

Occurance and identification of *Prototheca* species in cow's milk in Poland



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BACKGROUND

Prototheca spp. are unicellular, achlorophyllic yeast-like algae that normally live as saprophytes and are ubiquitously distributed in nature. Of the seven currently postulated species, five (*P. zopfii*, *P. wickerhamii*, *P. blaschkeae*, *P. cutis* and *P. miyajii*) are described as opportunistic pathogens in humans and animals being the causative agents of protothecosis. The most prevalent form of animal protothecosis is bovine *mastitis*, aetiologically linked to *P. zopfii* genotype 2 and *P. blaschkeae*. The disease, whose incidence has recently been increasing worldwide, usually runs a chronic course and is characterized by a dramatic reduction in milk production of dairy cows. This entails important economic losses to the dairy industry.

The aim of the study was to investigate the prevalence of *Prototheca* spp. in milk samples collected from cows bred on different farms in 5 voivodeships of Poland between 2014 and 2015.

MATERIAL AND METODS

The survey included milk samples from 134 dairy cows originating from 7 dairy herds in 5 voivodeships of Poland. Samples with a positive California mastitis test (CMT) result (64 samples, 20 cows) were plated on the *Prototheca* Isolation Medium (PIM). In addition, 76 control milk samples (CMT-negative) collected from 19 healthy cows were used for PIM inoculation. The plates were incubated under aerobic conditions at 37°C for at least 72 h. Initial species identification was based on the micromorphology of the colonies and carbohydrate assimilation profiles, determined with the API 20C AUX system (Biomérieux®). Phenotype-based identification was then confirmed by molecular methods. Genomic DNA was extracted using Bacterial & Yeast Genomic DNA Purification Kit (EURx®). Species identification was carried out using genotype-specific PCR assays for *P. zopfii* genotype 1, *P. zopfii* genotype 2, and *P. blaschkeae*, as described by Roesler *et al.* (Int. J. Syst. Evol. Microbiol., 2006, 56:1419-2).

RESULTS

A total of 43 *Prototheca* strains were isolated, all of which came from *mastitis* cases (17 cows). From 3 *mastitis* cows (5 milk samples) *Prototheca* spp. were not cultured. Control milk samples (76) did not yield growth of *Prototheca* spp. Among 43 strains cultured, all but one were identified as *P. zopfii* genotype 2. The remaining one was described as *P. blaschkeae*.

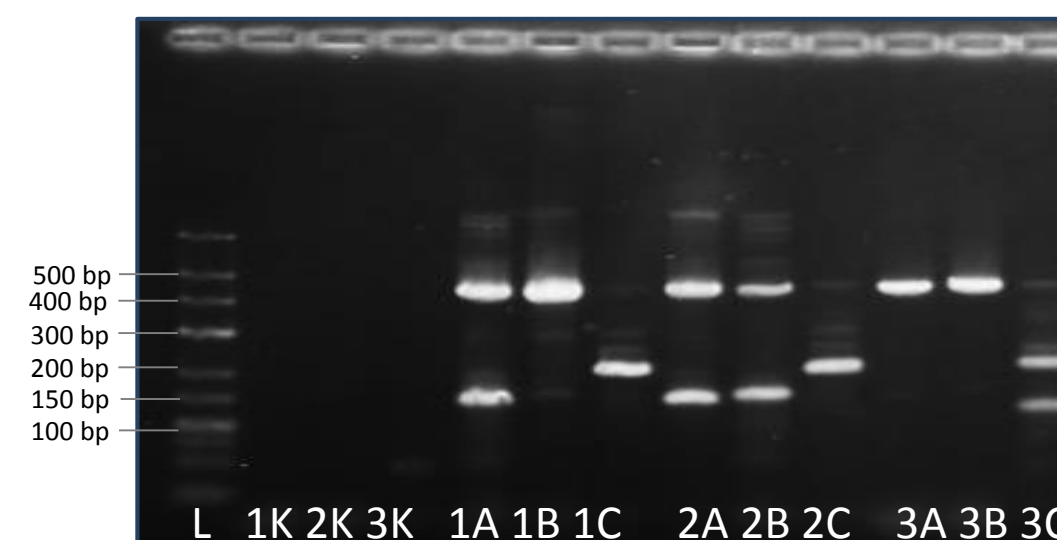


Fig. 1. Genotype-specific PCR method of identification for *Prototheca*. The amplified products were visualized on an agarose gel. Molecular pattern 1 refers to *P. zopfii* genotype 1, 2 - *P. zopfii* genotype 2, 3 - *P. blaschkeae*. A, B, C – PCR product in reaction with species-specific primers; K – control; L – ladder.

