Improving real-time PCR sensitivity to bovine *Tritrichomonas foetus*

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Introduction

Tritrichomonas foetus is a flagellate protozoon parasite and the causative agent in bovine trichomoniasis, a venereal disease that causes late-term abortions in affected cows and heifers. Bulls are asymptomatic carriers and are tested via preputial samples. These samples are analyzed using either microscopic analysis or a combination of DNA column extraction and real-time PCR (qPCR). Increased sensitivity of current testing methods will allow for early detection of *T. foetus* and decrease the number of false negatives. Our study set out to quantitatively define the sensitivity of current *T. foetus* testing methods in efforts to see how little organism would yield a positive result.

Objectives

A. Discern how much extracted DNA will yield a positive Ct on qPCR
B. Determine number of organism in sample will yield positive test result
C. Confirm the extent of current processing protocols on Ct level

Methods

Study A:

- Quantified contents of Biomed InPouch™ TF using Bright-Line® Hemacytometer
- Extracted DNA from each aliquoted sample using DNA column extraction
- Performed 1:10 serial dilutions of extracted DNA
- Calculated cycle threshold (Ct) using qPCR at threshold of 0.01

Study B:

- Quantified contents of InPouch™ using Hemacytometer
- Performed 2 independent 1:10 serial dilutions (A & B)
- Extracted DNA using DNA column extraction
- Calculated Ct using qPCR at threshold of 0.01

Study C:

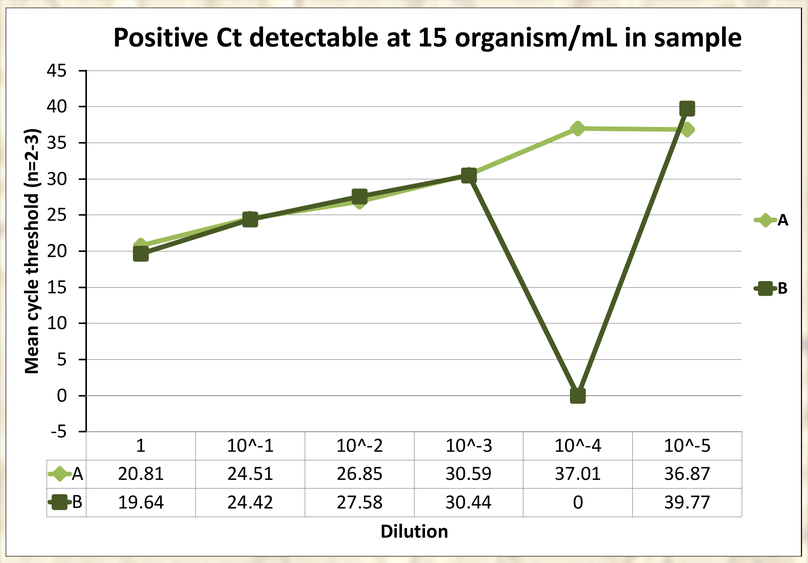
- Inoculated InPouch™ with 100µL stock *T. foetus* (n=12); store at RT (25°C) for 24 hours
- Place one group at 35°C for an additional 24 hours (n=6) while the other remains at RT (n=6)
- Collect sample every 24 hours

Data

Study A

Organism/reaction	Dilution	Ct
$2.32x10^3$	10^{-1}	20.09
$2.32x10^2$	10^{-2}	23.49
23.2	10^{-3}	26.89
2.32	10^{-4}	30.07
0.232	10^{-5}	34.06
0.0232	10^{-6}	36.65

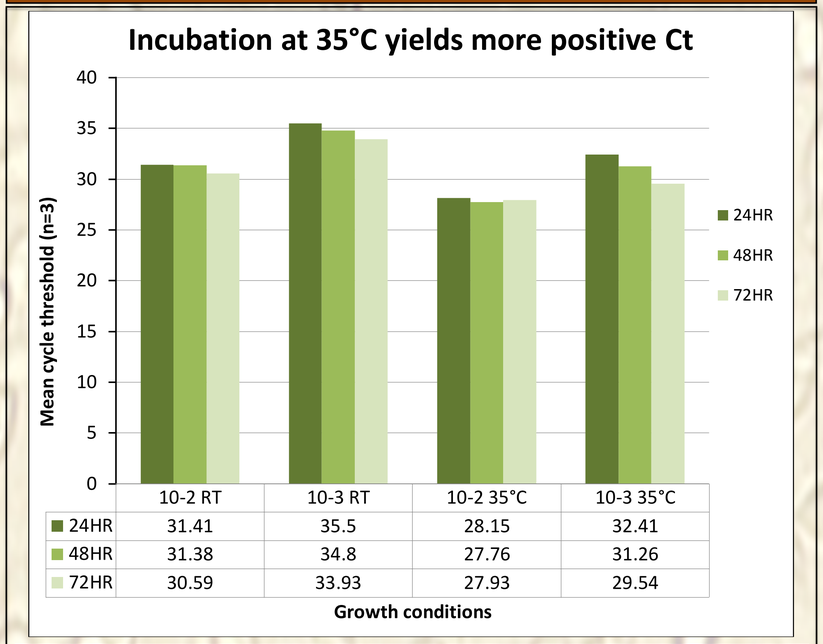
Study B



	1	10^-1	10^-2	10^-3	10^-4	10^-5
A	20.81	24.51	26.85	30.59	37.01	36.87
B	19.64	24.42	27.58	30.44	0	39.77

Dilution	Quant (organism/mL)
1	$1.50x10^4$
10^{-1}	$1.50x10^3$
10^{-2}	$1.50x10^2$
10^{-3}	15.00
10^{-4}	1.50
10^{-5}	0.15

Study C



	10-2 RT	10-3 RT	10-2 35°C	10-3 35°C
24HR	31.41	35.5	28.15	32.41
48HR	31.38	34.8	27.76	31.26
72HR	30.59	33.93	27.93	29.54

Dilution	Inoculum (organism/mL)
10^{-2}	$1.60x10^2$
10^{-3}	16.00

Conclusions

A. DNA of 0.0232 organism in reaction detectable on qPCR
B. 15 organism/mL in pouch for positive Ct after DNA column extraction and qPCR
    i. Can boost this by adding carrier RNA; carrier will increase the sensitivity in cases where there are low numbers of organism
C. Incubating samples overnight at 35°C will increase the sensitivity of the test because there is increased growth (log) when incubated at 35°C overnight

Current and future studies

We hope to continue our quantitative analysis of current *T. foetus* diagnostic methods and explore methods of increasing sensitivity of qPCR such as including carrier RNA in our DNA column extraction. Our lab is currently looking to increase the sensitivity of pooled samples.

References

McMillen, L and Lew, AE 2006. Improved detection of *Tritrichomonas foetus* in bovine diagnostic specimens using a novel probe-based real time PCR assay. Vet Parasit 141: 204-215.

Acknowledgements

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