Prevalence, Density, and Geographic Distribution of Two Novel *Rickettsia* Species in *Ixodes pacificus* from California by real-time PCR

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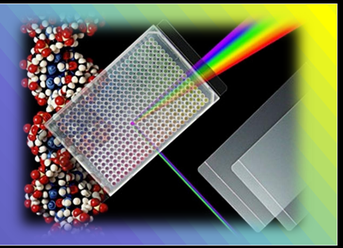

BACKGROUND

Ticks, as globally distributed, bloodsucking parasites, are major vectors of disease. Often housing symbiotic bacteria, the potential for transmission to vertebrates is great. Notable tick borne diseases include Lymes disease (*Borrelia*), Rocky Mountain Spotted Fever (*Rickettsia rickettsii*), erlichiosis anaplasmosis (*Anaplasma phagocytophilum*), relapsing fever (*Borrelia*), and Tularenia (*Francisella tularensis*).

Ixodid ticks are host to the most diverse collection of bacteria of any arthropod group (Jongejan and Uilenberg, 2004; Telford and Goethert, 2004). *Rickettsia* are Gram negative, obligate, intracellular bacteria found mainly in arthropods, but are also known to infect various vertebrates.

In both *I. pacificus* (dominant along the west coast) and *I. scapularis* (dominant along the east coast) ticks, *Rickettsia* bacteria have been detected (Philippe Parola, Christopher D. Paddock, and Didier Raoult, 2005). These strains remain incompletely characterized, however, little is known about correct species designation and prevalence. It is of high priority to investigate and monitor these newly discovered stains as virulence is yet undetermined and may be attained in the future.

Various methods for detection and quantification of intracellular bacteria have been used. Real-time PCR is a frequently used and practical tool for quantification of a particular intracellular bacterial gene. Real-time is rapid, highly sensitive (can detect acute infections), and reproducible. Also, this method is superior to conventional PCR in that, in addition to the necessity of two specific primers binding to the genomic DNA, a probe must also bind for detection, heightening discrimination.

MATERIALS AND METHODS

A total of 176 *I. pacificus* samples were collected from the Humboldt, Marin, Shasta, Orange, and Contra Costa counties by flagging roadside vegetation and stored in 95% ethanol until use. Following liquid nitrogen pulverization, DNA in tick samples was extracted with a DNeasy tissue & Blood kit (QIAGEN Valencia, CA, USA) with a modified protocol. The *ompA* and actin genes were detected by an MGB probe in real-time PCR for rickettsiae and tick cell quantification, respectively. Serially diluted, cloned DNA plasmids of *ompAs* and actin were used as a quantification standard. Rickettsiae burden per tick was calculated as a ratio of *ompA* to actin gene copy number in the same sample.

For log transformed data, one-way ANOVA and Tukey's, *t*-test, and chi squared tests were performed for statistical analysis.

RESULTS

Table 1. Prevalence of two *Rickettsia* phylotypes in *Ixodes pacificus* collected from five counties in northern California. 2009-2010

Category	Phylotype G021		phylotype G022	
	No. ticks examined	No.(%) infected	No. ticks examined	No.(%) infected
Humboldt	60	60(100%)	60	8(13.3%)
Marin	41	41(100%)	41	1(2.4%)
Shasta	45	45(100%)	45	1(2.2%)
Orange	31	31(100%)	31	8(25.8%)
Contra Costa	29	29(100%)	29	4(14.0%)
Total	176	176(100%)	176	22(12.5%)

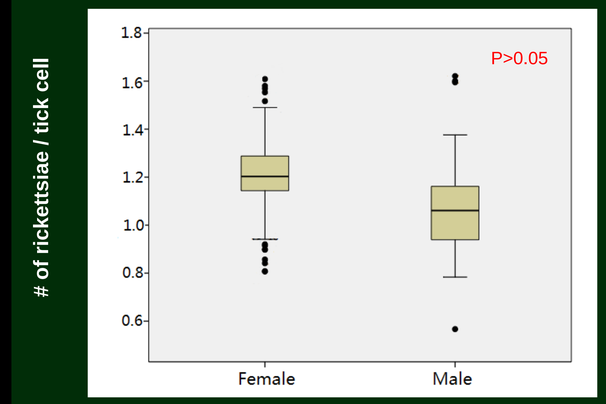

Figure 1. Density of *Rickettsia* phylotype G021 in *Ixodes pacificus* by sex.

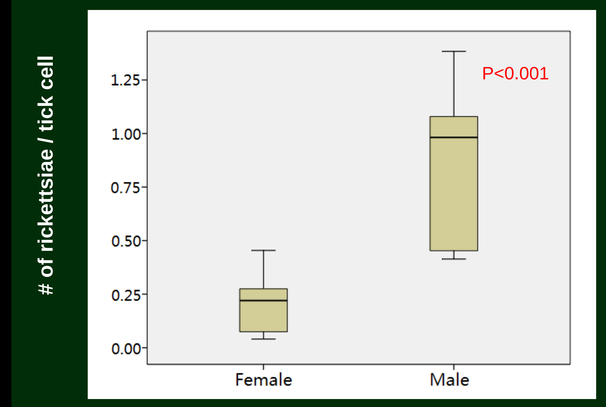

Figure 2. Density of *Rickettsia* phylotype G022 in *Ixodes pacificus* by sex.

