First genetic evidence of *Coxiella burnetii* in cases presenting with acute febrile illness, India

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Sir,

Q fever is a worldwide zoonosis that is caused by *Coxiella burnetii*, an obligate intracellular Gram-negative bacterium [1]. Infected domestic animals, particularly sheep, goats and cattle are the main sources of infection to humans [2]. The organism is shed in placentas, abortion products, urine, faeces and milk of infected animals. In humans, infection is mainly acquired via infective aerosols or contaminated dust [3]. Q fever is characterized by acute and chronic courses. Acute Q fever is often asymptomatic or manifests as a non-specific febrile, unrecognized illness [2]. Clinical presentation of chronic Q fever includes vascular infections, blood culture-negative endocarditis, osteoarticular involvement and chronic liver diseases [4]. Proper patient management can significantly reduce mortality due to chronic Q fever, making timely diagnosis of utmost importance. Although *C. burnetii* causing acute febrile illness has been reported worldwide [5, 6], its distribution in India is ill-defined.

In the past 60 years, there have only been about 25 publications on human and animal Q fever from India in international databases. The first confirmed human case of Q fever was reported in 1954 [7]. Subsequently, there are only a few reports of human Q fever in the country [8, 9]. The serological diagnosis in most of these reports was mainly based on less precise conventional tests like complement fixation test (CFT) and capillary agglutination test (CAT). These tests may fail to detect primary Q fever during the early phase of illness as well as in some chronically-infected patients, therefore, requiring acute and convalescent phase serum samples for reliable interpretation of results [10, 11]. Moreover, in India, paired serum samples are almost never sent to the laboratory and also the clinical suspicion of *C. burnetii* aetiology is almost imperceptible. Therefore, it is logical to assume that the infection by *C. burnetii* has remained grossly under-diagnosed in the Indian population largely due to the lack of facilities for culture and advanced diagnostic assays for this infectious agent. Only two references have shown the application of molecular techniques for diagnosis of Q fever in humans in recent years [12, 13]. To the best of our knowledge, there is no report of acute Q fever among patients with acute febrile illness in India employing molecular tools. Therefore, the objective of this study was to detect the presence of *C. burnetii* using molecular methods in acute febrile subjects in India in an area where clinical studies of zoonoses are scarce.

Diagnosis of Q fever is generally made serologically, relying on the demonstration of *C. burnetii* specific antibodies in both acute and convalescent sera. The most reliable test for serological diagnosis of Q fever is the immunofluorescence assay [2]. However, serological tests are not suitable for early diagnosis because of the lag phase in antibody response of 2 to 4 weeks after onset of clinical symptoms [2]. Moreover, serological cross-reactivity with other pathogens (*Legionella micdadei*, *Mycoplasma pneumoniae*, *Ricketsia* species, *Chlamydia phila* species, cytomegalovirus and Epstein–Barr virus) has been reported in Q fever patients [14]. Molecular techniques have proven advantageous in almost all early acute Q fever patients, yet to develop an antibody response [15]. Several PCR based diagnostic assays were developed to detect *C. burnetii* DNA in cell cultures and clinical samples. These assays used conventional PCR [16, 17], nested PCR [18, 19] and real-time PCR [15, 20, 21]. These assays have generally targeted singular chromosomal genes like *coml* or *hptB* or the transposase gene of insertion element *JS1111* [22] which is present in 20 copies in the genome of the *C. burnetii* Nine Mile RSA493 strain [23].

