FEMS Microbiology Letters, 363, 2016, fnw088

doi: 10.1093/femsle/fnw088
Advance Access Publication Date: 7 April 2016
Letter to the Editor

LETTER TO THE EDITOR – Pathogens & Pathogenicity

False detection of *Coxiella burnetii*—what is the risk?

Talima Pearson*, Jill H. Cocking, Heidie M. Hornstra and Paul Keim

Microbial Genetics and Genomics, Northern Arizona University, 1395 S. Knoles Dr. Bldg. 56, Flagstaff, AZ 86011, USA

*Corresponding author: Microbial Genetics and Genomics, Northern Arizona University, 1395 S. Knoles Dr. Bldg. 56, Flagstaff, AZ 86001, USA. Tel: 9285234290; E-mail: Talima.Pearson@nau.edu
Editor: Arnoud van Vliet

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

Received: 18 February 2016; Accepted: 5 April 2016

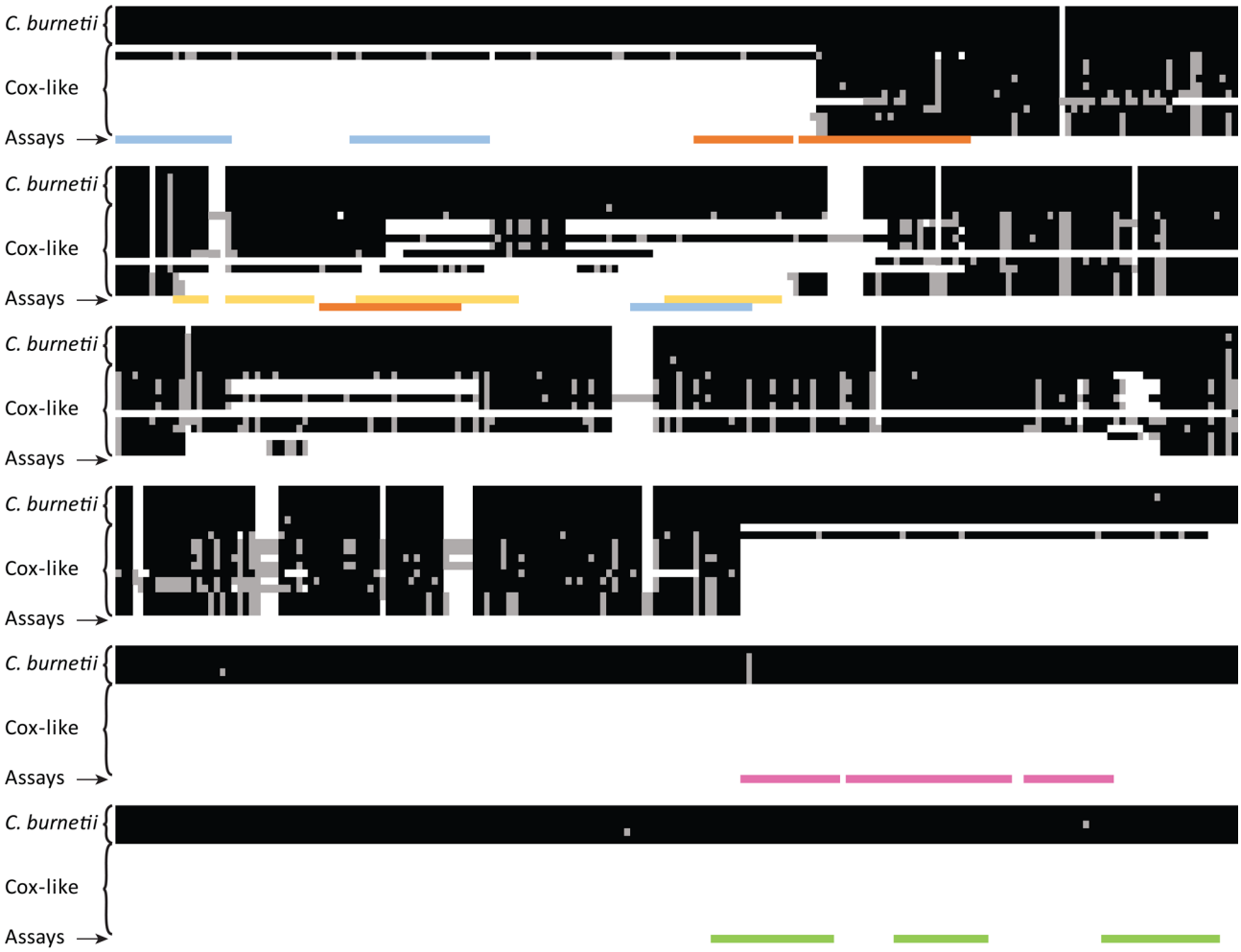

Figure 1. Alignment of 1146bp of IS1111 including sequences from 5 *C. burnetii*, 12 *Coxiella*-like bacteria, and primers and probes from five assays. Assay colors correspond to (Klee *et al.* 2006) (blue); (Kim *et al.* 2005) (yellow); (Loftis *et al.* 2006) (pink); (Panning *et al.* 2008) (green); and (de Bruin *et al.* 2011) (orange). Other formats for this alignment are available as supplemental files.

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.

REFERENCES

Bauer AE, Olivas S, Cooper M *et al*. Estimated herd prevalence and sequence types of *Coxiella burnetii* in bulk tank milk samples from commercial dairies in Indiana. *BMC Vet Res* 2015;**11**:186.

de Bruin A, de Groot A, de Heer L *et al*. Detection of *Coxiella burnetii* in complex matrices by using multiplex quantitative pcr during a major Q fever outbreak in the Netherlands. *Appl Environ Microb* 2011;**77**:6516–23.

Duron O. The IS1111 insertion sequence used for detection of *Coxiella burnetii* is widespread in Coxiella-like endosymbionts of ticks. *FEMS Microbiol Lett* 2015;**362**, DOI:10.1093/femsle/fnv132.

