Parasitology Research (2019) 118:3449–3457
https://doi.org/10.1007/s00436-019-06473-w

PROTOZOOLOGY - ORIGINAL PAPER

Food safety assessment and risk for toxoplasmosis in school restaurants in Armenia, Colombia

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Received: 28 April 2019 / Accepted: 24 September 2019 / Published online: 28October 2019

Abstract

We assessed the risk for toxoplasmosis in 10 school restaurants in Armenia (Quindio, Colombia). We analyzed the presence of *Toxoplasma gondii* DNA in the food, water, and living and inert surfaces of school restaurants, and we correlated these findings with the results of food safety inspection scores and with the prevalence of specific anti-*T. gondii* antibodies in children who ate at these restaurants. Of the 213 samples, 6.1% were positive using PCR to test for *T. gondii* DNA. Positive samples were found in meat, water, cucumber, guava juice, inert surfaces, and living surfaces. In 60% (6/10) of the public school restaurants, there was at least one PCR *T. gondii*-positive sample. In 311 serum samples from children who attended the restaurants, 101 (33%) were positive for IgG and 12 (3.9%) for IgM anti-*T. gondii.* The median of the compound score for the fulfillment of inspection for food safety conditions was of 60.7% (range 50–72). Higher *T. gondii* PCR positivity in surfaces, food, or water at each restaurant was correlated with lower inspection scores for water supply and water storage conditions. Lower scores in physical infrastructure and disinfection procedures and higher scores in furniture were correlated with a higher prevalence of IgG anti-*T. gondii* in children who ate at those restaurants. Inspection scores can identify restaurants with a higher risk for the presence of *T. gondii*.

Keywords *Toxoplasma* · Foodborne protozoa · PCR · Molecular detection

Introduction

Foodborne toxoplasmosis was prioritized within the foodborne parasites by the WHO and FAO, as the fourth most dangerous parasite for humans, due to its impact on human health and large prevalence worldwide (FAO/WHO 2014). Foodborne toxoplasmosis can be acquired through contaminated water (De Moura et al. 2006), undercooked meat (Franco-Hernandez et al. 2016) or unwashed raw vegetables (Robertson 2016). Inspection scores of sites of production or preparation of food, such as restaurants, are a vital instrument of public health authorities and have made proof to identify sites with high risk for food contamination with bacteria (Lee and Hedberg 2016). However, no evaluation exists of the concordance between inspection scores for parasites such as *Toxoplasma gondii* on food. For food industry policies and for health inspection authorities, it is important not only to identify the risk factors involved in foodborne toxoplasmosis but how hygienic measures can reduce such risks and if food safety evaluation tools, such as questionnaires about food safety

Julio César Luna and Alejandro Zamora contributed equally to this work.

Section Editor: Berit Bangoura

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s00436-019-06473-w) contains supplementary material, which is available to authorized users.

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compliance, can identify restaurants that have a risk for foodborne toxoplasmosis.

We chose to analyze the elements involved in food preparation (that can be modified) for school restaurants in Armenia regarding the *T. gondii* DNA presence as an indicator of the potential contamination of food, live and inert surfaces, and water. We selected a representative sample of public school restaurants in Armenia, Quindio, in Colombia, and we collected all the information related to the hygienic conditions of providers of school students' meals and of the seroprevalence of *T. gondii* in school children.

