbiol., 2006, 56:1419-2).

Results: CMT-positive milk samples, collected from 32 (32/38; 84.2%) cows, yielded 69 (69/152; 45.4%) *Prototheca* isolates. For 6 (6/38; 15.8%) cows with mastitis no *Prototheca* cultures were obtained. Among isolates cultured, 67 (67/69; 97.1%) were identified as *P. zopfii* genotype 2, and the remaining two were described as *P. blaschkeae* (2/69; 2.9%). All control milk samples (280) were negative for the presence of *Prototheca* algae.

Conclusions: This study clearly shows the predominance of *P. zopfii* genotype 2 as the causative agent of protothecal mastitis among dairy herds in Poland. *P. zopfii* genotype 2 seems to possess an epidemiological advantage over *P. blaschkeae* in the transmission of the diseases.

Keywords: *Prototheca*, mastitis, dairy herds, algae