Quantification of rickettsial load during the acute phase of experimental bovine anaplasmosis

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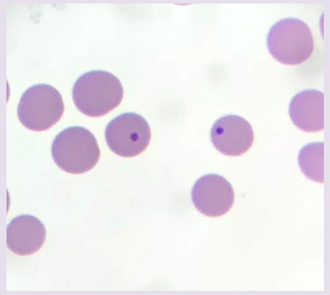
Abstract

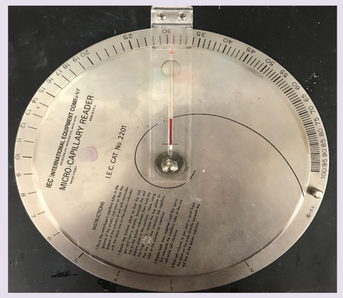
Anaplasma marginale is a prototypical tick-borne pathogen, which is the primary etiologic agent of bovine anaplasmosis. The disease has been characterized in depth, with well-established acute and subsequent subclinical phases. Although ascending levels of parasitemia (percent infected erythrocytes) are well established during the incubation through acute phases of bovine anaplasmosis, there are data indicating the highest rickettsial load in peripheral blood of a related pathogen. *Ehrlichia canis*, is not always correlated with the acute phase of canine monocytic ehrlichiosis. This discrepancy could could be due to differences in pathogen numbers within infected host cells. Thus, **the central hypothesis of this study is that total rickettsial load in the peripheral blood is directly correlated with parasitemia during the onset of acute bovine anaplasmosis**. Six calves were experimentally infected with *A. marginale*, and peripheral blood was monitored throughout transitions from incubation, acute and subclinical phases of experimental anaplasmosis *marginale*.
A related experiment will test the working hypothesis that different anticoagulants (*i.e.*, EDTA, heparin and sodium-citrate) will not affect qPCR after silica-based template isolation. This study is expected to demonstrate that parasitemia is a direct reflection of rickettsial load. Moreover, these results are expected to determine the utility of these methods for *A. marginale* transmission studies.

Background

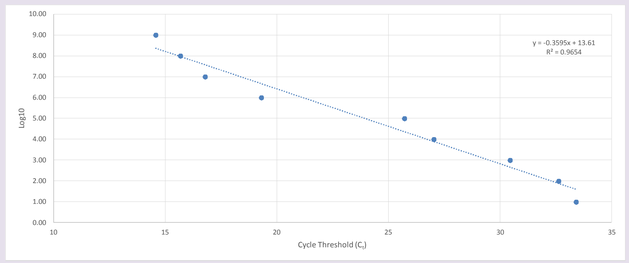
The reason for this experiment was to see how much of a bacterial load was in a cows during the time of infection. A standard curve was produced from a dilution series in order for us to compare the copy numbers we read from the qPCR. As we understood how much bacterial load we had in a cow we then compared it to parasitemia which is shown in **Figure 3**.

