Global metabolomic analysis of blood from mice infected with *Brucella abortus*

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**ABSTRACT.** To better understanding *Brucella abortus* infection, serum metabolites of *B. abortus*-infected and -uninfected mice were analyzed and twenty-one metabolites were tentatively identified at 3 and 14 days post-infection (d.p.i.). Level of most lysophosphatidylcholines (LPCs) was found to increase in infected mice at 3 d.p.i., while it was decreased at 14 d.p.i. as compared to uninfected mice. In contrast, acylcarnitines were initially reduced at 3 d.p.i then elevated after two-weeks of infection, while hydroxysanthine was increased at 14 d.p.i. in infected mice. Our findings suggest that the significant changes in LPCs and other identified metabolites may serve as potential biomarkers in acute phase of *B. abortus* infection.

**KEY WORDS:** *Brucella abortus* infection, lysophosphatidylcholine, metabolomics profiling, serum, ultra-high performance liquid chromatography-quadrupole-time-of-flight mass spectrometry
Blood metabolites from *Brucella*-infected or uninfected mice were analyzed as previously described [8] with some modifications. Briefly, blood proteins were precipitated with cold methanol. The supernatants were dried using a Centrivap Speedvac concentrator (Labconco Co., Kansas City, MO, USA). The residues were resolved by 20% methanol with terfenadine and abscisic acid as internal standards. After centrifugation, sample supernatants were analyzed by ultra-high performance liquid chromatography-quadrupole-time-of-flight (UPLC-Q-TOF) mass spectrometry (MS) (Xevo, Waters, Milford, MA, USA). An Acquity UPLC BEH C18 column (2.1 × 100 mm, 1.7 µm; Waters) filled with blood metabolites extracts was equilibrated with 0.1% formic acid in water. The metabolites were then eluted with a gradient from 1 to 100% acetonitrile containing 0.1% formic acid. The eluted metabolites were analyzed using Q-TOF MS with a positive electrospray ionization mode. The capillary and sampling cone voltages were set at 2.5 kV and 20 V, respectively. The cone gas and desolvation flow rate were 30 l/hr and 900

**Fig. 1.** Partial least-squares discriminant analysis (PLS-DA) score plots of blood metabolites for ultra-high performance liquid chromatography-quadrupole-time-of-flight mass spectrometry (UPLC-Q-TOF MS) data at different collection times. The quality of the PLS-DA models was evaluated by R²X, R²Y, Q², and P-values and validated by 200 permutation tests.
The quality of PLS-DA models was evaluated using three parameters (R², X, and Q²). Partial least-squares discriminant analysis (PLS-DA) was used to visualize the differences among sample groups.

The peaks were collected using peak-to-peak baseline noise of 1, noise elimination of 6, a peak-width at 5% height of 1 sec and a signal-to-noise ratio of 3. The collected and aligned data were normalized by each internal standard. Metabolites were tentatively identified and VIP values of all identified metabolites were above 1.0, indicating as major metabolites contributing to the separation on the PLS-DA scores plot of the data set analyzed by ultra-high performance liquid chromatography-quadrupole-time-of-flight mass spectrometry (UPLC-Q-TOF MS).

The profiles of blood metabolites from Brucella-infected mice were analyzed using UPLC-Q-TOF MS and compared to those of uninfected mice. The differences in blood metabolite profiles of sample groups were visualized by PLS-DA scores plot (Fig. 1). The sample groups were clearly separated along the first two-component PLS-DA score plots with statistically acceptable quality parameters (R²=0.258, X²=0.521, and Q²=0.258; P-value <0.0003) and cross-validation values (R² intercept <0.2 and Q² intercept <−0.2) analyzed by the permutation test (n=200). Normalized chromatogram intensities of metabolites were statistically analyzed using one-way analysis of variance (ANOVA) with Duncan’s test (P<0.05) or t-test (P<0.05) using SPSS 17.0.0.0 (SPSS Inc., Chicago, IL, USA). Identified metabolites were also visualized in a heat map representing the z-score transformed data of identified metabolites in a blue-red color scale, with red indicating a decrease and blue indicating an increase in metabolite levels.

The normalized chromatogram intensities of all the identified metabolites were relatively compared (Fig. 2). Interestingly, at 3 d.p.i., the levels of most blood LPCs were increased during Brucella infection, but at 14 d.p.i., their levels were lower except LPC (14:0 and 20:2) than those of uninfected mice suggesting a potential complicated role of the phospholipid in initiating immune response.

### Table 1. Identification of major metabolites contributing to the separation among sample groups on the partial least-squares discriminant analysis (PLS-DA) scores plot of the data set analyzed by ultra-high performance liquid chromatography-quadrupole-time-of-flight mass spectrometry (UPLC-Q-TOF MS)