Materials and methods

School restaurants selected for the study and analyzed samples

Armenia is the capital of the "Departmento del Quindío" and is located within Colombia's central mountain range at an altitude of 1480 m, and has 301,226 inhabitants, according the projections and results from the last general census data in 2005 (DANE 2018). Armenia is divided into 10 administrative zones, called "communes," and has 46 public school restaurants. For this study's purpose, one restaurant was randomly selected from each commune, resulting in a sample of 10 public school restaurants (21% of the total). By choosing one school by commune, the representativity of the geographical and socioeconomical diversity of the city was assured. The sampling days were randomly chosen, and all the food and water samples were taken in triplicate. The food samples corresponded to those that were prepared in the restaurant on the day of collection. We took 12 samples of raw beef meat and nine of raw chicken meat (50 g each), nine of raw and unwashed egg, three of lettuce, three of cabbage, nine of cucumber, nine of carrot, and six of tomato (200 g of principal raw vegetables from a salad before being mixed). Thirty samples of boiled water were used to prepare the juice (200 ml each), 10 samples of faucet water (4 l each), six of banana juice, 12 of guava juice, six of mango juice, and six of tamarillo (local Colombian fruit) juice (200 ml each). Samples from 52 food contact surfaces were taken using a swab and saline solution 0.9% (the number varied depending on the number of utilities or supplies used during food preparation). Thirty-one live surface samples were collected using a swab in saline solution 0.9% (palms of the hands of the people who handled food). In total, 213 samples from food and live and inert surfaces from the public school restaurants were collected. In consequence, in this study, we obtained the prevalence of *T. gondii* DNA present in food, surfaces, and water at each restaurant, as long as samples from each component of the menu and surfaces and water involved in food preparation were analyzed.

Serological testing for anti-*T. gondii* antibodies in children attending public schools of Armenia

The targets of the study were students of the public school restaurants aged 4 to 12 years, where the food samples were collected. Sample size was determined using Epi Info v7.2 software, where the total population of children attending public schools in 2017 was of 12,277 according to the data of the "Secretaría de Educación de Armenia." The expected prevalence of IgG anti-*T. gondii* in children in Colombia was estimated to be ~ 30%, according to data from the national study of toxoplasmosis (Cañón-Franco et al. 2014). A sample of 302 children was necessary to obtain inferences about the prevalence in this population with margin of error 5% and confidence level 90%. We prepared public meetings with the parents and guardians of children and requested voluntary permission from the children to gain access to venous blood sampling; if they agreed, they then signed an informed consent. The parents of children were interviewed by using questionnaires that included general demography data including age, followed by the main water supply and habits at home related to food preparation. Other factors included habit of meat consumption (well done or undercooked), type of meat, and cat contact (yes or not). A total of 5 ml of peripheral venous blood samples was obtained from each child by a laboratory technician in the presence of the children's parents. All serum samples were analyzed for anti-*T. gondii* IgG and IgM antibody titers using ELFA commercial assays according to the manufacturer's recommendation (bioMerieux, Marcy L'Etoile, France) and read in a Mini Vidas immune diagnostic assay system (bioMerieux, Inc., NC, USA).

DNA extraction method and PCR for *Toxoplasma*

The DNeasy Blood & Tissue Extraction Kit (Qiagen, Germany) with mechanical lysis and a zirconium beads was applied five times for DNA extraction in the vegetable and juice samples after agitation in a stomacher 400-W BagMixer (Interscience-France) at 260 rpm for 30 s with glycine 1 M and a pH of 5.5 (Cook et al. 2007) and a wash solution (PBS 1X, 100 μL of Tween 80 and 10 g of sulfamic acid) to a pH of 7.5, respectively, and a formalin-ether concentration method for water eluates was applied (also for inert and live surfaces samples), as described by Triviño-Valencia et al. (2016) and Lora-Suarez et al. (2016). Meat samples were processed for analysis as described by Alvarez et al. (2015) and Franco-Hernandez et al. (2016). The meat was cut until pieces of approximately 5 g; they were then ground in a mortar with 2 ml of a lysis solution from the DNA extraction kit from Wizard Genomics (Promega, Madison, WI, USA) and with 50 μl of proteinase K over the course of 10 min, and then kept at room temperature for 30 min. Next, it was incubated at 37 °C for 30 min. After that, we added 250 μl of a saline solution buffer with a pH of 7.4, and the sample was centrifuged at 1000*g* over

10 min; the supernatant was then discarded. The cellular proteins were then removed using the reagents and procedures of the Wizard Genomic DNA Purification Kit (Promega, Madison, WI, USA), and genomic DNA was concentrated by adding 80 μl of a Tris-EDTA rehydration solution and stored first at 4 °C overnight and then frozen at − 80 °C until use. Oocysts from the *T. gondii* ME49 strain (clonal type II), cat 19, TX 586, collected June, 2014 (USDA, Belstville), were used as controls of the DNA extraction procedure.

