

# Real time PCR values for mastitis pathogens – relations to milk quality and herd characteristics in Danish dairy herds

Torben Bennedsgaard, Aarhus University  
Jørgen Katholm, Knowledge Centre for Agriculture, Cattle  
Denmark

This project has been subsidised by the European Union's Agricultural Fund for Rural Development and the Danish Ministry of Food, Agriculture and Fisheries.



**KNOWLEDGE CENTRE FOR AGRICULTURE**

# SAMPLES

- Bulk tank samples collected October 2009-January 2010
- 4258 herds (all Danish dairy herds)
- Data on Somatic cell count available from all deliveries in 90% of herds. The rest once per week.
- Total Bacteria Count measured every two weeks.

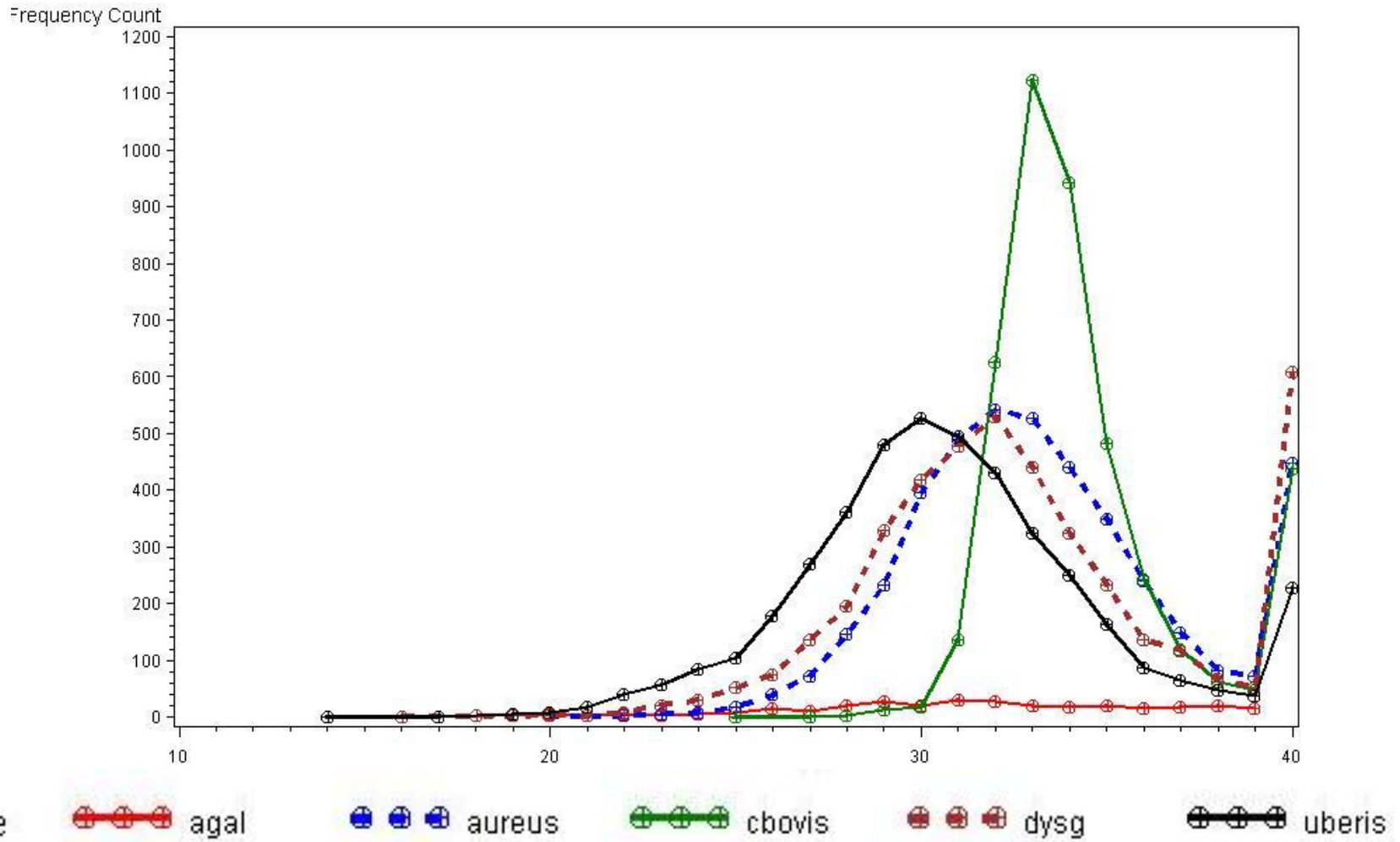
# PCR-analysis

- PathoProof® by Thermo Fisher Scientific
- 11 bacteria plus beta-laktamase
- Measured as Ct value. Low value = high concentration of bacteria.
- Ct=40 as cut-off = no infection.

