



# Detection of major mastitis pathogens by real-time PCR in absence of major-pathogen growth



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## INTRODUCTION

- Determination of causative agent is important for mastitis treatment and prevention in dairy cows
- Standard culture is a convenient method for most routine labs, but sensitivity might be moderate
- In Flanders, 5,093 quarter milk samples from clinical mastitis were examined during 2013 and showed
  - No growth in 17 % of samples, and
  - Minor pathogens, only, in 12 % of samples
- This leads to frustration of dairy farmers/veterinarians, and to aversion from lab analysis



## AIM

To investigate the use of a real-time PCR-test for the detection of major pathogens in quarter milk samples from bovine clinical mastitis from which no major pathogen could be cultured (culture negative or minor pathogen only).

## MATERIALS AND METHODS

- Routine quarter milk samples from bovine clinical mastitis
  - Identification of mastitis pathogens by
    - Standard culture based on NMC procedures, and
    - Real-time PCR (Pathoproof<sup>®</sup>, Complete 16-kit, Thermo Scientific, Finland) (Table 1)
  - Analyzed in Flanders Milk Control Centre
  - Data handling:
    - Samples in which no major pathogen was cultured were further analyzed
    - Contaminated samples (> 2 pathogens) excluded
- => 75 samples included

Targets of Pathoproof <sup>®</sup> Complete 16-kit	
<i>Staphylococcus aureus</i> /species	<i>Klebsiella</i> species
<i>Streptococcus uberis</i>	<i>Serratia marcescens</i>
<i>Strep. dysgalactiae</i>	<i>Mycoplasma bovis</i> /species
<i>Strep. agalactiae</i>	<i>Trueperella pyogenes</i>
<i>Enterococcus</i> species	Yeasts
<i>Corynebacterium bovis</i>	<i>Prototheca</i> species
<i>Escherichia coli</i>	Beta-lactamase gene

Table 1: targets of Pathoproof<sup>®</sup> Complete 16-kit

		Standard culture				Total	Total
		No growth	Minor pathogen*				
Real-time PCR	Negative	17	27.9%	1	7.1%	18	24.0%
	Minor pathogen*	9	14.8%	10**	71.4%	19	25.3%
	Major pathogen	35	57.4%	3	21.4%	38	50.7%
	Total	61	100%	14	100%	75	100%

Major pathogen	Number (%)
<i>Mycoplasma bovis</i> /species	11 (28.9)
<i>Escherichia coli</i>	9 (23.7)
<i>Streptococcus uberis</i>	9 (23.7)
<i>Enterococcus</i> species	3 (7.9)
<i>Streptococcus dysgalactiae</i>	1 (2.6)
Yeasts	1 (2.6)
<i>Prototheca</i> species	1 (2.6)
Mixed infection***	3 (7.9)
<b>Total</b>	<b>38</b>

Table 2: real-time PCR classification of quarter milk samples in which major pathogens are absent based on standard culture and detected pathogens by real-time PCR.  
\* Only minor pathogens (*Corynebacterium bovis* or non-aureus staphylococci) detected; \*\* Same pathogen detected with both methods; \*\*\* 2 major pathogens present.

## RESULTS

- DNA of major pathogen(s) was detected by real-time PCR in 50.7 % of samples where major-pathogen growth was absent (Table 2)
- Besides *Mycoplasma* (unable to detect with standard culture), *Escherichia coli* and *Streptococcus uberis* are frequently present
- Although real-time PCR has high sensitivity, minor pathogens cultured could not be recovered by PCR in one sample
- A larger sample size is needed to evaluate the exact surplus of this test

**Real-time PCR (Pathoproof<sup>®</sup> PCR, Complete 16-kit) has an added value over standard culture for detection of major pathogens in quarter milk samples from bovine clinical mastitis from which no major pathogen could be cultured.**