To amplify the *T. gondii* DNA, a conventional nested PCR was used for this study, as previously described (Triviño-Valencia et al. 2016; Franco-Hernandez et al. 2016; Zamora-Vélez et al. 2016). This test amplified a 97-bp fragment of the B1 repetitive gene of *T. gondii*, which is tandemly arrayed as 35-fold repetitive (Genebank accession number AF179871). The primers for the first PCR were Toxo N1 (694 to 714 nt) at 5′-GGA ACT GCA TCC GTT CAT GAG-3′ and Toxo C1 (887 to 868 nt) at 5′-TCT TTA AAG CGT TCG TGG TC-3′. The second PCR was performed with the primers Toxo N2 (757 a 776 nt) at 5′-TGC ATA GGT TGC CAG TCA CTG-3′ and Toxo C2 (853 a 831 nt) at 5′-GGC GAC CAA TCT GCG AAT ACA CC-3′. All primers were synthetized by the Invitrogen Corporation (USA). All PCR amplifications were performed in the Applied Biosystems Veriti Thermal Cycler machine (Termo Fisher Scientific, USA). PCR products were gel-purified from low-melt agarose gels for sequencing. DNA recovery from gels was performed by using the Wizard PCR SV and PCR clean up system kit (Promega, WI). Sequencing was done by using the service by Macrogen (Korea) in 3730XL DNA sequencer with the same primers as the PCR amplifications. Sequences were aligned with Clustal W in Molecular Evolutionary Genetics Analysis (MEGA) software, Version 5.05 (available at: http://www.megasoftware.net/).

Evaluation of food safety conditions in school restaurants

To evaluate the food safety conditions of public school restaurants, we applied the checklist instrument for restaurant inspection from the Colombian National Institute for Food and Drug Control Resolution 2674 of 2013 (INVIMA, Bogota, Colombia, https://www.minsalud.gov.co/sites/rid/Lists/BibliotecaDigital/RIDE/DE/DIJ/resolucion-2674-de-2013.pdf), which included five topics with 26 items: (i) the physical conditions of restaurant infrastructure, including material of kitchen working zones and the material of the walls, ceiling, and floor; (ii) equipment, furniture, utensils, and the temperature that vegetables and meat are stored at; (iii) water supply, including water storage conditions; (iv) property conditions, including disinfection procedures, such as the frequency of washing hands, washing hands after meat manipulation, disinfectants used for surface decontamination, disinfectants used for food utensils, and the frequency that equipment and furniture are cleaned; and (v) cooks knowledge and training in handling food. This instrument gives a scale by which to rate each one of the food safety practice items (2 = full, 1 = partial, or 0 = no compliance). Based on these scores, the percent of the fulfillment of conditions within each of the five conditions was finally established. A ranking of 100% indicated that a restaurant complied with all the items within each topic; lower percentages indicated partial fulfillment of conditions within each topic. Members of the research group were instructed to standardize the evaluation process. All data were collected and used for this study and were not part of a routine inspection.

Statistical analysis

Results were expressed as the median [min–max] for continuous variables and *N* (%) for categorical variables. Differences in proportions were analyzed using the chi-square test or Fisher's exact test when appropriate. Mantel–Haenszel statistics was used to test for independence between a dichotomous factor variable and a dichotomous response variable, conditional on covariate patterns defined by one or more layer (control) variables. Differences in means were compared using a Student's *t* test or a non-parametric test if not normally distributed. Stratified analysis was applied for controlling confounding factors (Tripepi et al. 2010).

