BULK TANK MILK SURVEILLANCE FOR CONTAGIOUS MASTITIS PATHOGENS: COMPARISON OF TWO COMMERCIAL REAL-TIME PCR TEST KITS

Erik Rattenborg[1], Carl O. Paulrud[1], Søren K.S. Jensen[2], and Jørgen Katholm[3]

[1]Knowledge Centre for Agriculture, Cattle, Aarhus N, Denmark
[2]Eurofins, Vejen, Denmark
[3]DNA-Diagnostic, Risskov, Denmark

Introduction

As part of the Danish surveillance program for *Streptococcus agalactiae,* bulk tank milk (BTM) samples from all dairy herds are tested annually. Since 2009 the bulk tank samples has been analyzed using real-time PCR PathoProof™ Mastitis assay (Thermo Fisher Scientific, Vantaa, Finland). In 2009 and 2010 the analyses were carried out with the Complete 12 Kit and in 2011-2013 with the Complete 16 Kit. In September 2014 the real-time PCR Mastit 4B (DNA-Diagnostic, Risskov, Denmark) was made commercially available. The objective of this study was to compare the PathoProof™ Mastitis Complete 16 Kit with the Mastit 4B kit regarding the detection of four species of bacteria tested by both tests (*Staphylococcus aureus*, *Streptococcus agalactiae*, *Streptococcus uberis* and *Mycoplasma bovis*). The comparison reveals essential knowledge that may be used in designing the future surveillance program.

Material and Methods

As part of the annual screening of all BTM samples in Denmark in October 2014, initially 328 samples were collected for the comparison study. All samples were transported on ice to the laboratory Eurofins (Vejen, Denmark) and there analyzed at the day of arrival. Analyses were carried out using two commercially available real-time PCR tests, PathoProof™ Complete 16 Kit and Mastit 4B. The results, Ct values, of both tests for the four bacteria *Staph. aureus*, *Strep. agalactiae*, *Strep. uberis*, and *Mycoplasma bovis* were submitted to the Danish Knowledge Centre for Agriculture, Cattle, for statistical analyses. For both tests a cut off value of $Ct \leq 37$ was used as positive. The results were analyzed by common statistical methods calculating Kappa values for test agreements and McNemar's test for independence.

Results

The results of the analyses are shown in the 2x2 tables 1A-D. In table 1A the results of the comparison between the two tests for *Strep. agalactiae* are shown. The prevalence according to the PathoProof test is 8.5%. The prevalence according to the Mastit 4B test is 7.9%. Both tests are positive in 5.8% of the samples. This reveals a Kappa value of 0.68 with a 95% confidence interval between 0.53 and 0.83 which can be considered as a moderate to high agreement. There is no significant dependency in the table the p-value of the McNemar's test being 0.62.

In table 1B the test results for *Strep. uberis* are shown. The prevalence according to the PathoProof test is 53.7%. The prevalence according to the Mastit 4B test is 73.2%. Both tests are positive in 48.2% of the samples. This reveals a Kappa value of 0.37 with a 95% confidence

interval between 0.28 and 0.46 which can be considered as a low agreement. This is the only test with significant dependency in the table the p-value of the McNemar's test being less than 0.01.

In table 1C the test results for *Staph. aureus* are shown. The prevalence according to the PathoProof test is 69.5%. The prevalence according to the Mastit 4B test is 66.5%. Both tests are positive in 56.1% of the samples. This reveals a Kappa value of 0.45 with a 95% confidence interval between 0.35 and 0.56 which can be considered as a moderate agreement. There is no significant dependency in the table the p-value of the McNemar's test being 0.26.

In table 1D the test results *Mycoplasma bovis* are shown. The prevalence according to the PathoProof test is 1.5%. The prevalence according to the Mastit 4B test is 2.1%. Both tests are positive in 0.6% of the samples. This reveals a Kappa value of 0.32 with a 95% confidence interval between -0.03 and 0.67 which can be considered as a low agreement. There is no significant dependency in the table the p-value of the McNemar's test being 0.48.

Table 1A, B, C, and D: Comparison of real-time PCR results[1] from tests with PathoProof Mastitis Complete 16 Kit and Mastit 4B for A: *Strep. agalactiae*, B: *Strep. uberis* C: *Staph. aureus* and D: *Mycoplasma bovis*.

A: *Streptococcus agalactiae*

Pathoproof	Mastit 4B	
	Positive	Negative
Positive	19	9
Negative	7	293

B: *Streptococcus uberis*

Pathoproof	Mastit 4B	
	Positive	Negative
Positive	158	18
Negative	82	70

C: *Staphylococcus aureus*

Pathoproof	Mastit 4B	
	Positive	Negative
Positive	184	44
Negative	34	66

D: *Mycoplasma bovis*

Pathoproof	Mastit 4B	
	Positive	Negative
Positive	2	3
Negative	5	318

[1]Cut off value of Ct $\leq$37 used as positive.

Discussion

Concerning the test for *Strep. agalactiae* in BTM the agreement between the two tests is moderate to high in this study. However, the difference in the number of positive samples between the two tests is minor and not significant.

Concerning the test for *Staph. aureus* the agreement between the two tests is moderate in this study. Whether this indicates a slightly higher sensitivity of the PathoProof test or a lower specificity is not known. This requires further investigation, eg. sequencing of the involved strains of bacteria. Concerning the test for *Strep. uberis* the agreement between the two tests is moderate. The Mastit 4B test seems somewhat more sensitive, but again whether this could be a sign of a lower specificity needs further investigation. Concerning the test for *Mycoplasma bovis* the agreement between the two tests is low. The direction of the performance for this organism cannot be assessed with these results.