samples were sent to the Laboratory of Human Parasitology and Immunology for blood processing, plasma separation and storage for later serological analysis methods. Each serum sample was obtained from the whole blood, after centrifugation at 350 × *g*, for 15 minutes. The resulting supernatant was stored at −20°C.

Indirect Fluorescent Antibody Test (IFAT)

The indirect fluorescent antibody test (IFAT) technique for the screening of anti-*T. gondii* antibodies, of the IgG class, was carried out according to the methodology described by Camargo [23]. Human anti-IgG serum produced from rabbit inoculations was employed conjugated with fluorescein isothiocyanate (Sigma Chemical Corp., USA). For each serum sample, dilutions at 1:16, 1:64, 1:256, 1:1024 and 1:14096 were prepared. All reactions had a previously known positive and negative control serum. The reading of the plates was made in a fluorescence microscope (Nikon™, Japan). Dilutions greater than or equal to 1:16A were considered positive results.

ELISA immunoenzymatic test

Anti-*T. gondii* IgG antibodies were determined by the methodology of Camargo [24] and Uchôa *et al*. [39]. Plates with flat-bottom wells (Corning®, USA) sensitized with 1 μg/mL of *T. gondii* soluble antigen extract were used for OPD ELISA; and those sensitized with 10 μg/mL of *T. gondii* soluble antigen extract were used for ABTS ELISA. Plates were sensitized with antigen by incubation at 4°C for 18 hours, with 100 μL of antigen solution per well. After washing, plates were blocked by incubation for one hour at 37°C with 250 μL of PBS-T per cavity (0.05% of Tween 20 in phosphate-buffered saline solution – PBS) containing 3 g of skimmed milk powder (Molico®, Nestlé, Brazil). Afterward, plates were washed five times in PBS-T for five-minute periods. Plates containing serum with human-peroxydase-conjugated anti-IgG dilutions (Sigma®, USA) were incubated for the test in stove for one hour. Following both incubations, plates were washed three times in PBS-T for five minutes. The color reaction was carried out subsequently in the dark for 15 minutes at room temperature with 100 μL of the OPD chromogenic solution (o-phenylenediamine 0.05%, Sigma® + citric acid 1% + Na_2HPO_4 1.45% in H_2O, addition of 10 μL de H_2O_2 30% for each 20 mL of the solution) and was quenched by the addition of 25 μL of 2 M sulfuric acid solution. For the OPD chromogenic solution, the optical density (OD) was determined by the reading at 492 nm, and for the ABTS chromogenic solution, by the reading at 414 nm and 612 nm, in a plate spectrophotometer (700 Plus Spectrophotometer, Fendo, Brazil). For OPD ELISA and ABTS ELISA, in both the first analysis and the one six months after, the cutoff point was obtained from the mean of results from negative control sera evaluated by IFAT plus two standard deviations.

IgG avidity

The same procedure was used for IgG analysis through in-house ELISA, according to the methodology described by Hedman *et al*. [40]. The plate was coated by the addition, for 24 hours at 4°C, of 100 μL of *T. gondii* protein antigen to the wells at 1 μg/mL concentration, in an 8 M urea chaotropic (Sigma®) solution in PBS. After blocking possible free sites, the wells received 100 μL of PBS-T diluted serum samples (1/100, 1/200, 1/400, 1/800). Plates were incubated for one hour at 37°C and washed five times in PBS-T. For each dilution, two wells received additional ten-minute incubation at 37°C with 6 M urea neutral chaotropic solution (Sigma®) in PBS-T. Wells containing the control solutions were kept in PBS-T. Afterwards, the antibody development was carried out with the use of human anti-IgG peroxydase conjugate (Sigma®).

The estimation of effective titer of each serum was carried out by means of the isolated dilutions of each sample in a log-log linear regression model. The titers of total or high avidity chaotropic-resistant antibodies were achieved by means of log-log regression, with the use of the values able to generate 1.0 absorbance in ELISA (Log = 0). Avidity (AVT) was determined by the percentage of chaotropic resistant titers. Samples with AVT values higher than 30% were considered as high avidity. The optical density (OD) was determined by the reading at 492 nm in a plate spectrophotometer.

Statistical analysis

The correlation rate was used for the analysis among the interspecific titers and the analysis of association among the positive results and variables related to risk factors (gender, direct contact with soil, contact with cats, consumption of raw or undercooked meat and raw vegetables). Values were submitted to the chi-square analysis, with the help of the Epi Info version 6.04 statistics software [41]. A reliable 95% range was considered and $p\text{-value} < 0.05$ was considered statistically significant. In order to check the agreement among ELISA and OPD or ABTS chromogen and IFAT diagnosis tests, the results were reviewed by the kappa test (K), and by sensitivity, specificity, positive predictive value and negative predictive value. Statistical comparisons were made using the GraphPad Prism version 5.00 software for Windows (Graphpad Software, USA).

Ethics committee approval

The present study was approved by the Research Ethics Committee of Marília Medical School (FAMEMA – Marília, SP, Brazil) under protocol n. 449/08. It included