To establish the predictive positive and negative value of the percent scores of fulfillments of food safety conditions for restaurants (obtained by food safety inspection instrument) that helped identify restaurants with *T. gondii* PCR-positive results in food and surfaces, a receiver operating curve was calculated. The SPSS software (version 14.0, Lead Technologies Inc, USA) was used to analyze the statistical tests. Values below $P < 0.05$ were considered to be statistically significant.

Bioethics aspects

The protocol of this study was approved by the institutional ethical committee for the Faculty of Health Sciences of the Universidad del Quindio (Act 35 of May 14, 2012). All parents of the children accepted participating in the study and signed the informed consent. Institutional consent was also obtained from the school restaurants. Clinically relevant results were returned to the patients.

Results

Positivity of nested PCR for *T. gondii* in food, water, and live and inert surfaces

Of the 213 analyzed samples, 6.1% were positive using PCR for *T. gondii* DNA. The number of positive samples was higher in living surfaces and water samples and less common in vegetables

Table 1 Prevalence of DNA-positive samples by conventional nested PCR that amplified the B1 repeated *T. gondii* sequence from food, water, juice, and inert and live surfaces, collected in 10 public school's restaurants in the city of Armenia (Colombia) during the year 2018

Type of sample	Number of samples	Restaurant number where the samples were positive	PCR-positive samples (% of total)
Raw chicken meat	9	-	0 (0%)
Raw beef meat	12	II	1 (8%)
Egg	9	-	0 (0%)
Faucet water	10	III, IV	2 (20%)
Boiled water for juice	30	III, X	2 (6%)
Lettuce	3	-	0 (0%)
Cabbage	3	-	0 (0%)
Cucumber	9	X	1 (11%)
Carrot	9	-	0 (0%)
Tomato	6		0 (0%)
Banana juice	6	-	0 (0%)
Guava juice	12	III	1 (8.3%)
Mango juice	6	-	0 (0%)
Tamarillo juice	6	-	0 (0%)
Inert surfaces	52	III, VIII	2 (3.8%)
Live surfaces	31	III, IV, IX, X	4 (12.9%)
Total	213		13 (6.1%)

and fruits (Table 1). Interestingly, the only positive sample for fruit juice (guava juice) was in the same restaurant where the water sample for juice preparation was positive.

In 60% (6/10) of the public school restaurants, there was at least one PCR *T. gondii* DNA-positive sample. The higher frequency was in restaurant III, with 5/25 (20%) positive samples; followed by restaurant X with 3/24 (15%) positive samples; and restaurant IV, with 2/21 (9.5%) positive samples. One DNA-positive samples were detected in restaurants II (1/22), VIII (1/23), and IX (1/20) and all samples were negative at restaurants I (n = 24), V (n = 20), VI (n = 19), and VII (n = 18). When Mantel–Haenszel statistics were applied to test what variable (the presence of *T. gondii* on a surface, in meat, in vegetables, in fruits, or in water) was responsible for differences between restaurants, the water appeared to be the main reason for the differences (p = 0.003).

We performed sequencing of all positive samples and compared them with the B1 sequence of the RH strain of the lab, to demonstrate that there was not contamination during PCR amplification (Fig. 1). All good quality sequences had differences at

Water for juice (X)	A	T	G	A	G	T	A	T	C	T	G	T	G	C	A	-	C	T	T	T	G	G	T	G	T	A	T	T	C	G	C	A	G	A	T	T	G	G	T	C
Water for juice (III)	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	-	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
RH positive control	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Live surface (X)	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	A	.
Live surface (IX)	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	G	.
Live surface (III)	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Inert surface (VIII)	-	A	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	A	.
Inert surface (III)	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	-	-	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Live surface (IV)	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Guava juice (III)	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Faucet water (IV)	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	-	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Faucet water (III)	-	-	-	-	-	-	-	-	-	-	A	A	T	G	C	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	A
Cucumber (X)	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.
Beef meat (II)	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	-	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.

