Quantitation of Viable *Coxiella burnetii* in Milk Using a Liquid Medium-based MPN-PCR Assay

Key Words: Quantitation; *Coxiella*; Milk; MPN-PCR

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Supplemental File

Materials and Methods

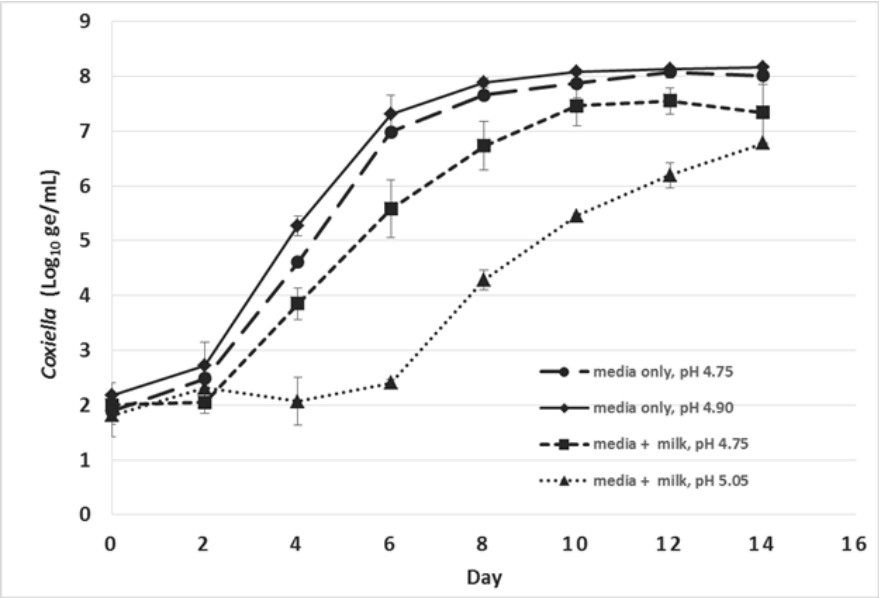
Modifications of ACCM-2 Enrichment for Improved Growth of Coxiella *in Milks*

In efforts to improve growth of *Coxiella* from certain milks either the pH or the ratio of milk to ACCM-2 medium was adjusted. For trials involving pH adjustment, milk was diluted 1:10 with ACCM-2 and the pH was adjusted to 4.75 with filter-sterilized 0.1 N HCl (Sigma-Aldrich, St. Louis, MO) prior to inoculation with *Coxiella*, to match the optimal pH for growth in pure culture. Overall, the pH levels in the milks mixed 1:10 with ACCM-2 prior to adjustment were 5.05, 5.07, 4.90 and 5.03 for bovine, goat, camel, and water buffalo milks, respectively. The effect of the initial milk to medium ratio was determined by diluting the milk 1:10, 1:100 and 1:1000 with pH 4.75 ACCM-2 medium without additional pH adjustment. In both cases, flasks were inoculated at ~4 log ge of *Coxiella* (equal to 3 log ge/ml milk) and two trials were performed with triplicate flasks in each trial.

