

Diagnostic of intra-mammary bacterial infections – comparison between a PCR assay and culturing

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Why PCR assay?

- Quicker
- Can identify DNA from non viable bacteria
- More objective
- Can analyse milk samples where a preservative has been added



Why not?

- Identifies DNA from both living and dead bacteria
- More difficult to interpret if contaminated (?)



The projekt

Aim

- to determine the diagnostic properties of a multiplex real-time PCR assay, when analyzing whole udder samples, compared to culturing of quarter milk samples
- to investigate associations between bacterial findings and test-milking somatic cell counts (SCC)



The projekt

M&M

- Quarter milk samples were collected aseptically from the foremilk
 - Analyzed by routine bacteriology
- Whole udder samples were taken according to normal test-milking procedure
 - Analyzed using PathoProof™ Mastitis PCR Assay



How do you compare two tests?

- One is the "golden standard"
- Bayesian latent class analysis
 - Information from all studied tests are used
 - Previous knowledge about the tests are used or best guess (expert opinion)
 - Sensitivity
 - Specificity
 - prevalence

	Disease +	Disease -
Test +		
Test -		

Bayesian latent class analysis

- Is influenced by what you put in
- Different results depending on your input
- Good method to take in to account previous knowledge

Bacterial findings

	BC+/PCR+	BC+/PCR-	BC-/PCR+	BC-/PCR-
All	246	12	594	103
<i>S. aureus</i>	54	20	47	834
<i>Str. dysgalactiae</i>	28	5	99	823
<i>Str. uberis</i>	17	14	38	886
<i>Str. agalactiae</i>	1	1	11	942
<i>E. coli</i>	1	3	77	847
CNS	102	11	614	228

Bacterial findings and SCC

	SCC < 100'		SCC ≥ 100'	
	BC+	PCR+	BC+	PCR+
All	20%	49%	80%	51%
<i>S. aureus</i>	12%	17%	88%	83%
<i>Str. dysgalactiae</i>	0%	29%	100%	71%
<i>Str. uberis</i>	16%	18%	84%	82%
<i>Str. agalactiae</i>	0%	54%	100%	46%
<i>E. coli</i>	25%	52%	75%	48%
CNS	32%	49%	68%	51%

Performance of the test

	Bacteriological culture				PCR assay			
	Se	95% PPI	Sp	95% PPI	Se	95% PPI	Sp	95% PPI
<i>S. aureus</i>	0,76	0,62; 0,89	0,98	0,968; 0,991	0,91	0,81; 0,98	0,966	0,946; 0,985
<i>Str. dysgalactiae</i>	0,82	0,72; 0,90	0,99	0,989; 0,999	0,93	0,81; 0,99	0,898	0,876; 0,918
<i>Str. uberis</i>	0,83	0,73; 0,90	0,99	0,979; 0,999	0,85	0,60; 0,98	0,96	0,947; 0,975
<i>Str. agalactiae</i>	0,26	0,18; 0,35	1,00	0,994; 1,00	0,95	0,86; 0,99	0,99	0,982, 0,997
<i>E. coli</i>	0,28	0,13; 0,48	1,0	0,991; 1,00	0,69	0,37; 0,92	0,92	0,904; 0,945
CNS	0,58	0,54; 0,63	0,88	0,86; 0,89	0,97	0,86; 1,00	0,28	0,25; 0,31

Conclusions (so far)

- You find more bacteria with PCR...
- ...but not all seems related to mastitis
- Whole udder milk samples could be analysed with PCR for screening purposes



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