Supplementary Methods S3

1. Onderstepoort Veterinary Institute (OVI), South Africa, Indirect Fluorescent Antibody Test (IFAT) protocol

The IFATs were carried out at the University of Cambridge, UK.

The quality of the serum samples was excellent, with only a few samples with slight to moderate haemolysis. The samples were frozen at -80^{o}C immediately after the blood protein assays had been performed and have remained at -20^{o}C or -80^{o}C. All of the samples had been through at least one freeze-thaw cycle (for Fluorescent Antibody Virus Neutralization assays for Rabies Virus) prior to undertaking the IFATs for this study.

Antigen slides of whole *Babesia rossi* protozoa in canine erythrocytes (with a parasitaemia of 2-5%) were provided by the OVI. Each glass (microscope) antigen slide contained 12 wells. The slides were stored at -20^{o}C until they were thawed for the IFATs at the University of Cambridge. The IFATs were carried out in accordance with the protocol used by the OVI, as follows.

The antigen slides and test and control sera were thawed at room temperature immediately prior to use. The test and positive control sera were diluted to 1:40 and 1:80, and the negative control sera diluted to 1:40 in phosphate-buffered saline (PBS, without calcium and magnesium). The antigen slide was fixed in cold acetone for 1 min and then allowed to air dry at room temperature. Twenty five microlitres of one test serum at 1:40 dilution was loaded into one well on the antigen slide. This was repeated in sequence for nine other test sera at 1:40 dilution. Thus, the sera of 10 dogs, diluted 1:40, were tested per antigen slide. The remaining two wells on each antigen slide contained the positive and negative control sera. The dilution of the positive control sera corresponded to the dilution of the test sera. The negative control sera were always tested at 1:40 dilution. The slides were incubated in a humid chamber at 37^{o}C for 1 h. After incubation the serum was removed from the slides, which were then fully immersed in PBS for 10 min with constant, gentle stirring. The slides were transferred to a wash of distilled water for 5 min; then were removed

from the wash and allowed to air dry. Twenty-five microlitres of fresh conjugate, at 1:80 dilution in 0.02% Evans Blue solution, were then loaded into each well, sufficient to cover the well. The Sigma-Aldrich anti-dog IgG (whole molecule) fluorescein isothiocyanate (FITC) conjugate antibody developed in rabbit was divided into 50 µL aliquots, sufficient to prepare enough fresh conjugate solution for each day of testing, and stored according to the manufacturer's instructions. Evans Blue was reconstituted in PBS according to the manufacturer's instructions and stored protected from light. The slides were incubated in a humid chamber at 37^{o}C for 1 h. After incubation the conjugate was removed from the slides; then the slides were washed in PBS for 10 min with stirring. The wash container was covered in aluminium foil to protect the slides from light. Slides were allowed to dry in light-proof containers. Slides were read immediately using a Zeiss Axio Observer Fluorescence Microscope (Observer A1 Inverted Microscope) at a magnification of 40x. Each well was covered with a drop of glycerine and a cover slip prior to microscopy. Each well was thoroughly examined and at least six high-resolution, digital images, representative of each well (including the control wells), were recorded.