Supplemental Figure 1. SDS polyacrylamide gel of fractionated canine immunoglobulins. Immunoglobulin fractions were analyzed by SDS polyacrylamide gel electrophoresis by the method of Laemmli[15] on a 10.5% acrylamide gel loaded with 10.5 μg of protein per lane. The gel was stained with Coomassie Brilliant Blue R-250. Immunoglobulin fractions are labelled W, X, Y, and Z according to the convention of Mazza et al.[14] SeeBlue Plus2 prestained markers (ThermoFisher Scientific) were run in the lane marked 'Std'. The apparent molecular weights (MWapp) are indicated on the left.

Supplemental Figure 2. Multiplex bead assay detection of antibodies to recombinant HSP1-GST and HSP2-GST fusion proteins. Multiplex bead assays were conducted as described in the Materials and Methods using magnetic beads covalently coated with either HSP1-GST (left) or HSP2-GST (right). Biotinylated monoclonal secondary antibody 4E3D9 was used to detect responses in sera from dogs previously infected with GW (n = 39) or in dog sera from a non-endemic region of Chad (n = 41). Individual values are indicated by open circles. Note that two negative values for the HSP1-GST assay and three negative values for the HSP2-GST assay are not plotted as they fell below the range of the graph. Boxes include values between the 25th and 75th percentiles. Whiskers and closed circles represent the 10th and 90th and the 5th and 95th percentiles, respectively. Median values are indicated by a horizontal line within the boxes. Distributions that show statistically significant differences (P<0.05) using the Kruskal-Wallis one-way analysis of variance on ranks are indicated by brackets with asterisks.

Supplemental Figure 3. Receiver-operating characteristic (ROC) curve results of responses from HSP1-GST multiplex bead assays using different detection reagents. Curves were constructed using MFI-bg responses of samples collected from individuals with previous GW infection (n = 39) or from individuals from a non-endemic region of Chad (n = 41). Antibody responses were detected with either an anti-IgG Fc polyclonal antibody (anti-IgG, black dashed line), an anti-IgG_1 polyclonal antibody (anti-IgG_1, red line), an anti-IgG_2 monoclonal antibody (anti-IgG_2, red dashed line), or the 4E3D9 monoclonal antibody (anti-IgG_4, black line) as described in the Materials and Methods section. The optimal threshold for the HSP1-GST assay with the 4E3D9 antibody was 94.5 MFI-bg with a sensitivity of 71.8% and a specificity of 73.2%.

Supplemental Figure 4. ROC curve results of responses from HSP2-GST multiplex bead assays using different detection reagents. Curves were constructed as described in Supplemental Figure 3. The optimal threshold for the HSP2-GST assay with the 4E3D9 antibody was 96 MFI-bg with a sensitivity of 69.2% and a specificity of 82.9%.