Fig. 1 Alignment of 13 *T. gondii* B1 sequences amplified from food and surfaces samples obtained at the school restaurants in Armenia (Quindio, Colombia) during 2018 and the RH strain used as control during amplifications. The source of visually curated good quality sequences is described together with the number assigned to the restaurants where the sample was obtained. The region of 40 nt is located at the chromosome IX of *T. gondii* VEG strain (GenBank: LN714499.1) coding for glycerol-3-phosphate dehydrogenase—B1 repeated sequence (nt 5186929 to nt 5186968)

Table 2 Prevalence of IgG and IgM *T. gondii* antibodies as determined by ELISA assay in children that assisted to the public school's restaurants in the city of Armenia (Colombia) during the year 2018

Restaurant	I	II	III	IV	V	VI	VIII	IX	X	Total
Proportion of children with IgG anti-*Toxoplasma* n/N (%)	7/10 (70%)	4/8 (50%)	2/4 (50%)	14/38 (36.8%)	1/2 (50%)	20/55 (36.4%)	14/56 (25%)	4/48 (8.3%)	35/90 (38.9%)	101/311 (32.5%)
Proportion of children with IgM anti-*Toxoplasma* n/N (%)	2/10 (20%)	0/8 (0%)	1/4 (25%)	3/38 (7.9%)	0/2 (0%)	2/54 (3.7%)	0/56 (0%)	1/48(2.1%)	3/90 (3.3%)	12/311 (3.9%)

the region analyzed, indicating that they were unique, excepting three of them that are the same that the control sequence. Overall, there were no indications of generalized contamination.

Prevalence of *T. gondii* antibodies in children that assisted to school restaurants in Armenia

In total, 311 serum samples were collected from children. There were 101 (33%) children with IgG anti-*T. gondii*-positive and 12 (3.9%) with IgM anti-*T. gondii*-positive tests (Table 2). In one school (number VII), it was not possible to collect serum samples from children because the school's director denied to permit sampling. The lowest IgG prevalence was in school IX, with 8.4%, which was statistically significant compared with the other schools ($p = 0.0001$).

When the questionnaires about habits at home were analyzed in relation with IgG prevalence, we found that no habits at home were related to a higher frequency of IgG or IgM anti-*Toxoplasma*, but a lower socioeconomical stratum was related in statistically significant manner with higher prevalence of IgG anti-*Toxoplasma* and age lower than 5 years with a higher frequency of IgM anti-*Toxoplasma* (Supplementary Material).

Inspection scores for the food safety conditions of school restaurants and the correlation with the positivity of *T. gondii* DNA and the results of *T. gondii* serology

The median of the compound score for fulfillment of inspection for food safety conditions was of 60.7% for the 10 restaurants with a range of 50–72 (Table 4). The lower compliance was for physical infrastructure conditions with a mean of fulfillment of 23% (range of 0–50). A Kolmogorov–Smirnov test indicated the normality in data distribution for the inspection scores (Table 3), and a Pearson test was performed to determine if significant correlations between each inspection items, if the compound score and the positivity in the presence of *T. gondii* DNA using PCR at each restaurant, or if the prevalence of anti-*T. gondii* IgG or IgM in children who attended those restaurants existed (Table 4). Higher *T. gondii* PCR positivity in surfaces, food, or water at each restaurant was correlated with lower inspection scores for water supply and water storage conditions. Lower scores at the physical infrastructure conditions of each restaurant or property and higher scores in equipment or furniture were significantly correlated with a higher prevalence of specific IgG antibodies (Table 4). When stratified analysis was performed to control for lower socioeconomical level of children, the effect of exposure to restaurants with lower scores for infrastructure or property and high in equipment, was only significant for children with higher socioeconomical level. Conversely, the stratified analysis indicated that the relation between IgM anti-*T. gondii* and lower scores in disinfection was related to a lower age and not to the scores of the restaurants (Supplementary Material).