Methods



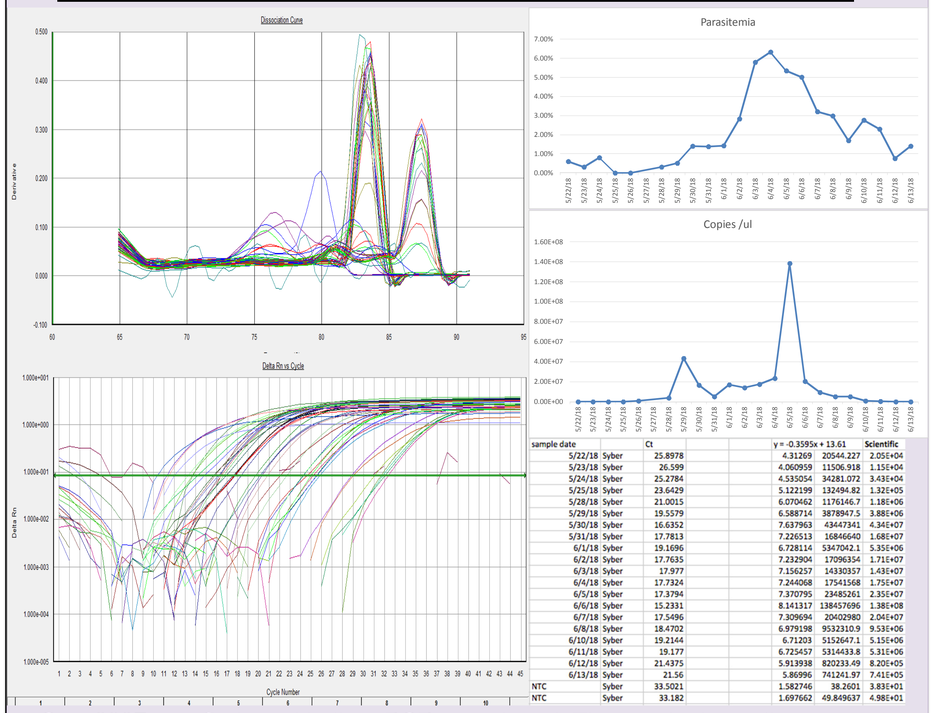


Parasitemias and packed cell volumes were measured with standard methods. Template was isolated with a silica-based spin-column kit (Roche, Indianapolis)(**Figure 1**).



Quantitative PCR(qPCR) will be used to measure rickettsial loads (**Figure 2**).

Quantitative PCR of rickettsial load compared to parasitemia



sample date		Ct		y = -0.3595x + 13.61	Scientific
5/22/18	Syber	25.8978		4.31269	20544.227	2.05E+04
5/23/18	Syber	26.599		4.060959	11506.918	1.15E+04
5/24/18	Syber	25.2784		4.535054	34281.072	3.43E+04
5/25/18	Syber	23.6429		5.122199	132494.82	1.32E+05
5/28/18	Syber	21.0015		6.070462	1176146.7	1.18E+06
5/29/18	Syber	19.5579		6.588714	3878947.5	3.88E+06
5/30/18	Syber	16.6352		7.637963	43447341	4.34E+07
5/31/18	Syber	17.7813		7.226513	16846640	1.68E+07
6/1/18	Syber	19.1696		6.728114	5347042.1	5.35E+06
6/2/18	Syber	17.7635		7.232904	17096354	1.71E+07
6/3/18	Syber	17.977		7.156257	14330357	1.43E+07
6/4/18	Syber	17.7324		7.244068	17541568	1.75E+07
6/5/18	Syber	17.3794		7.370795	23485261	2.35E+07
6/6/18	Syber	15.2331		8.141317	138457696	1.38E+08
6/7/18	Syber	17.5496		7.309694	20402980	2.04E+07
6/8/18	Syber	18.4702		6.979198	9532310.9	9.53E+06
6/10/18	Syber	19.2144		6.71203	5152647.1	5.15E+06
6/11/18	Syber	19.177		6.725457	5314433.8	5.31E+06
6/12/18	Syber	21.4375		5.913938	820233.49	8.20E+05
6/13/18	Syber	21.56		5.86996	741241.97	7.41E+05
NTC	Syber	33.5021		1.582746	38.2601	3.83E+01
NTC	Syber	33.182		1.697662	49.849637	4.98E+01

Cow 2897 was first used in the qPCR experiment. The graph shows the comparison between bacterial load and parasitemia (**Figure 3**)

Different anticoagulants (*i.e.*, EDTA, heparin and sodium0-citrate) will not affect qPCR after silica-based template isolation

EDTA

Heparin

Sodium-citrate

This experiment will be done at a later date, but these are the tubes we used to extract blood from each cow (**Figure 4**)

Conclusions

In conclusion, cow 2897 was successfully tested with qPCR then compared to its parasitemia and the outcome was that they both correlate to each other.

What's next?

We are working on finding out the hypothesis of different anticoagulants and how they will not affect qPCR after silica-based template isolation

Acknowledgements

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