Clinical mastitis-related *Prototheca* infections among dairy herds in Brazil: a large-scale study

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Prototheca spp. are unicellular, non-photosynthetic algae (*Chlorophyta*, *Trebouxiophyceae*), known to cause opportunistic infections in vertebrates (protothecosis). The most common and emerging form of protothecal disease in animals is bovine mastitis, which incurs heavy economic losses to the global dairy industry, due premature culling and early replacement of infected cows.

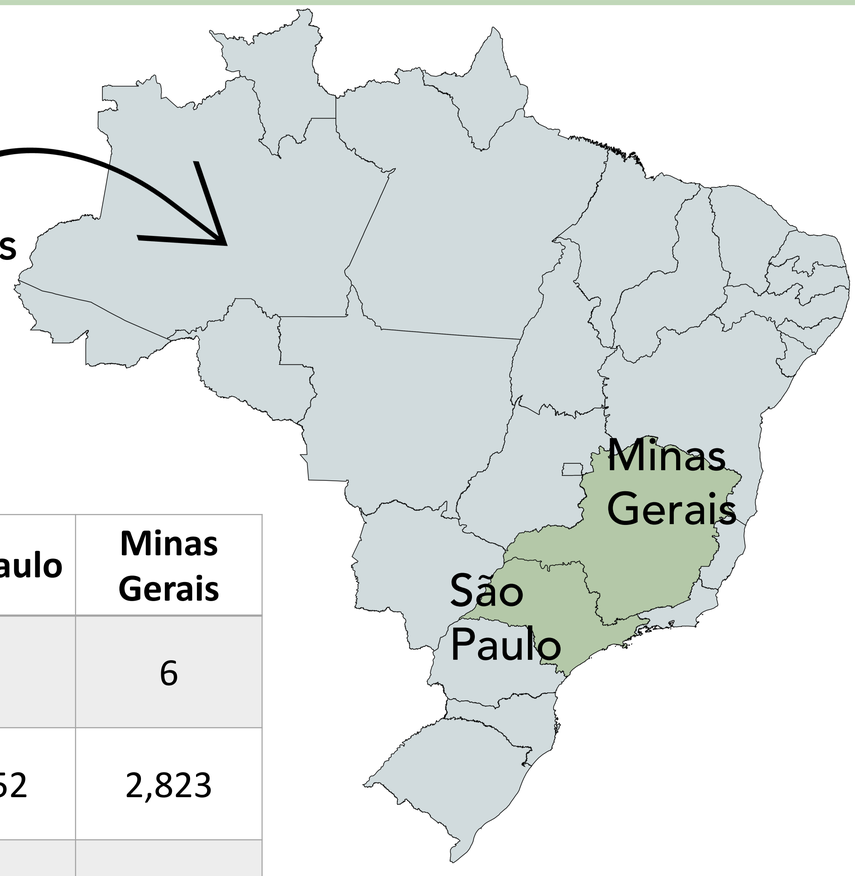
The purpose of this large-scale study was to investigate the occurrence of *Prototheca* mastitis among 4,275 cows on ten dairy herds located in São Paulo and Minas Gerais states, in the southeast region of Brazil, where dairy farming is extensively practiced.

METHODOLOGY

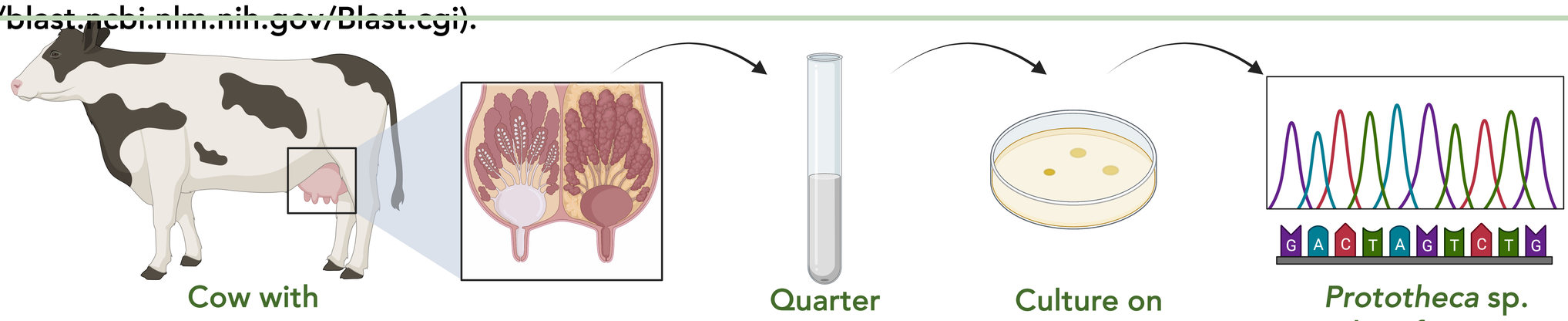
The animals were from medium-scale farms (20–200 hectares), with different average sizes of herds and breeding with similar nutrition, management, technical level, and sanitary conditions, including mandatory vaccinations. All cows from eligible farms that experienced clinical mastitis between 2017-2019 were sampled.