## Herds with NoCt and median of herds with Ct<40

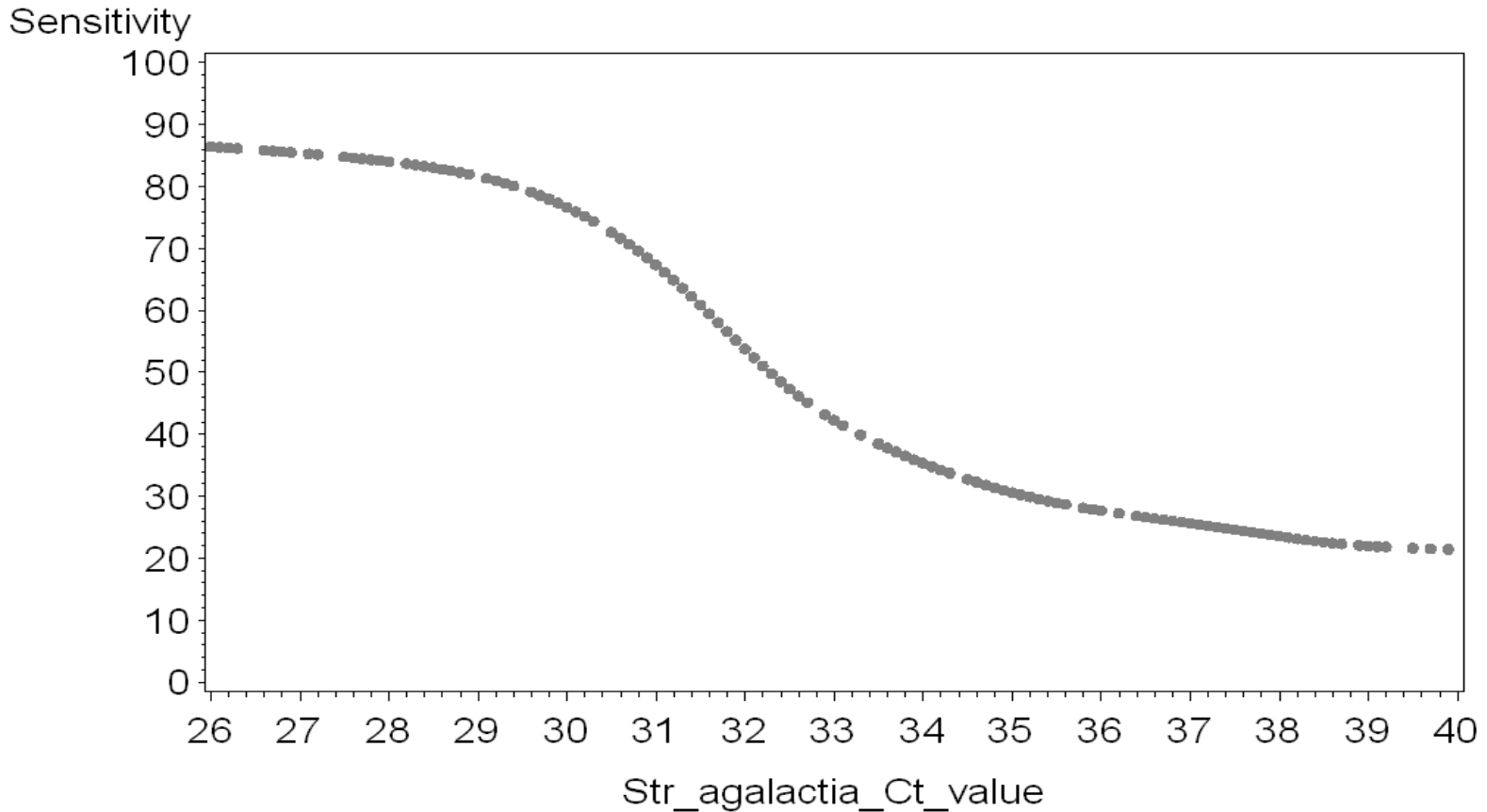
Bacteria gene	NoCt	Median (of herds with ct<40)
<i>Staph aureus</i>	9	32.5
<i>Staph spp</i>	0	29.8
<i>Beta lactamase</i>	22	34.8
<i>Str. agalactiae</i>	93	31.5
<i>Str. dysgalactiae</i>	14	31.6
<i>Str. uberis</i>	5	30.3
<i>Klebsiella spp</i>	87	36.5
<i>E. coli</i>	39	35.8
<i>Coryne bovis</i>	10	33.7

