

Development of a LAMP based assay to identify bacterial species causing clinical mastitis in cattle

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Hannah Dairy Research
Foundation

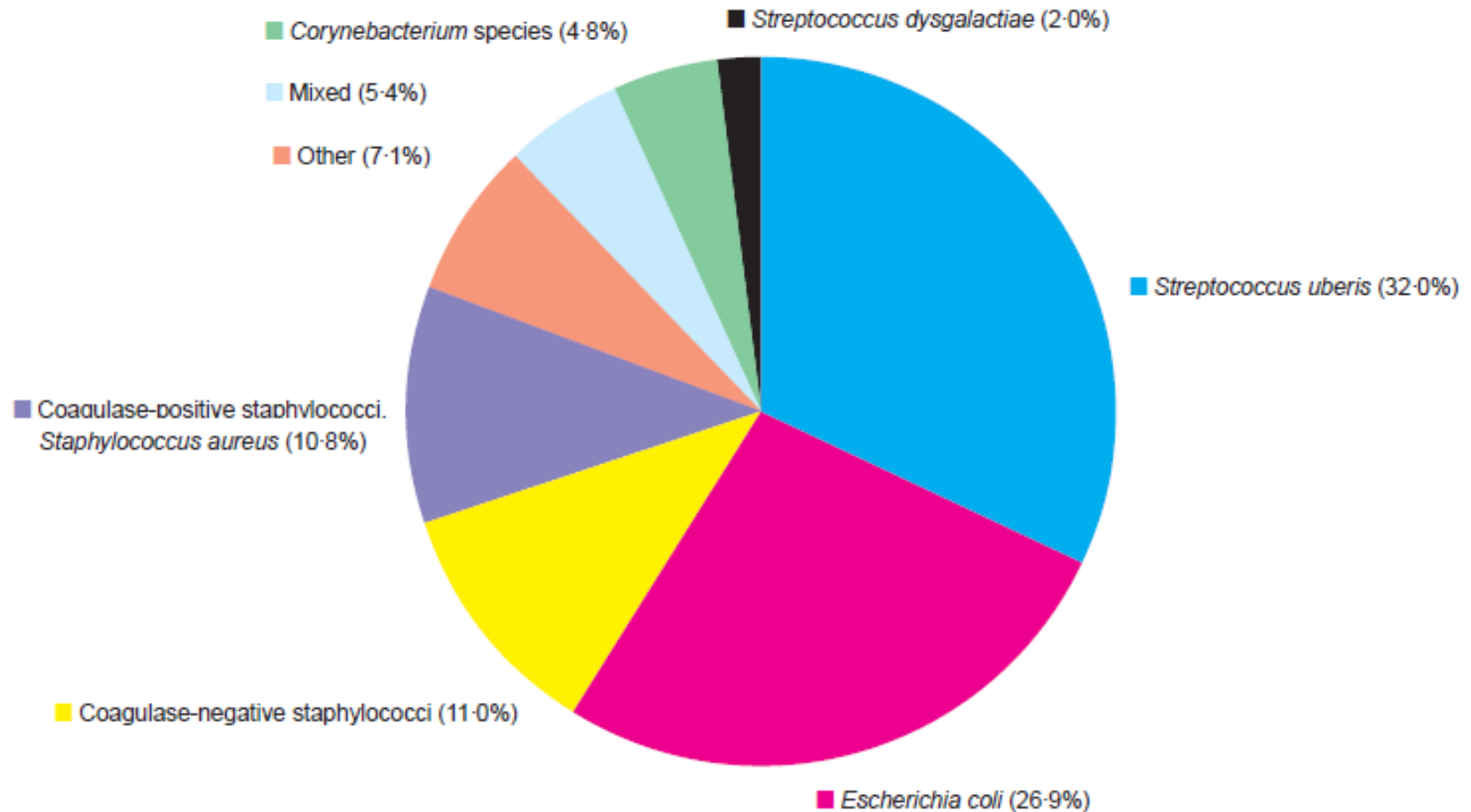


Mastitis

- Inflammation of the mammary gland caused by a bacterial infection
- Clinical or subclinical
- Requires treatment (Antimicrobials, NSAIDs)