PCR

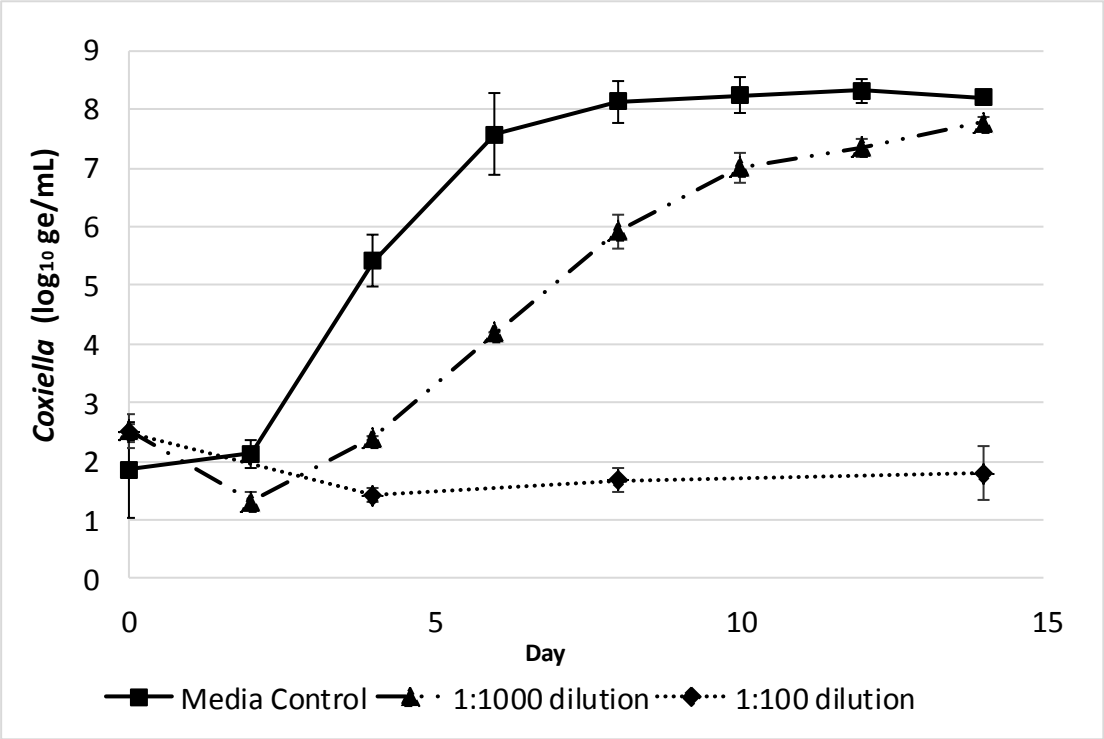
The forward primer 3'-AATTTCATCGTTCCCGGCAG-5', reverse primer 3'-GCGGCGTTTACTAATCCCCA-5' and MGB probe 3'-FAM-TGTCGGCGTTTATTGG-5' were manufactured by Applied Biosystems (Foster City, CA). Amplifications were run using the Quantitect PCR Probe mix (Qiagen, Valencia, CA) with 5 mM $MgCl_2$ and 5 µl templates in 20 µl volumes. Reactions were run on a Roche LightCycler 480 (Indianapolis, IN) with a cycling profile of a denaturation cycle at 96 °C for 15 min. followed by amplification using 45 cycles of 96 °C for 10 sec., and 60 °C for 30 sec.

Supplemental Figure 1



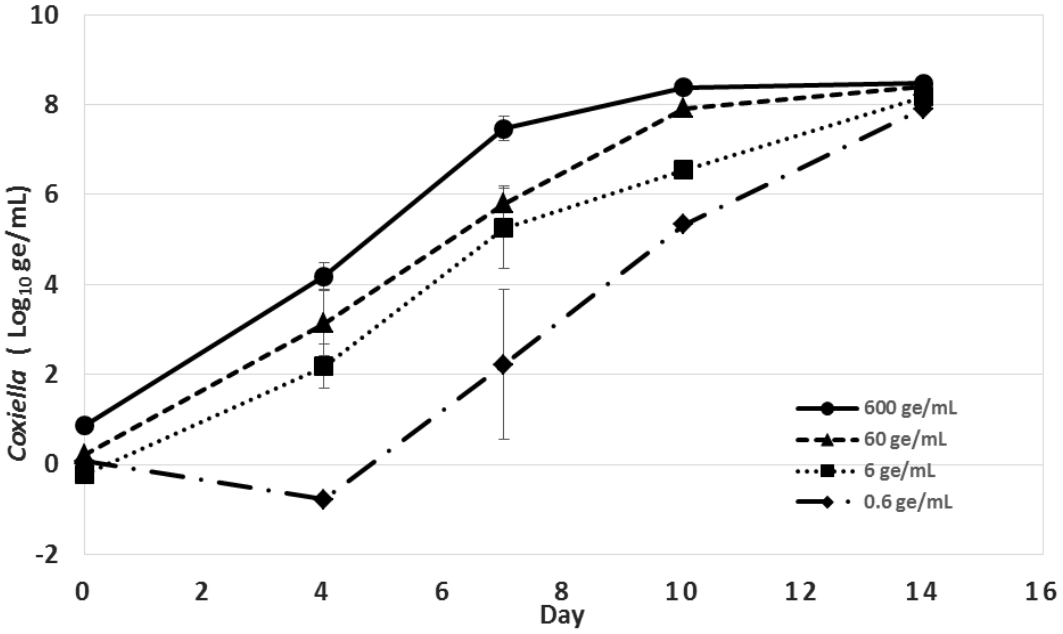
Supplemental Figure 1. Effect of pH adjustment on growth of *Coxiella* in ACCM-2 media alone or bovine milk and media. *Coxiella* genome equivalents (ge)/ml were measured by qPCR. Data points represent the average and standard deviations of 6 replicate flasks from two trials.

