**S2 Table. Calcium**

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| **Authors**  | **Sample (N)** | **Methods** | **Definition of Leptospirosis** | **Definition of Micronutrient** | **Main Findings** |
| ***Laboratory*** |
| [[56](#_ENREF_56)] | Mouse macrophage line (J774A.1) and human macrophages (THP-1) | Ca2+ concentrations in infected macrophages were compared to uninfected macrophages. Cells were treated with Ca2+ chelators to determine source of Ca2+ | J774A.1 or THP-1 cells were infected with*L. interrogans icterohaemorrhagiae* serotype *Lai* strain Lai at a density of 100 leptospires per cell | Changes in Ca2 calculated by: (fluorescence intensity in 500 infected cells) / (fluorescence intensity in the same number of cells before infection) x100% | *L. interrogans* infection significantly elevated Ca2+ levels (p<0.05) due to extracellular Ca2+ influx and intracellular Ca2+ release. High Ca2+ induced macrophage apoptosis and necrosis (p<0.05). Calcium chelators reduced the Ca2+ elevation during infection |
| [[57](#_ENREF_57)] | Human kidney cells *in vitro* | Ca2+-binding mutants of LipL32 (lipoprotein 32) were created to analyze the role of the Ca2+-binding cluster in LipL32 | *L. shermani* Lipl32 gene transformed into *E. coli* | Ca2+ binding integrity to LipL32 assessed by CD (circular dichroism) spectrometry using a probe for detecting Ca2+ -binding proteins | Ca2+ binding of LipL32 essential for regulating interaction with TLR2 (toll-like receptor 2) for inflammatory response induction |
| [[58](#_ENREF_58)] | *E. coli* strain B834 | CD spectra assessed binding affinity of LipL32 to Ca2+ and fibronectin F30 (human fibronectin fragment) in *E. coli* | *L. shermani* LipL32 gene transformed into *E. coli* | Ca2+ and fibronectin F30 binding affinity assessed by CD spectrometry | Ca2+ promotes LipL32 binding to fibronectin. Binding affinity for F30 was stronger for Ca2+ bound LipL32 than for Ca2+-free LipL32 (Kd values = 0.29 ± 0.01 μM and 1.15 ± 0.06 μM, respectively; p<0.0001, R2=0.99) |
| [[59](#_ENREF_59)] | *E. coli* BL21 | LipL32 mutants (D163-168A, Q67A, and S247A) were created to assess affinity for Ca2+ and human plasminogen and fibronectin | LipL32 gene isolated and transformed into *E. coli* cells | Binding affinity to Ca2+ and to human plasminogen/fibronectin assessed | Wild type and mutant LipL32 bound to plasminogen and fibronectin with similar affinities both with and without Ca2+; Ca2+ not required for interaction between LipL32 and host extracellular matrix proteins |
|  [[89](#_ENREF_89)] | Isolated Lig proteins | Lig proteins isolated to assess binding affinity to Ca2+ and to fibronectin | Lig proteins isolated | Effect of Ca2+ on binding affinity of Lig proteins to fibronectin | Ca2+ binding increases conformational stability of LigBCen2 (binds to host extracellular matrix proteins) Midpoint of LigBCen2 unfolding increased from 50.7 ± 0.9 to 54.8 ± 0.5 ºC when Ca2+ was added.Ca2+ increases binding affinity (Kd = 63 nM compared to apoprotein Kd = 272 nM) |

N/A, not applicable; micronutrient cutoffs not provided.