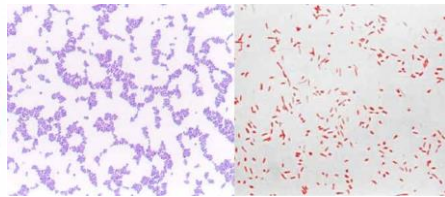
Mastitis Etiology



(Bradley *et al.*, 2007)

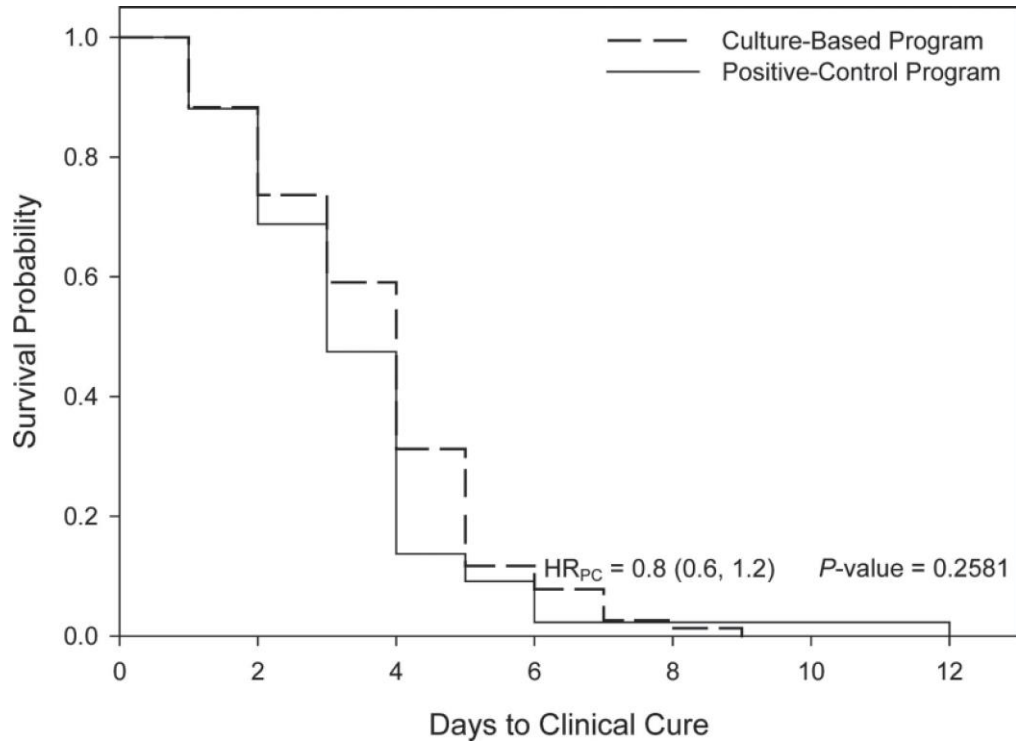
Diagnostic tools available

- Bacteriological culture
- PCR
- On farm culture (selective media)



<http://extension.msstate.edu>

Clinical Mastitis Selective Treatment (on farm culture)