A total of 198 blood samples from patients presenting with less than two weeks of fever, with chills, headache, myalgia, shortness of breath and nausea etc. at SDM College of Medical Sciences & Hospital, Dharwad, Karnataka from June 2013 to December 2013 were collected aseptically by venous puncture after obtaining permission from the Institutional Ethical Committee. The patients included in the study belonged to both sexes and ranged from 3 to 80 years of age (mean age 26±18). The serum samples were tested
for diagnosis of fever using tests ordered by the treating clinicians for conditions viz. brucellosis (Rose Bengal Plate Test and tube agglutination; IVRI, India), dengue (Dengue Duo NS1 Ag and IgG/IgM; Standard Diagnostics), malaria (MAL CARD; J Mitra), typhoid (Widal tube test; Span Diagnostics), antistreptolysin O (ASLO) titre estimation (ASO Latex Test; Span Diagnostics), hepatitis B (HBsAg; Diagnostics), and scrub typhus (Weil Felix test; Tulip Enterprises) and hepatitis C infection (HCV Tri-dot; Diagnostic Enterprises). The serum samples negative for the suggested tests were stored at –20 °C until further testing for Q fever. Genomic DNA was isolated from serum samples using a DNeasy Tissue kit (Qiagen) according to the manufacturer’s instructions. Extracted DNA from serum samples was subjected to TaqMan and SYBR green real-time PCR based on amplification of a 70 bp fragment of repetitive sequence IS1111 [15] and 154 bp fragment of another multicopy gene target IS30A spacer [24] respectively. Genomic DNA of C. burnetii Nine Mile RSA493 strain was used as positive control for PCR and nuclease free water as negative control. The PCRs were carried out in a StepOne Real-time PCR system (Applied Biosystems) using TaqMan Universal Master Mix II (Invitrogen) according to the manufacturer’s instructions. The results were analysed with StepOne software v2.3 (Applied Biosystems).

Out of 198 samples tested, nine (4.5 %) were found positive by both real-time PCRs. Serum samples that were found positive by both techniques were further tested by nested PCR targeting repetitive element IS1111 of C. burnetii [25] aiming to amplify a 520 bp fragment in the first amplification and a 297 bp fragment in the second. Four samples tested positive by nested PCR. This difference in positivity could be attributed to the sensitivity of real-time PCR in amplifying small amplification products [15]. Amplicons of nested PCR were confirmed by double pass sequencing which showed 100 % sequence identity with the reference strain of C. burnetii (GenBank accession nos LT699836 to LT699839). These results along with demographic, clinical and laboratory findings are summarized in Table 1. Upon statistical analysis we did not find any special predilection of the infection to either age (P=0.13) or sex (P=0.37). This is in agreement with another finding where age or sex related differences were not detected in a study on human Q fever [26]. Findings of the present study suggest that patients with acute fever should be screened by molecular methods in the absence of facility for culture and serology with paired sera.

In conclusion, the present study suggests that C. burnetii is present among patients with acute febrile illness in India. These cases are not frequent but the severity of chronic Q fever must be taken into account. So it is imperative that local physicians be made aware of the possibility of this pathogen in human infections and they should be sensitized to suspect Q fever in patients with compatible clinical signs. We recommend that C. burnetii serum PCR should be routinely included in the diagnostic testing of a patient with suspected acute Q fever. In addition, further studies should be carried out on a bigger sample size from diverse geographical locations employing molecular methods to understand the epidemiology of Q fever in India.

**Table 1.** Demographic, clinical and laboratory details of nine C. burnetii patients determined by molecular methods

<table>
<thead>
<tr>
<th>Sample no.</th>
<th>Age/sex</th>
<th>Duration of fever (days)</th>
<th>Brief clinical details</th>
<th>IS1111 TaqMan real-time PCR</th>
<th>IS30A SYBR green real-time PCR</th>
<th>IS1111 nested PCR</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>21/M</td>
<td>13</td>
<td>Myalgia, nausea, malaise and vomiting</td>
<td>+</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>2</td>
<td>27/F</td>
<td>10</td>
<td>Chills, dry cough and myalgia</td>
<td>+</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>3</td>
<td>31/M</td>
<td>10</td>
<td>Nausea, malaise, myalgia and vomiting</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>4</td>
<td>33/M</td>
<td>11</td>
<td>Dry cough, shortness of breath and hepatosplenomegaly</td>
<td>+</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>5</td>
<td>35/F</td>
<td>7</td>
<td>Myalgia, chills, nausea and hepatosplenomegaly</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>6</td>
<td>38/M</td>
<td>6</td>
<td>Myalgia, chills and cough</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>7</td>
<td>52/M</td>
<td>7</td>
<td>Dry cough and shortness of breath</td>
<td>+</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>8</td>
<td>63/M</td>
<td>6</td>
<td>Breathlessness and hepatosplenomegaly</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>9</td>
<td>61/M</td>
<td>5</td>
<td>Nausea, malaise and vomiting</td>
<td>+</td>
<td>+</td>
<td>–</td>
</tr>
</tbody>
</table>

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Conflicts of interest
The authors declare that there are no conflicts of interest.

References

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