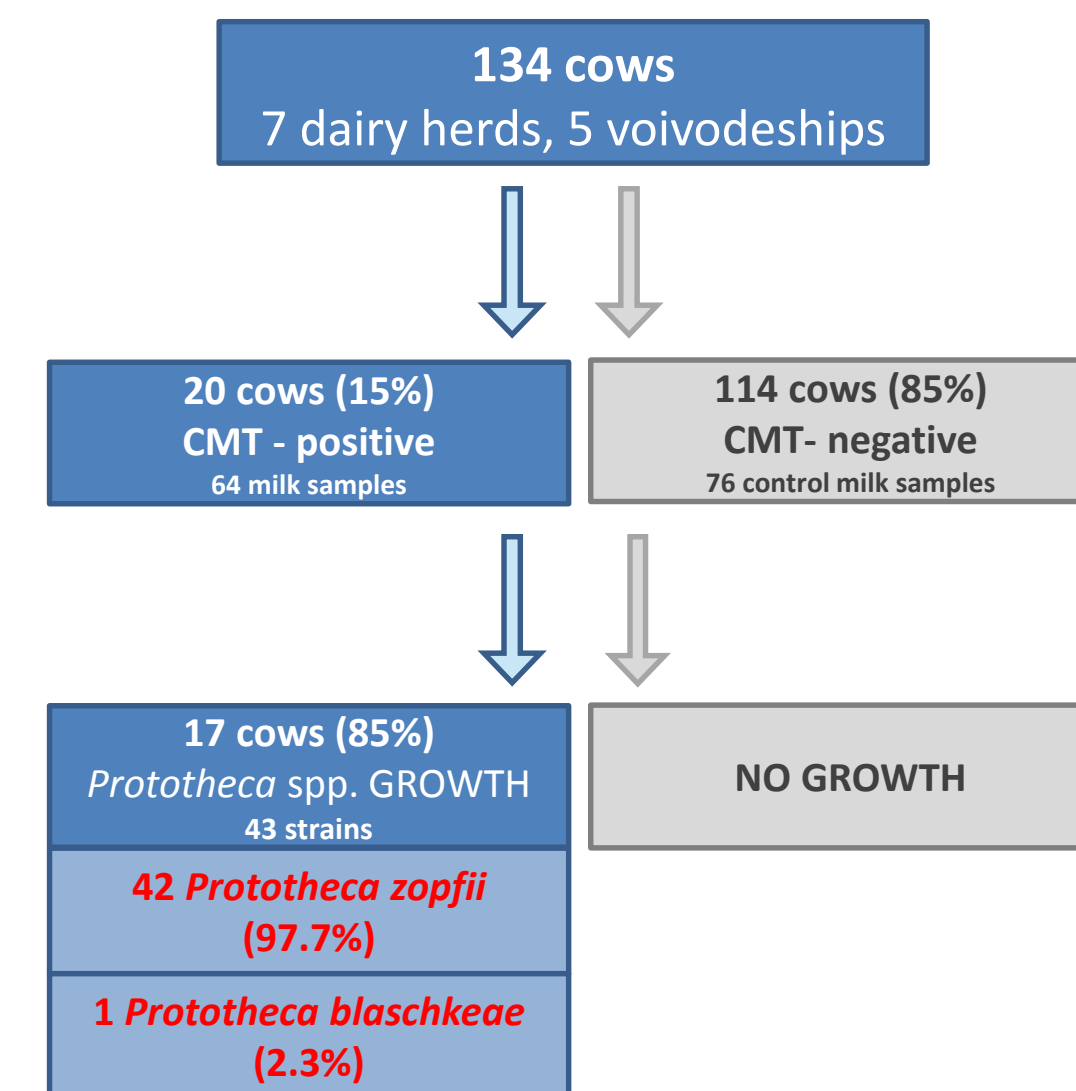


Fig. 2. Results of a total group identified during a following survey.

CONCLUSIONS

The study shows the predominance of *P. zopfii* genotype 2 as the causative agent of protothecal *mastitis* in dairy cows in Poland. This may be linked to an enhanced infectivity of *P. zopfii* genotype 2 towards bovine mammary tissue.