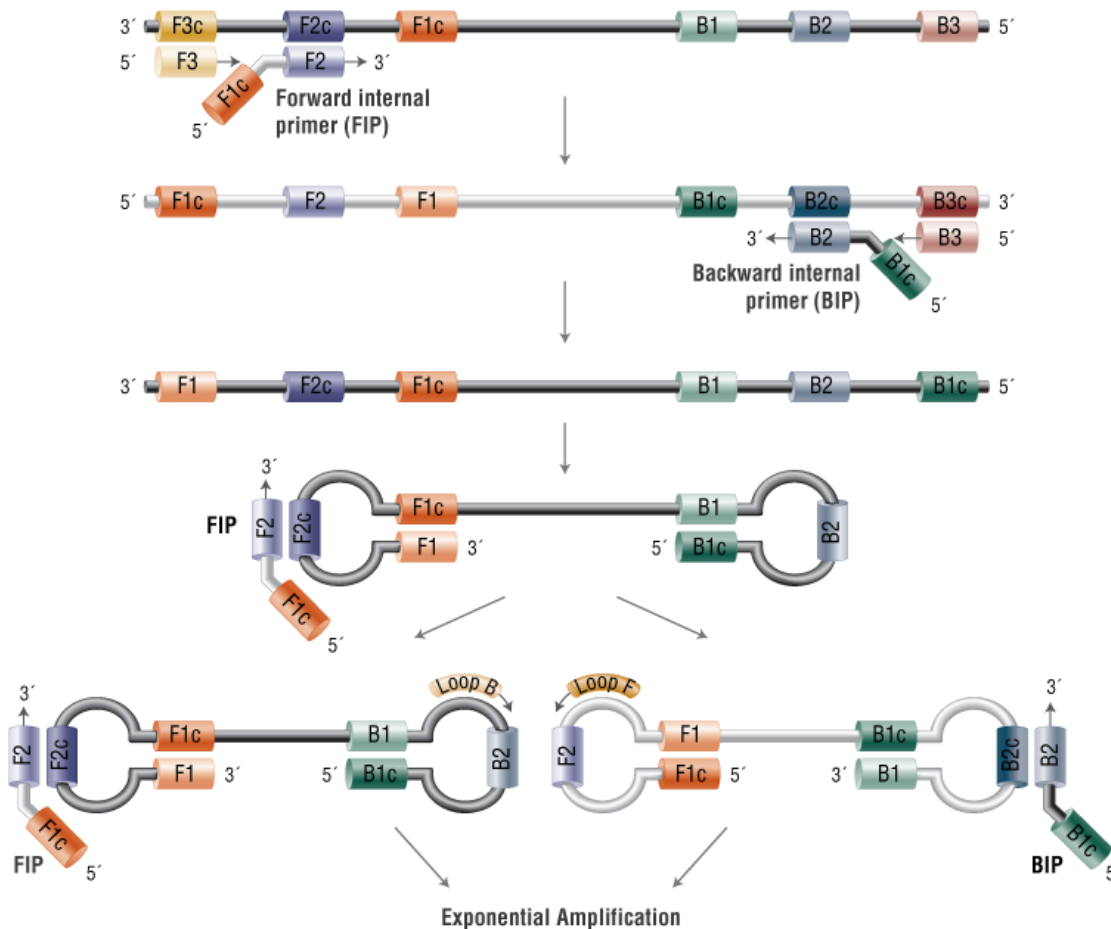


Reduction of antimicrobial usage without reducing the cure rate

(Lago *et al.*, 2011)

LAMP as pen side test?

Loop-mediated isothermal amplification



PCR vs LAMP

PCR	LAMP
Requires temperature cycling	Isothermal – single temperature (~60 °C)
Typically >1hr	Typically <30 min
yield ~ 0.2 µg	yield ~ 10–20 µg
Not amenable to visual detection	Amenable to visual detection based on turbidity colour change
Sensitive to sample matrix inhibitors	Tolerant to sample matrix inhibitors
Can be multiplexed	Difficult to multiplex

Project plan

1. Identify candidate target genes
2. Design and produce LAMP primers to target candidate genes identified in collaboration with Mast Group Ltd
3. Test the LAMP primers with DNA extracted from representative isolates
4. Test the LAMP reaction with DNA extracted from milk
5. Test alternative DNA extraction methods and the use of crude samples, where no DNA extraction takes place
6. Test alternative reporting methods, which do not require complex or expensive equipment (i.e. colour change or precipitation which may be evaluated with the naked eye)