Table 3 Percent of fulfillment of compliance with items of food safety conditions at the checklist INVIMA instrument by public school's restaurants in the city of Armenia (Colombia) during the year 2018

School restaurant code	I. Physical conditions of restaurants infrastructure	II. Equipment, furniture, utensils, and temperature of storing of vegetables and meat	III. Water supply, including water store conditions	IV. Property conditions including disinfection procedures	V. Food manipulators knowledge and training in food handling	Median compound score
I	0	71.4	71.4	42.90	62.5	62.5
II	0	71.4	59.5	52.40	62.5	59.5
III	16.6	64.3	50	50	70.8	50
IV	16.7	64.3	63.1	76.2	83.3	64.3
V	16.7	71	72.6	78.6	79.2	72.6
VI	16.7	66.7	64.3	78.6	70.8	66.7
VII	25	57.1	55.4	85.7	75	57.1
VIII	38.9	57.1	58.9	92.9	70.8	58.9
IX	50	57.1	58.9	92.9	68.8	58.9
X	50	57.1	54.8	92.9	66.7	57.1
Mean	23	63.7	60.89	74.31	71	60.76
Standard deviation	18	6.2	7.15	19	6,69	6.17
Range	0–50	57–71	50–72	42–92	62–83	50–72
One sample Kolmogorov–Smirnov test	0.6243	0.5344	0.9124	0.6146	0.7479	0.8987

Predictive value of inspection scores for *T. gondii* DNA positivity

As the lower inspection scores for water supply and water store conditions was significantly associated with higher positivity of *T. gondii* DNA in the restaurants, we performed a receiver operating analysis to establish which percentage scores were able to identify the restaurants with a higher risk for *T. gondii* presence and which were its predictive values (Table 5). Item III showed us that when the percent of fulfillment of conditions of ≤ 55% was selected (Fig. 2), it had a sensitivity of 61.5% and a specificity of 87%, with a negative predictive value of 97% and a positive predictive value of 23%.

Discussion

We found that a majority of school restaurants in Armenia show the presence of *Toxoplasma* DNA in water, inert and living surfaces, and food. This presence could be correlated with a lower fulfillment of food safety conditions, particularly with the water supply and water storage conditions. The instrument that we used to check the food safety conditions was the Colombian official instrument for the inspection of restaurants and food processing installations. This questionnaire poses nine questions related to the existence of written protocol about the quality and handling of water; the existence of registries for the drinkability of water, such as chlorine concentration; the conditions of water reservoirs;

Table 4 Pearson correlation test between items and compound scores of the checklist INVIMA instrument and PCR positivity for *T. gondii* DNA at ten public school's restaurants or anti-*T. gondii* IgG and IgM in children that assisted to the public school's restaurants, of the city of Armenia (Colombia) during the year 2018. Two-tailed statistically significant correlation ($p < 0.05$) are showed in italics

		Percent of samples with *Toxoplasma* DNA positivity by PCR at each restaurant $N = 10$	Percent of prevalence of anti-*Toxoplasma* IgG positivity in children at each restaurant $N = 9$	Percent of frequency of anti-*Toxoplasma* IgM positivity in children at each restaurant $N = 9$
I. Physical conditions of restaurants infrastructure	Pearson correlation	0.27	*− 0.79*	− 0.37
	Sig. (2-tailed)	0.43	*0.0106*	0.3138
II. Equipment, furniture, utensils, and temperature of storing of vegetables and meat	Pearson correlation	− 0.28	*0.79*	0.21
	Sig. (2-tailed)	0.4321	*0.0112*	0.5713
III. Water supply, including water storage conditions	Pearson correlation	*− 0.69*	0.37	− 0.17
	Sig. (2-tailed)	*0.0252*	0.3160	0.6555
IV. Property conditions including disinfection procedures	Pearson correlation	− 0.09	*− 0.81*	*− 0.70*
	Sig. (2-tailed)	0.7916	*0.0069*	*0.0355*
V. Food manipulators knowledge and training in food handling	Pearson correlation	− 0.009	− 0.22	− 0.14
	Sig. (2-tailed)	0.9787	0.5578	0.7186
Median compound score	Pearson correlation	*− 0.66*	0.11	− 0.47
	Sig. (2-tailed)	*0.0342*	0.7620	0.1964