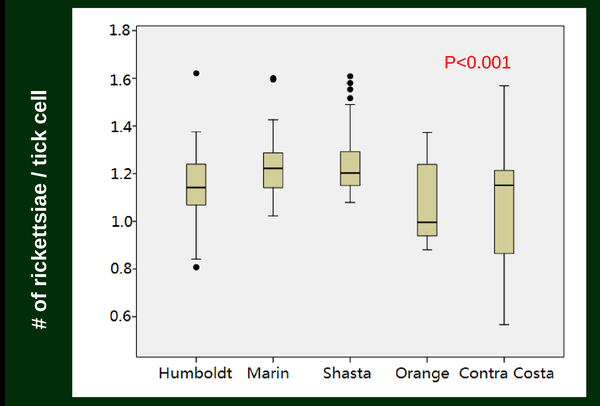

Figure 3. Density of *Rickettsia* phylotype G021 in *Ixodes pacificus* by counties in California.

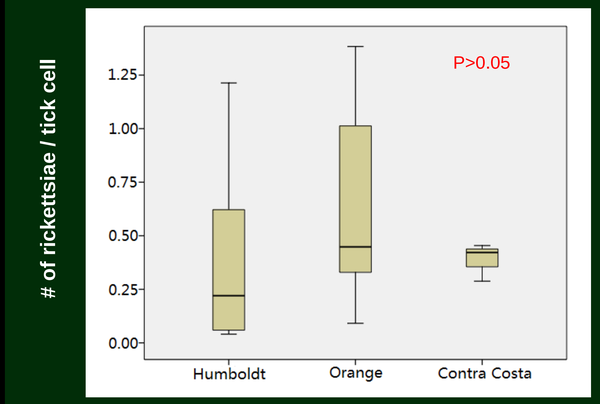

Figure 4. Density of *Rickettsia* phylotype G022 in *Ixodes pacificus* by counties in California.

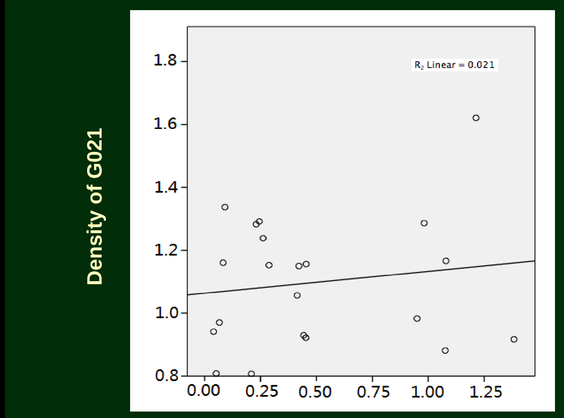

Figure 5. *Rickettsia* phylotype G021 as a function of phylotype G022 in *Ixodes pacificus*.

Prevalence of rickettsial phylotypes in *I. pacificus* varied from 2.2% to 25.8% in California. Values for G021 prevalence rates (%) proved nonsignificant for males or females in the Humboldt, Marin, Shasta, Orange, and Contra Costa counties ($p>0.05$). There was also no significant difference in G022 prevalence rates (%) for males or females in the Humboldt, Orange, and Contra Costa counties ($p > 0.05$). The female ratio of G021 to tick cell was significantly greater than that of the males for counties combined ($p<0.05$) with a median of 17.78 bacteria per tick cell. The ratio of G022 to tick cell in males was significantly greater than that of females ($p < 0.01$) with a median of 4.90 bacteria per tick cell. For the density of G021 per tick cell, Orange, Humboldt, and Contra Costa counties proved statistically similar ($p>0.05$) and Marin and Shasta counties grouped together ($p>0.05$). The same groups were observed when comparing the density of G022 per tick cell. However, difference of densities of G021 per tick cell has statistical significance ($P<0.001$) between counties of Orange, Humboldt, and Contra Costa and counties of Marin and Shasta. There is a moderate correlation between the density of two phylotypes in *I. pacificus*

CONCLUSIONS

I. pacificus novel rickettsial phylotypes were detected in all counties surveyed, G021 as a confirmed symbiont and G022 as an incidental bacterium. The disparity in G021 and G022 bacterial load in females (with higher G021 Rickettsial loads) versus males (with higher G022 Rickettsial loads) may be the result of different transmission routes. That counties grouped consistently for both phylotypes may indicate a correlation between G021 and G022 bacterial loads, with coninfection dynamics dictating phylotype counts. No difference was observed in prevalence rates for either phylotype between counties, probably largely due to insufficient sample numbers for the Marin and Shasta counties, which prevented their incorporation in statistical analysis for the G022 phylotype. An alternate explanation may be a stability in phylotype competition and resulting rates across California. Symbiotic bacteria have the potential for tick-borne disease control and this relationship should be further investigated.

REFERENCES

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Aknowledgements

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