Duron O, Sidi-Boumedine K, Rousset E *et al*. The importance of ticks in Q fever transmission: what has (and has not) been demonstrated? *Trends Parasitol* 2015;**31**:536–52.

Hornstra HM, Priestley RA, Georgia SM *et al*. Rapid typing of *Coxiella burnetii*. *PLoS One* 2011;**6**:e26201.

Jourdain E, Duron O, Severine B *et al*. Molecular methods routinely used to detect *Coxiella burnetii* in ticks cross-react with *Coxiella*-like bacteria. *Infect Ecol Epidemiol* 2015;**5**:29230.

Kim SG, Kim EH, Lafferty CJ *et al*. *Coxiella burnetii* in bulk tank milk samples, United States. *Emerg Infect Dis* 2005;**11**:619–21.

Klee SR, Tyczka J, Ellerbrok H *et al*. Highly sensitive real-time PCR for specific detection and quantification of *Coxiella burnetii*. *BMC Microbiol* 2006;**6**:2.

Loftis AD, Reeves WK, Szumlas DE *et al*. Rickettsial agents in Egyptian ticks collected from domestic animals. *Exp Appl Acarol* 2006;**40**:67–81.

Panning M, Kilwinski J, Greiner-Fischer S *et al*. High throughput detection of *Coxiella burnetii* by real-time PCR with internal control system and automated DNA preparation. *BMC Microbiol* 2008;**8**:77.

Pearson T, Busch JD, Ravel J *et al*. Phylogenetic discovery bias in *Bacillus anthracis* using single-nucleotide polymorphisms from whole-genome sequencing. *P Natl Acad Sci USA* 2004;**101**:13536–41.

Pearson T, Hornstra HM, Hilsabeck R *et al*. High prevalence and two dominant host-specific genotypes of *Coxiella burnetii* in U.S. milk. *BMC Microbiol* 2014;**14**:41.

Pearson T, Hornstra HM, Sahl JW *et al*. When outgroups fail; phylogenomics of rooting the emerging pathogen, *Coxiella burnetii*. *Syst Biol* 2013;**62**:752–62.

Pearson T, Okinaka RT, Foster JT *et al*. Phylogenetic understanding of clonal populations in an era of whole genome sequencing. *Infect Genet Evol* 2009;**9**:1010–9.

Vilcins IME, Old JM, Deane E. Molecular detection of *Rickettsia*, *Coxiella* and *Rickettsiella* DNA in three native Australian tick species. *Exp Appl Acarol* 2009;**49**:229–42.