Table 5 Diagnostic predictive values of the score percent of fulfillment of conditions at the item III. Water supply, including water store conditions, of the checklist INVIMA instrument to identify restaurants with PCR-positive samples for *T. gondii* DNA at ten public school's restaurants of the city of Armenia (Colombia) during the year 2018

Percent score of fulfillments of conditions at the item III. Water supply, including water store conditions	Number of samples PCR positives at the restaurant with the selected score range	Number of samples PCR negatives at the restaurant with the selected score range	Sensitivity	Specificity
≤ 50%	5	20		
> 50%	8	180		
Total	13	200	38	90
≤ 55%	8	26		
> 55%	5	174		
Total	13	200	61.5	87
≤ 60%	11	121		
> 60%	2	79		
Total	13	200	84	39.5
≤ 65%	13	159		
> 65%	0	41		
Total	13	200	100	20.5
≤ 70%	13	159		
> 70%	0	41	100	20.5
Total	13	200		

and the production of ice from drinking water. In consequence, the lower percentages of scores in water storage conditions indicated that restaurants failed to have adequate conditions for drinkable water, and this was correlated with a higher presence of *T. gondii* DNA. The PCR assay detected that the restaurants where water had a presence of *T. gondii* also had positive samples on food and inert surfaces. This suggests that water is a major source for contamination in those restaurants, as logically, water is used in all food preparation and surface cleaning steps. The presence of *T. gondii* on inert surfaces has been reported recently as a finding in metagenomics studies on automated teller machine keypads in New York City (Bik et al. 2016) and suggests that the disinfection of surfaces can play an important role in preventing *T. gondii* infection. In support of this, our finding that lower compliance of disinfection procedures was associated with a higher prevalence of IgG anti-*T. gondii* in children who attended these restaurants indicates that this is an important food safety condition that should be surveilled in restaurants.

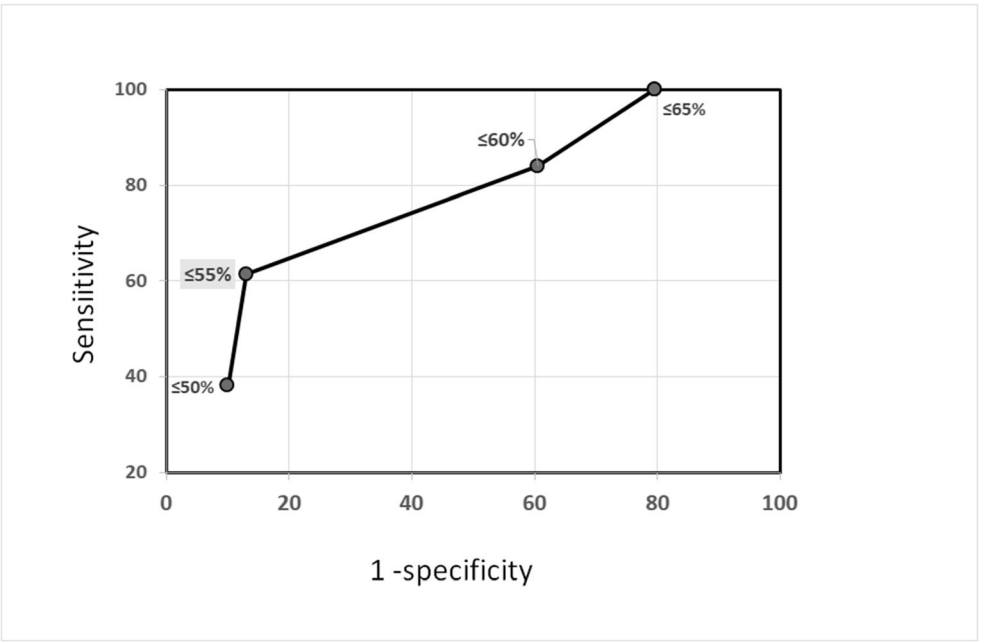

Fig. 2 Receptor operating curve for the diagnostic predictive values of the score percent of fulfillment of conditions at the item III Water supply, including water store conditions, of the checklist INVIMA instrument to identify restaurants with PCR-positive samples for *T. gondii* DNA. The best score percent of fulfillment of conditions at the item III as cutoff (≤ 55%) is highlighted

