A comparison of different DNA extraction protocols in the detection of *Borrelia burgdorferi* sensu stricto and *Anaplasma phagocytophilum* from *Ixodes pacificus* ticks by real-time PCR

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**Background:**
We approached this study in the two following steps:
1) Primarily we compared four protocols for the extraction of DNA from *Ixodes pacificus* ticks.
2) Secondarily two optimal protocols were then evaluated for sensitivity for *Borrelia burgdorferi* sensu stricto and *Anaplasma phagocytophilum*.

Extraction of DNA from ticks is an important step towards molecular diagnosis of tick-borne disease because this extracted DNA can be used in specific PCR reactions that target pathogens including the agents of Lyme disease and anaplasmosis.

The objectives of the present study were to optimize and compare PCR based detection methods for the pathogens of anaplasmosis and Lyme disease.

**Material and Methods:**
We compared the protocols for sensitivity by comparing the mean CT (threshold cycle) when PCR is performed, using the tick DNA to amplify the 18S rRNA target that occurs in all eukaryotes.

The four DNA extraction protocols used in the first step of the study are:
1) Pestle crushing, proteinase K digestion and DNA extraction using Qiagen tissue kit protocol
2) Pestle crushing and boiling in TE Buffer (One step tick extraction)
3) Pestle crushing and boiling in ammonium hydroxide
4) Fine crushing with bead beater, proteinase K digestion and DNA extraction using the Qiagen tissue kit protocol (Valencia, CA, USA)

For each protocol, 12 tick samples were extracted.

For the second step in this study 24 ticks were cut longitudinally in half. DNA extraction of one half of the tick was done with protocol 1 and DNA from the other half of the tick was extracted with protocol 3.

**Results**
- Only protocol 4 was significantly less sensitive than the other 3 protocols.
- DNA extraction efficiency using protocol 3 was significantly higher than DNA extraction efficiency using protocol 1 (p<0.001).
- Both halves of one tick were found infected with *Anaplasma phagocytophilum*. CT (Qiagen)=25.2 and CT (ammonium hydroxide)=21.8
- Both halves of two ticks were found infected with *Borrelia burgdorferi*. CT (Qiagen)= (1) 32.4; (2) 29.9 and CT (ammonium hydroxide)=(1) 30.8; (2) 27.8