| No. | RT (min) | Compound | Exact mass (M+H) | MS fragments | VIP | P-value |
|-----|----------|----------|-----------------|--------------|-----|---------|
| 1   | 1.09     | Hydroxyxanthine | 169.04 | 141, 124 | 1.20 | 1.58E-04 |
| 2   | 3.26     | Unknown  | 362.20 | 344 | 0.96 | 3.06E-02 |
| 3   | 6.3      | Linoleoylcarnitine | 424.34 | 85 | 1.89 | 5.13E-06 |
| 4   | 6.38     | Lpc (c14:0) | 468.31 | 450, 184, 104 | 1.41 | 5.91E-03 |
| 5   | 6.41     | Lpc (c20:5) | 542.32 | 524, 184, 104 | 2.00 | 1.38E-06 |
| 6   | 6.49     | Lpc (c18:3) | 518.32 | 184, 104 | 1.96 | 2.04E-06 |
| 7   | 6.51     | Palmitoylcarnitine | 400.34 | 85 | 1.92 | 6.31E-06 |
| 8   | 6.57     | Lpc (c16:1) | 494.32 | 476, 184, 104 | 1.72 | 2.63E-04 |
| 9   | 6.58     | Vaccenylcarnitine | 426.36 | 85 | 1.91 | 1.76E-05 |
| 10  | 6.67/6.81| LPC (C18:2) 2M | 1,039.67 | 520, 184, 104 | 1.55 | 4.55E-03 |
| 11  | 6.72     | LPC (C20:3) | 526.29 | 184, 104 | 1.59 | 1.33E-03 |
| 12  | 6.73     | LPC (C22:6) 2M | 1,135.67 | 568, 184, 104 | 1.73 | 2.96E-04 |
| 13  | 6.76     | LPC (C15:0) | 482.32 | 184, 104 | 1.89 | 9.80E-06 |
| 14  | 6.78     | LPC (C20:4) 2M | 1,087.67 | 544, 184, 104 | 1.84 | 7.61E-06 |
| 15  | 6.93     | LPC (C22:5) | 570.36 | 184, 104 | 1.87 | 1.69E-05 |
| 16  | 6.94     | LPC (C17:1) | 508.34 | 184, 104 | 1.66 | 5.11E-04 |
| 17  | 7.07     | LPC (C20:3) | 1,091.70 | 546, 184, 104 | 1.88 | 2.40E-06 |
| 18  | 7.3      | LPC (C18:1) 2M | 1,043.70 | 522, 184, 104 | 1.79 | 4.02E-05 |
| 19  | 7.33     | LPC (C22:4) | 572.37 | 184, 104 | 1.82 | 8.14E-05 |
| 20  | 7.47     | LPC (C20:2) | 548.37 | 184, 104 | 1.30 | 1.56E-03 |
| 21  | 7.52     | LPC (C17:0) | 510.36 | 184, 104 | 1.59 | 1.49E-03 |

a RT, retention time. b Variable importance in the projection (VIP) values were determined by PLS-DA. c P-values were processed by ANOVA with Duncan’s test.
responses upon *B. abortus* infection. In contrast to LPCs, the levels of acylcarnitines were reduced during *Brucella* infection at 3 d.p.i. then showed to increase at 14 d.p.i., while an increased hydroxysanthine level was only observed at 14 d.p.i. in *Brucella*-infected group. Acylcarnitines are also suggested to possess immunomodulatory function [12] indicating pathology of murine brucellosis possibly associated with changes in LPCs and acylcarnitines release by host cells.

Metabolic shift is a prominent feature of *B. abortus* infection in human macrophage-like cells suggesting that *Brucella* invasion may disrupt or interfere with host metabolism, and likely enable metabolic modification in the bacteria itself [5]. Therefore, identification of metabolic differences between infected and uninfected host may suggest alternatives to upgrade diagnosis tools. Importantly, as products of body metabolism, the type and concentration of metabolites may infer the involvement of defined or undefined pathways associated with *Brucella* pathogenesis from which further examinations may provide insights into host-pathogen interactions for subsequent designing of efficient novel treatment, vaccine as well as diagnosis. In the present study, we observed that LPCs are among the groups of detected metabolites contributing to the separation between *Brucella*-infected and uninfected animals. LPC, an endogenous phospholipid, is generated by the action of phospholipase A2 during low density protein oxidation. LPC was found to decrease during early *Acinetobacter baumannii* infection in mice, and its therapeutic efficacy against severe infections was associated with an elevation of the anti-inflammatory interleukin (IL)-10 that suppresses pro-inflammatory cytokines [14]. As other bacterial infection and viral infection may also exert significant changes in LPCs level, it is likely that the dynamics of detected LPCs in our study may not be specific to *B. abortus* infection. Study on LPCs and carnitines dynamics to determine whether these metabolites can also be found in other *Brucella* infection models such as human and cattle is encouraged to consolidate our findings. Additionally, LPC was suggested to promote phagosome maturation—a major killing mechanism of macrophage against intracellular pathogens [9] including *Brucella* spp. The metabolism of acylcarnitines stabilizes intracellular sugar and lipid metabolism by a series of reactions involving three components transporting system such as carnitine palmitoyltranseras I (CPTI),

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**Fig. 2.** Normalized chromatogram intensities and heat map of identified blood metabolites analyzed using ultra-high performance liquid chromatography-quadrupole-time-of-flight mass spectrometry (UPLC-Q-TOF MS). (A) Chromatogram intensity of detected metabolites was normalized with a proper internal standard. (B) The heat map was drawn by R with ggplot2 and the blue-red color shows the z-score transformed raw data of blood metabolites with significant differences among *Brucella*-infected and uninfected groups. Red and blue colors represent a decrease and an increase of metabolite level, respectively.
the carnitine acylcarnitine translocase and CPTII [11]. Acylcarnitines are capable of inducing cyclooxygenase-2 expression and secretion of a number of pro-inflammatory cytokines in culture macrophages suggested to be dependent on TLR4/MyD88 signaling pathway [12].

Overall, findings in this study suggested a number of compounds which may potentially serve as biomarkers for murine brucellosis detection during the acute phase of infection. Nonetheless, further work remains significant. Specifically, animals’ age, sex, genetics and infections may invoke wide range of heterogeneity, thus affecting metabolic status. Furthermore, how major detected metabolites contribute in the differences among B. abortus-infected and uninfected groups such as LPC and acylcarnitines affect host immune responses and dictate disease outcome requires further investigation.

CONFLICT OF INTEREST. The authors declare no conflict of interest.

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