Armenia has a prevalence of *T. gondii* antibodies in the general population that is greater than 50% (Cañón-Franco et al. 2014). This study confirms this high prevalence exists since infancy and suggests that school restaurants can contribute to this early infection. A higher prevalence of IgG was associated with restaurants with lower compliance with disinfection procedures, indicating that children who attend public school restaurants have a higher risk of acquiring the infection as a foodborne disease. The stratified analysis for the confounding factor of lower socioeconomical level (for IgG anti-*T. gondii*) confirmed that lower scores for physical infrastructure and disinfection procedures, increased the risk for *T. gondii* infection, but only for children of high socioeconomical level, in other words, the results suggest that children of low socioeconomical level are being infected in home and those of high socioeconomical level at school restaurants. Curiously, higher scores related to furniture increased the risk of children to have more IgG anti-*T. gondii*. This score increases if plastic material is used in food preparation; therefore, it is possible that the recommendation of this material should be changed as part of the current norms, instead the use of metallic material should be privileged. The control of the age as confounding factor for the correlation between IgM anti-*T. gondii* and score of restaurants demonstrated that these were not related. As most of the children analyzed in this study have been exposed many years to the school restaurants, IgG anti-*T. gondii* is a better indicator for the risk than IgM anti-*T. gondii*.

The infection sources in school restaurants for children, according to our findings, could be water (contaminated fruit juices), meat, and some vegetables. We found 8% positivity in beef meat, and previously, in large studies on various types of meat, we found that PCR can be positive in 32.4 to 52.7% of meat samples (Lora-suarez et al. 2007; Alvarez et al. 2015; Franco-Hernandez et al. 2016). Additionally, in water samples, we found a positivity of 58.6% using the same technique (Triviño-Valencia et al. 2016). One sample, from a cucumber, had a positive result in our study; there are no previous studies in Colombia about the detection of *T. gondii* DNA in vegetables samples.

PCR for DNA detection does not determine viability or infectivity. Indeed, there is not available method for routine verification for *T. gondii* or other protozoa on food (Rousseau et al. 2018). We used PCR to detect *T. gondii* because it is sensitive enough to detect low quantities of parasites and are accessible for routine analyses. While this method can overestimate the presence of infective parasites by detecting all populations (live and infectious, live and non-infectious, or dead) they offer information of the maximum occurrence and about the level of food contamination. Food should be free from the presence of protozoa and any contamination (by viable or non-viable protozoa) indicates failure in good agricultural or food processing practices (Rousseau et al. 2018).

The instrument of restaurant inspection we applied identified the critical factor that increased the risk for the presence of *Toxoplasma* DNA. Although water seems to have a prominent role, other sources are crucial and the low positive predictive value of the score for water indicates that this cannot be the only item to be used to evaluate the risk for *T. gondii* presence at restaurants. These findings support the application of the food safety inspection instrument as a public health tool (da Cunha et al. 2016; Lee and Hedberg 2016). Future studies should analyze how the application of the procedures can impact the reduction of toxoplasmosis in the school children population.

In conclusion, this study identified the potential sources and the critical points where public health authorities can intervene in school restaurants to reduce the risk of foodborne toxoplasmosis.

Acknowledgments We thank Dr. Dubey (USDA, Belstville) who kindly donated *T. gondii* oocysts.

Funding information This work was funded by the Universidad del Quindio and Colciencias Grant Number: 111372553376.

Compliance with ethical standards The protocol of this study was approved by the institutional ethical committee for the Faculty of Health Sciences of the Universidad del Quindio (Act 35 of May 14, 2012).

Conflict of interest The authors declare they have no conflict of interest.

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