Field Evaluation of the Pathoproof Mastitis PCR Assay for the Detection of *Staphylococcus aureus* Infected Cows Using DHI Samples

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Introduction

Staphylococcus aureus is one of the most important etiological agents of chronic, clinical, and subclinical mastitis (4). A number of factors such as its cyclical nature of shedding make the detection of this pathogen difficult, and often require that cows be sampled on multiple occasions (5). Furthermore, issues related to the cost of sampling and impact of sample handling on quality of culture results has limited the widespread use of routine milk culture as a monitoring tool for mastitis control. Due to the limitations of culture-based testing for etiological agents such as *S. aureus*, the need exists for an alternative or complementary test. This study investigated the utility of a commercial PCR test (PathoProof Mastitis PCR Assay) (1) as a diagnostic tool for the identification of *S. aureus* directly from metered DHI milk samples.

Materials and Methods

Five herds located in Southern Ontario were chosen to participate in this study. These herds had a known history of intra-mammary infections due to *S. aureus*. Milk samples were collected from a total of 230 lactating cows based on a defined protocol. Both hand-stripped composite samples and a standard DHI metered milk samples were taken from each cow. The hand-stripped composite samples were collected following NMC recommended aseptic sampling techniques (3). These milk samples were analyzed using conventional bacterial culture methods at the Animal Health Laboratory, University of Guelph, Canada. As part of the normal laboratory protocol, if no growth is observed after 24 hours on the bacterial culture the sample that has been incubated overnight is re-plated. This plate is incubated and read after 24 hours.

The metered milk sample was collected through a milk metering device into a standard sample vial containing the preservative Bronopol, as is routinely performed by DHI field staff during milk recording sampling. This sample was analyzed using the PathoProof Mastitis PCR Assay. All PCR analyses were performed at the Finnzymes laboratory in Finland.

Agreement between the culture results from hand-stripped composite sample and the PCR result from the metered preserved composite sample was evaluated using the Kappa statistic. Assuming that culture is the 'gold standard' for *S. aureus* identification in milk samples, the relative sensitivity and specificity of PCR were calculated and reported.

Results and Discussion

There was a very high level of agreement between these two sets of results (Kappa=82%). Considering the composite culture as the gold standard, the PCR assay had a relative sensitivity of 94.1% and a relative specificity of 94.8% in identification of *S. aureus* from metered DHI milk samples. In 12 cows there was disagreement between the two methods and sample types. Follow-up was performed to determine these animals' true *S. aureus* status. For two animals, results obtained by culture were positive but those obtained by PCR were negative, while for the other 10 cases the result obtained by culture was negative but by PCR positive. Of these latter 10 cows, 7 were re-visited, sampled and cultured (these 7 were still in the lactating herd; the other 3 had either been dried off and treated with intramammary antibiotic or had been culled). These repeat cultures identified three cows as *S. aureus* positive, suggesting that for these cows the initial negative culture results were likely false-negatives and that PCR may have a higher sensitivity than composite culture. The other four cows were culture negative at the repeat visit, suggesting that either the initial PCR positive result was a false positive, that the cows had cured and were no longer infected with *S. aureus*, or that the cows were still infected but shedding very low numbers of bacteria in milk.

A concern regarding the use of metered milk samples for PCR testing was that the potential existed for carry-over of *S. aureus* DNA from one sample to the next via the milking equipment or meter (2). In all except a single case could the possibility of carry-over be ruled out.

This study suggests that the individual cow *S. aureus* results that can be obtained rapidly by PCR analysis using existing DHI samples was of equal diagnostic value as those obtained by traditional culture methods.

References

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