Quarter milk samples from a total of 4,275 cows with clinical signs of mastitis were collected aseptically and cultured on sheep blood agar (SBA) for the isolation of pathogens. The *Prototheca*-like colonies were subcultured and subjected to species identification by *CYTB* gene-based sequencing. Assembled sequences were analysed with the Prototheca-ID web application (https://prototheca-id.org) and BLAST database (https://blast.ncbi.nlm.nih.gov/Blast.cgi).

Total:
-10 farms
-4,275 cows with diagnosed mastitis



Characteristic	São Paulo	Minas Gerais
No. of tested farms	4	6
No. of cows with mastitis	1,452	2,823
Total no. of cows	1,725	2,507
Milk production – L/day	53,700	78,926

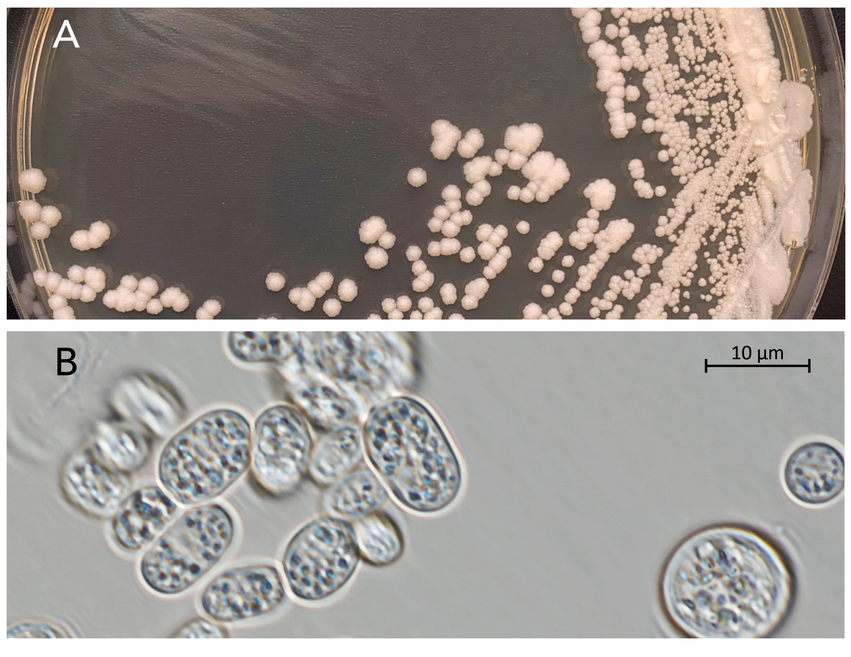


Cow with mastitis → Quarter milk → Culture on SBA → *Prototheca* sp. identification

RESULTS

Among all cows sampled, 44 (1.03%) yielded as many strains, which were phenotypically compatible with *Prototheca* species, were cultured from 3 dairy farms under the study. All cultured isolates were confirmed as *P. bovis*, upon molecular identification.

Overall, the study demonstrates the importance of bovine mastitis due to *Prototheca* algae in Brazil, pointing to *P. bovis* as the major etiological agent.



P. bovis SAG 2021 (T) strain: A – colonies on SDA medium; B – microscopic morphology (x 100).