LETTER TO THE EDITOR – Pathogens & Pathogenicity

False detection of Coxiella burnetii—what is the risk?

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The insertion sequence IS1111 is used for molecular detection of Coxiella burnetii but also found in Coxiella-like endosymbionts of ticks, presenting a risk of false positive detection of C. burnetii. Limited IS1111 sequences from Coxiella-like bacteria restrict in silico assessment of IS1111 assays. However, Coxiella-like bacteria detectable by IS1111 assays appear to be rare in tick populations, limiting the impact of false positives on C. burnetii prevalence estimations. C. burnetii can be distinguished from Coxiella-like bacteria using C. burnetii SNP genotyping assays. Such assays can be used for detection, but are best used as post hoc tests given the extreme sensitivity of assays that target the multiple copy IS1111.

Recently, Duron (2015) demonstrated the presence of IS1111 in Coxiella-like endosymbionts of ticks. This work adds to our knowledge of sequence diversity within IS1111 from Coxiella-like endosymbionts. Duron et al. (2015) also show that Coxiella-like endosymbionts are widespread among tick species. Detection assays for the Q fever pathogen, C. burnetii, often target portions of IS1111 (Kim et al. 2005; Klee et al. 2006; Loftis et al. 2006; Panning et al. 2008; de Bruin et al. 2011); the repetitive nature of IS1111 provides multiple targets for primer annealing, resulting in unparalleled detection sensitivity. Given the presence of IS1111 in Coxiella-like bacteria, Duron and colleagues (Duron 2015; Duron et al. 2015; Jourdain et al. 2015) raised the concern that these C. burnetii detection assays may lead to misidentification with Coxiella-like bacteria. Furthermore, these authors demonstrate that an IS1111 based assay does indeed amplify Coxiella-like bacteria (Jourdain et al. 2015). While these findings have an important bearing on C. burnetii research, current evidence suggests that in most situations, the overall risk of false detection of C. burnetii is low and can be mitigated with current methods.

Sequence alignment of haplotypes presented by Duron (2015) and Vilcins, Old and Deane (2009) that include primer and probe sequences from popular IS1111 qPCR assays demonstrate substantial gaps in our knowledge about diversity of this genomic region among Coxiella-like bacteria (Fig. 1 and Supplemental Files). In particular, the region (∼570bp) sequenced by Duron (2015) covers the primer and probe regions of only one assay. These 10 haplotypes are highly diverse and only 3 (from Rhipicephalus decoloratus, Bothriocroton auruginans and Ornithodoros maritimus) show matches at primer/probe regions likely to result in amplification. Thus, out of 115 tick samples known to be positive for endosymbiont bacteria (Duron 2015), only 4 would test positive with this assay. Jourdain et al. (2015) detected positive results in four additional tick species, but did not publish the primer/probe sequences. While we do not know the likelihood of other IS1111 assays detecting Coxiella-like bacteria, it is reasonable to expect that the diversity exhibited in this region can be extrapolated to flanking regions, causing highly variable amplification. Undoubtedly, additional diversity in IS1111 exists among Coxiella-like bacteria, and future sequencing will provide better estimates of the degree to which various assays differentiate between C. burnetii and Coxiella-like bacteria. Current evidence therefore suggests that cross-reactivity does indeed occur for one assay at an unknown region (Jourdain et al. 2015), is likely for another (Fig. 1), and possible for others. The works by Duron (2015); Duron et al. (2015); Jourdain et al. (2015) are based on tick species known to harbor Coxiella-like endosymbionts. Unless environmental surveys for C. burnetii contain a high proportion of these species, few samples are likely to test positive for Coxiella-like bacteria using IS1111 assays. However, given the low prevalence of C. burnetii in ticks, a few false positives can have a large proportional impact on prevalence estimations.

For positive identification of C. burnetii, Duron and colleagues (Duron 2015; Duron et al. 2015; Jourdain et al. 2015) suggest testing other targets in addition to IS1111. They demonstrate that other targets (GroEL/htpB, p1, scvA) are also contained in
Coxiella-like bacteria and will occasionally be amplified. Importantly, they point out that like IS1111, the presence/absence of other genomic targets in Coxiella-like bacteria is not well characterized and sequencing such regions can serve as a means for differentiating C. burnetii from other Coxiella. Unfortunately, gene sequencing requires an established reference database for interpretation of results. As a simpler alternative, we have used SNP signatures developed into sensitive qPCR assays (Hornstra et al. 2011; Pearson et al. 2014; Bauer et al. 2015). SNPs have a low mutation rate and such evolutionary stability and strictly clonal reproduction in C. burnetii minimizes the likelihood of the same nucleotide mutating again in either independent lineages or among descendants (Pearson et al. 2013). A single SNP can therefore define a lineage and can be combined with other lineage-specific SNP assays to accurately place an unknown sample on a phylogenetic tree and provide additional confidence in phylogenetic placement (Pearson et al. 2004, 2009). Genotyping assays must target single copy signatures to avoid phylogenetic problems inherent to comparing paralogs, and while such assays can also serve as detection assays, they will not be as sensitive as those that target highly repeated genomic regions. Using this system, a sample that is positive for IS1111 (Loftis et al. 2006) with a CT < 35 can be successfully genotyped (Olivas and Pearson unpublished data). Whether any of these genomic targets exists in near neighbors to C. burnetii is unknown, however their SNP signatures will still allow for the discrimination between C. burnetii and Coxiella-like bacteria.

Detection assays for any species suffer from the possibility of false positives due to sequence similarities among near neighbors. Duron and colleagues (Duron 2015; Duron et al. 2015; Jourdain et al. 2015) have provided valuable insights into possible limitations of popular detection assays for C. burnetii, yet much is unknown about the prevalence of cross-reactive signatures among near neighbors. Coupling sensitive detection assays with SNP genotyping methods provides an insurance mechanism for positive identification (and genotyping) of C. burnetii without the risk of false positives when dealing with samples obtained from ticks.

**SUPPLEMENTARY DATA**

Supplementary data are available at FEMSLE online.

**Conflict of interest.** None declared.
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