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Appendix

Materials and Methods

For *Toxocara* spp. and *S. stercoralis* helminth antibody detection, sera were exposed to antigen-coupled beads, using either recombinant *Toxocara canis* C-type lectin 1 (rTc-CTL-1) antigen for detection of antibodies to *Toxocara* spp (*1*). or a recombinant 31 kDA third stage *S. stercoralis* larval antigen (rSs-NIE-1) (*2*). For intestinal protozoa, two *Cryptosporidium parvum* immunodominant antigens (Cp17 and Cp23) and the *G. duodenalis* variant surface protein 3 (VSP3) (*4*) were employed. Serum was diluted 1:400 in Buffer B (1x PBS, 0.5% polyvinyl alcohol, 0.8% polyvinyl pyrrolidone, 0.5% casein [all Sigma, Burlington MA], 0.3% Tween-20, 0.02% sodium azide) containing 3 µg/mL of *Escherichia coli* extract.

For *Toxocara* spp. and *S stercoralis* antibody testing, the serum/buffer B/*E. coli* extract solution was incubated for 30 minutes, with shaking, at room temperature. For *Cryptosporidium* and *Giardia* antigens, the serum/buffer B/*E. coli* extract solution was incubated overnight at 4°C.

Antigen-specific IgG was detected by incubating specimens in duplicate with magnetic beads and then detecting with 50 ng per well of monoclonal mouse anti-human IgG and 20 ng per well of IgG4 (both Southern Biotech, Birmingham AL) and 250 ng per well of streptavidin-linked R-phycoerythrin reporter (Invitrogen, Waltham MA), as described previously (*4*). Between steps, the magnetic beads were washed three times with 0.05% Tween 20 in PBS, using a BioTek Plate washer (BioTek® Instruments, Winooski, VT). A MAGPIX® reader with xPONENT® software calculated the median fluorescence intensity (MFI) from each bead classification from each well. Background fluorescence from a blank with no serum was subtracted (MFI-bg, reported as MFI).

Samples were considered positive at above 8 MFI for *S. stercoralis* and 23.1 MFI for *Toxocara* spp. A *S. stercoralis* enzyme-linked immunosorbent assay based on crude larval

antigen (CrAg-ELISA) (*4*), with a positive cutoff of 1.7 IU/mL, was used to confirm the results for samples yielding positive results by the initial rSs-NIE-1 MAGPIX® assay. Cutoffs for Cp17 (85 MFI), Cp23 (377 MFI) and VSP3 (84 MFI) were extrapolated from in-house standard curves with cutoffs originally defined by receiver operator characteristic curves as described previously (*3*,*5*) Only samples reacting with both the Cp17 and Cp23 antigens were considered to be positive for prior exposure to *Cryptosporidium*.

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