# Distribution of Ct values



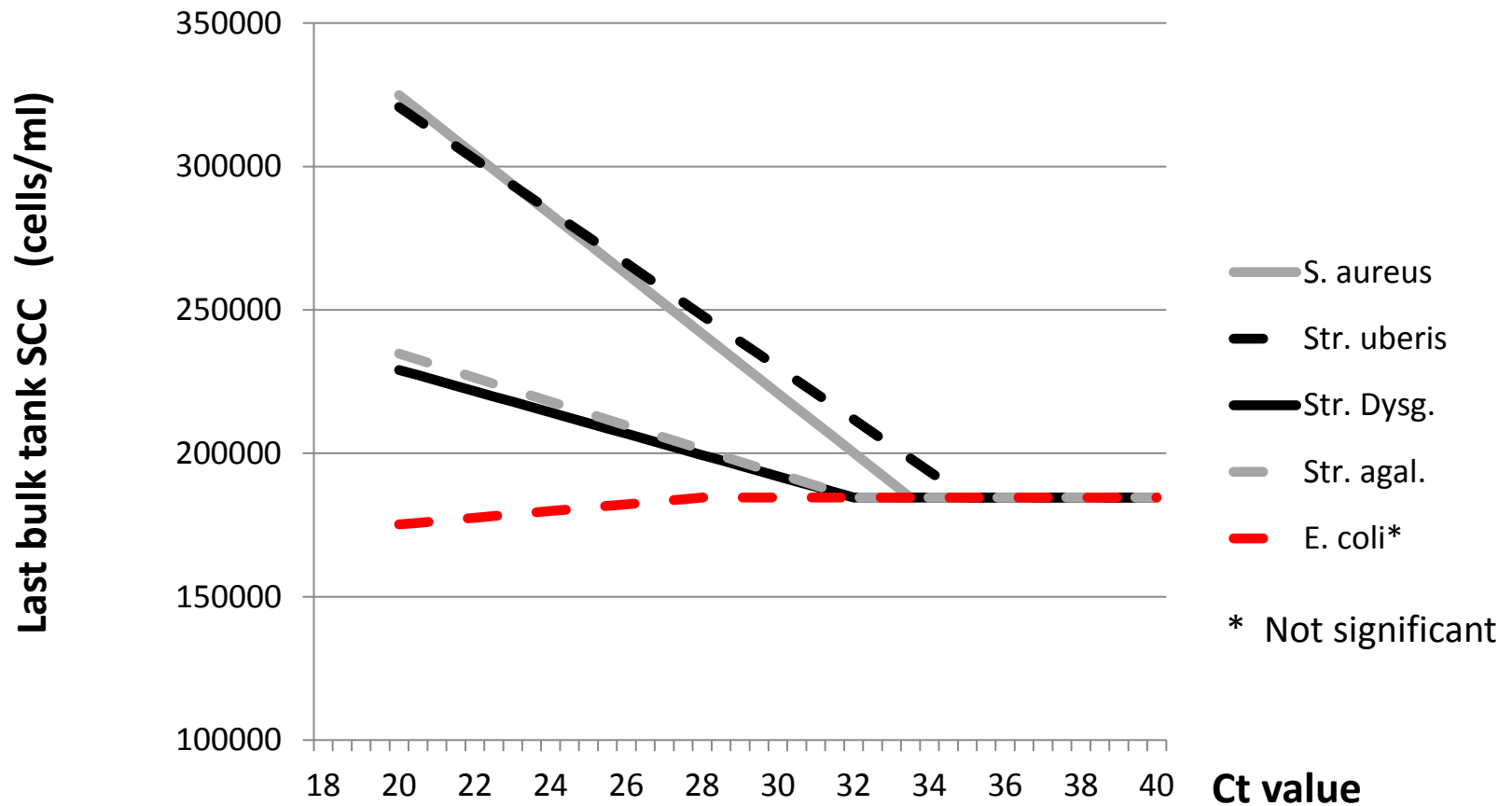
(Ct=40 for *Str. agalactiae* = 3928 outside axis)

