Supplemental Appendix A: Protocols for serological assays

Rose Bengal fast agglutination test (RBT)

The RB assay was used with Bengatest® antigen i.e. a concentrated suspension (4% v/v) of *B. abortus* Weybridge strain 19, heat and phenol (0.5%) inactivated, suspended in an acid buffer and stained with Rose Bengal. Equal quantities (30 µl) of serum and antigen were mixed in a well (4 min) on a glass plate and any degree of agglutination was considered a positive reaction.

Wright's Slow Agglutination Test with EDTA (SAT-EDTA)

For SAT-EDTA, the antigen (Antigen SAW®, Synbiotics code # ASAW) was a concentrated suspension of *B. abortus* (strain 1119/3), heat and phenol (0.5%) inactivated and suspended in a phenol-buffer at 0.5%. The assay was performed as described by Godfroid and Boelaert[18] with serum dilutions of 1/12.5, 1/25, 1/50, 1/100, 1/200, 1/400, 1/800, 1/1600, 1/3200, 1/6400, 1/12800 and 1/25600 in a constant volume (100 µl) of antigen. Quantitative results were given as International Units of Agglutination (IU/ml). A value equal to or above 100 IU/ml, corresponding to 75% transparency of dilution 1/50 was considered as a positive reaction.

Indirect Enzyme Linked Immunosorbent Assay (iELISA)

The assay was performed according to Godfroid and Boelaert (1995).[18] Smooth *B. abortus* Weybridge strain 19 lipopolysaccharide (LPS) antigen was incubated on polystyrene plates for 3.5 h at 37°C and overnight at 4ºC. Plates were washed 5 times with a washing solution (NaCl 0.9% + Tween 20 at 0.01%).

Then, 50 µl of 1/50 diluted serum in glycine-EDTA-Tween 80 buffer (BB) was added per well and the calibration curve was determined at dilutions 1/270, 1/540, 1/1080, 1/2160, 1/4320, 1/8640. After one hour incubation at ambient temperature, the solutions were discarded, plates were washed 5 times and 50 µl conjugate (Protein G-HRPO, Pirce CD47675, diluted at 1/1500 in G - HRPO + FCS at 2%) was added to each well and left to incubate at ambient temperature for 1 hour.

The same washing procedure was repeated and 100 µl substrate solution (i.e. *o*-PD Ortho-phenylendiamine tablets, SIGMA P-8287, one tablet of 10 mg dissolved in 25 ml citrate phosphate buffer SIGMA P-4809 + 5 µl H_2O_2 at 30%) was added to each well. Plates were left to incubate for 20 min in the dark at ambient temperature. Subsequently the reaction was stopped by adding 25 µl H_2SO_4 (2M) to each well. Optical densities (OD) were read by a spectrophotometer (STAT FX 2100), with filters between 492nm and 630nm. Mean OD values of the samples and the calibration curve were corrected by subtracting the mean BB from the mean OD.

A cut off value, above which a sample was considered positive, was set at or above 20 units. Calculation of the units was based on the reference values of the curve i.e. 1.87 U, 3.75 U, 7.5 U, 15 U, 30 U, and 60 U, for dilutions 1/270, 1/540, 1/1080, 1/2160, 1/4320, 1/8640 respectively.