Risk factors for *Prototheca* mastitis on Ontario dairy farms

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Introduction

Prototheca spp are unicellular, colorless algae that can cause incurable mastitis in dairy cows. *Prototheca* mastitis has been reported in many regions including North and South America, Europe, and Asia. Mastitis caused by these algae is rare, and knowledge about the risk factors for *Prototheca* mastitis is lacking. Anecdotal reports have suggested that improper milking hygiene, unsanitary environmental conditions, and the use of intramammary antimicrobial treatment may influence the development of the disease. However, scientific evidence in support of these risk factors has often been weak because of very small sample sizes or lack of comparison groups. In Ontario, mastitis caused by *Prototheca* spp appears to be an emerging disease, which leaves veterinarians and farmers with many questions and challenges. The aim of this case-control study was to formally assess herd-level risk factors for *Prototheca* mastitis on Ontario dairy farms.

Materials and Methods

Between January and May 2011, 23 Ontario dairy herds that had had ≥ 2 cases of *Prototheca* mastitis within the two previous years were enrolled in the study by their herd veterinarians. For each case herd, veterinarians also submitted four proposed control herds (matched to the case herd on herd size and barn type (free-stall or tie-stall)), of which one was randomly chosen to participate in the study. Case and control herds were visited once during milking. During the visit, composite milk samples were collected from each lactating cow and a management questionnaire was administered to the producer. The milk samples were submitted to the Animal Health Laboratory, University of Guelph (Canada). *Prototheca* spp and other mastitis pathogens were identified in accordance with the NMC guidelines. The questionnaire developed by the research team contained 54 multiple choice or short-answer questions regarding herd characteristics and management, milking technique and hygiene, medication use, and veterinary services. Statistical analyses were conducted with STATA 10.1 (Stata Corp, Texas, USA). Questionnaire data were screened for variability between case and control herds by means of descriptive statistics, univariate logistic regression, and Mann-Whitney *U* tests. Significant variables were tested for collinearity, and a multivariable logistic regression model was built by manual forward selection. Linearity of continuous variables was assessed and transformations performed accordingly. The Hosmer-Lemeshow goodness-of-fit test (five groups, $P = 0.3571$) and residual analyses were conducted to assess the model fit.

Results

Case and control farms all had Holstein-Friesian dairy cows and were similar to the majority of Ontario dairy herds in terms of herd size and milk yield. Of the 2, 428 (case, n = 1,229; control, 1,199) milk samples cultured, 83% yielded negative results. Sixty-four milk samples from 18 different case herds yielded *Prototheca* spp. The mean within-herd prevalence of *Prototheca* spp for case herds was 5.1% (range, 0% to 12.5%). The final multivariable model for herd-level risk factors associated with *Prototheca* mastitis included: intramammary injection of a non-intramammary drug (OR, 137; 95% confidence interval (CI), 5 to 3,464), number of different injectable antimicrobials used (OR, 2.82; 95% CI, 1.04 to 7.65), use of a teat sealant (external teat sealant OR, 80; 95% CI, 1.1 to 5,765.9; internal teat sealant OR, 34; 95% CI, 2.2 to 526.8), and having treated > three displaced abomasums in the previous 12 months (OR, 41; 95% CI, 2.7 to 628.3).

Significance

The results indicate that off-label intramammary administration of injectable drugs, antimicrobial treatment, and multiple and possibly unsanitary intramammary infusions were associated with an increased risk of *Prototheca* mastitis. Therefore, *Prototheca* spp is most likely an opportunistic pathogen. A producer-veterinarian relationship should be established, treatment protocols developed, and proper intramammary infusion hygiene maintained in order to minimize the risk of *Prototheca* mastitis.