The sensitivity of traditional culture compared to real-time PCR highly related to the Ct-value (correlated to bacterial concentration)



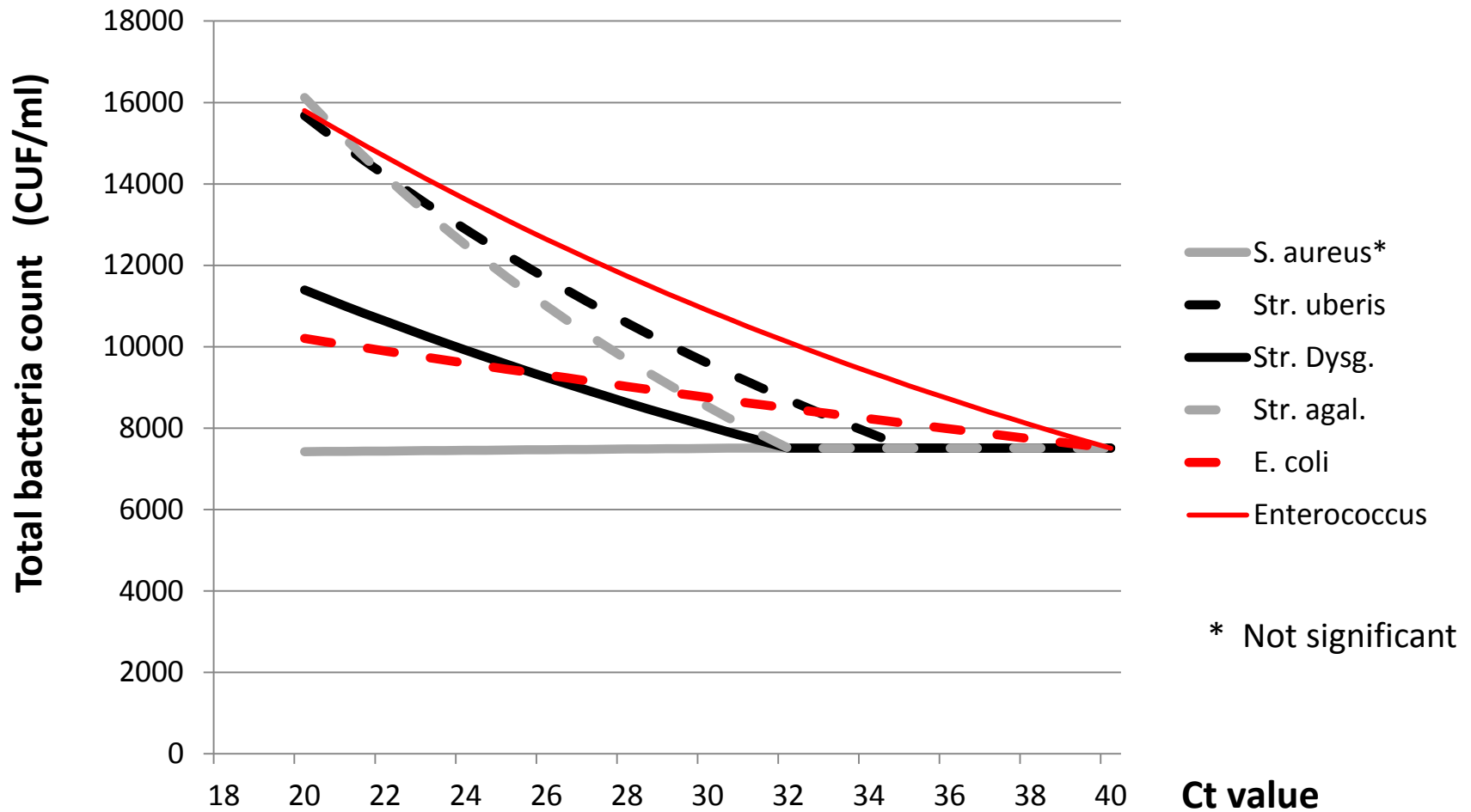
(Curve smoothed using a Generalized additive model)

