Supplementary Methods S2

1. Blood sample collection and laboratory analyses for the rabies control study

Blood sampling (and vaccine delivery) was door-to-door; and households were revisited repeatedly until the dog was caught, or it was apparent that the dog could not be caught or the owner would not be available to give consent. A dog was also excluded from the study if the owner declined consent, the dog did not remain calm during restraint, there was a high index of suspicion that the dog may bite, or it was apparent the dog had a clinical condition that might have deteriorated as a result of restraint. All the dogs were carefully restrained by experienced personnel using the correct equipment and under the direct supervision of the primary researcher. Blood sampling (and vaccination) were undertaken by the primary researcher. High-quality, sterile consumables (i.e. needle, syringe and blood tubes) were used for each vaccination and blood sample.

For each sample, 5-7 mL of blood were collected from the jugular or cephalic vein and divided into plain and ethylenediamine tetra-acetic acid (EDTA) containing vacutainer tubes. The vacutainer tubes were immediately coded by date, house number and dog identification, and placed in cool boxes with ice packs. Serum was separated by centrifugation within 8 h of collection and refrigerated at 4-6^{o}C. Biochemistry was performed on the serum samples within 48 h of collection. Serum total protein was measured using the biuret method and serum albumin using the bromocresol green method. The EDTA whole blood samples were refrigerated and complete blood counts (CBCs) performed within 48 h of collection by an automated cell counter (ADVIA 2120, Siemens, Munich, Germany) using impedance counting, flow cytochemistry, laser light scattering and validated veterinary software. Blood smears from the venous samples collected in July 2009, February 2010, August 2010 and February 2011 were prepared within 8 h of collection from the EDTA whole blood samples. Capillary blood smears, from a needle prick to the medial pinnae, were also prepared from approximately 120 randomly selected dogs at the time of blood sampling (of the

191 dogs by venipuncture) in February 2010 and all 135 dogs tested in July 2009. All of the venous and capillary smears were prepared using a Romanowsky stain (i.e. Diff-Quick®) and examined for blood parasites under high power magnification (50x or 100x) using a standard light microscope. Fifteen (of the 18) samples that were positive for blood parasites on blood smear evaluation from the four time points were frozen at -80°C within 48 h of collection. Polymerase Chain Reaction (PCR) - Reverse Line Blot (RLB) assays were undertaken on these samples in June 2012 and April 2018 using a method previously described (Matjila et al., 2004). The haematology, blood protein assays and PCR-RLB assays were undertaken by the Faculty of Veterinary Science, University of Pretoria, South Africa. The sera from all four time points, as well as March 2010, were also stored at -80°C within 48 h of collection.

Reference

Matjila, P.T., Penzhorn, B.L., Bekker, C.P.L., Nijhof, A.M., Jongejan, F., 2004. Confirmation of occurrence of *Babesia canis vogeli* in domestic dogs in South Africa. Veterinary Parasitology 122, 119–125.