Supplemental Figure 2

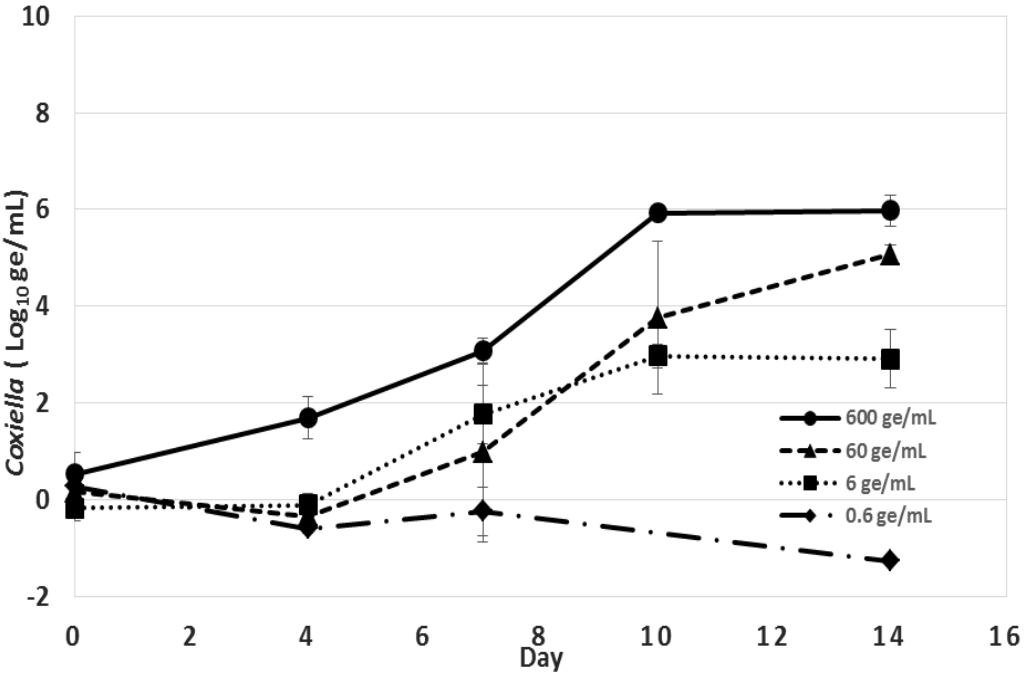


Supplemental Figure 2. Effect of additional initial dilution of milk with ACCM-2 media on growth of *Coxiella burnetii* from water buffalo milk. The standard dilution of milk into media for enrichments is 1:10 which did not allow growth. *Coxiella* genome equivalents (ge)/ml were measured by qPCR. Data points represent the average and standard deviations of 6 replicate flasks from two trials.

Supplemental Figure 3.



(A)



(B)

Supplemental Figure 3. ACCM-2 enrichment of *Coxiella burnetii* from ACCM-2 media (A) or media and milk (B) inoculated at initial levels of 0.6 – 600 ge/ml. *Coxiella* genome equivalents (ge)/ml were measured by qPCR. Data points represent the average and standard deviations of 6 replicate flasks from two trials.