# Low Ct values was associated with higher Bulk tank somatic cell count



Output from combined mixed linear model – estimate for each bacteria

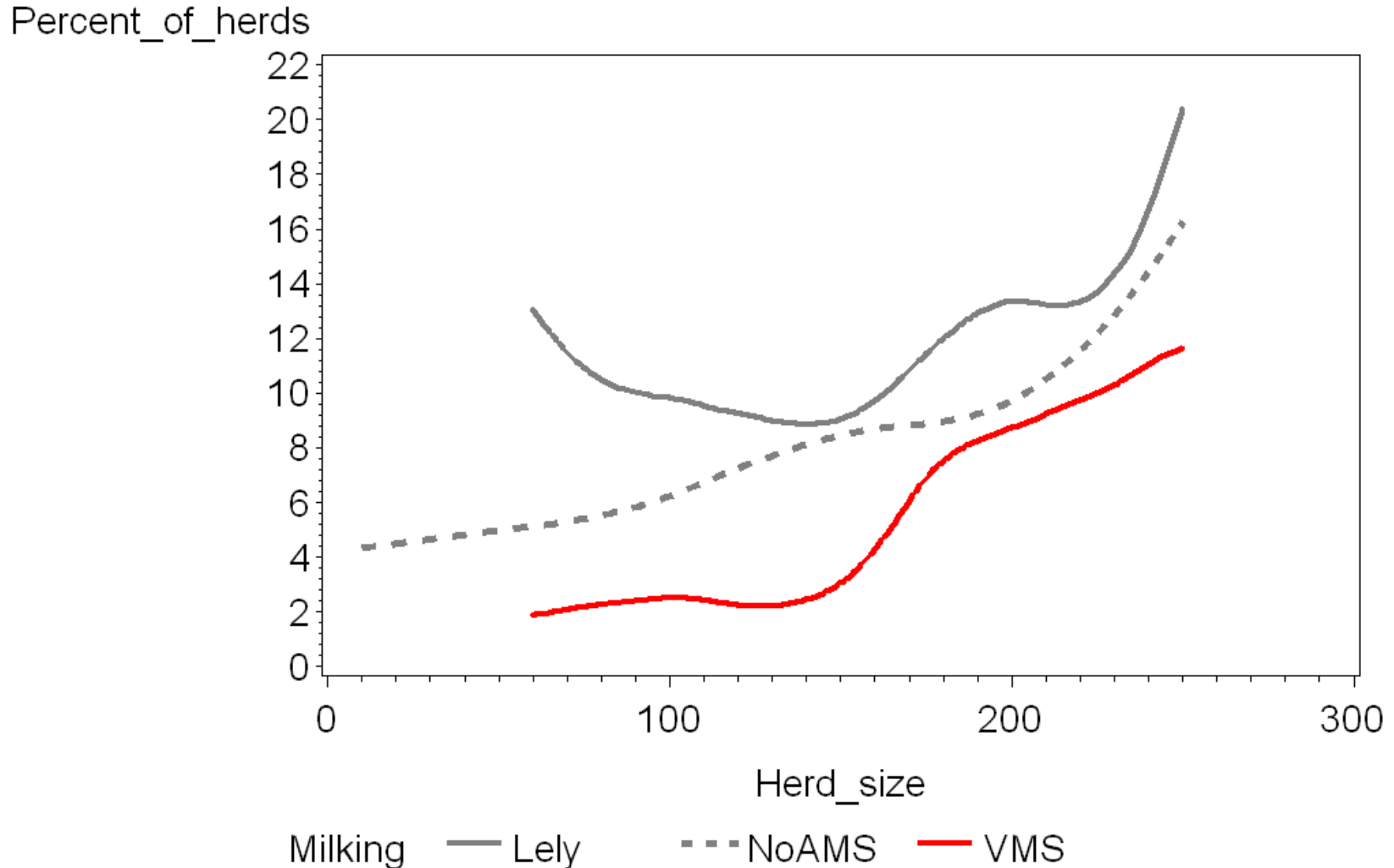
# Low Ct values was associated with higher total bacteria count



Output from combined mixed linear model – estimate for each bacteria



# Prevalence of *Str. agalactiae* increased with herd size depending on milking system.



(Curve smoothed using a Generalized additive model)

# Production system and *Str. agalactiae*

- Significantly fewer organic herds were positive for *Strep. agalactiae* compared to conventional herds ( 2.6 % compared to 8.2%).
- Only one out of 41 organic herds with AMS had a positive reaction for GBS (2.4 %).
- Not related to difference in herd size.



# Use of Real time PCR on bulk tank milk

- Low ct values of major mastitis pathogens are related to reduced milk quality
- Real time PCR are more sensitive than bacteriological culture in detecting *Str. agalactiae* in the bulk tank

