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Seroprevalence and national distribution of human toxoplasmosis in Mexico: analysis of the 2000 and 2006 National Health Surveys

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ABSTRACT

Global warming has had serious implications on dispersion of infectious diseases like toxoplasmosis. Since the frequency of *Toxoplasma gondii* largely depends on climatic conditions, we studied its prevalence by means of 3599 samples of the National Health Survey 2000 (NHS-2000) and 2916 of the National Health and Nutrition Survey 2006 (NHNS-2006) serum banks, obtained from 1–98 year old subjects of both genders and all states of Mexico. Anti-*T.gondii* IgG antibodies were determined by ELISA and confirmed by western blot. Crude, epidemiologically weighted and diagnosis-performance-adjusted prevalence values were calculated. Seroprevalence changes were compared between both surveys and among regions (north, center and coast). Also, correlations between changes in temperature or humidity and those in prevalence were measured. National crude prevalence was 60.1% and 62.6% for NHS-2000 and NHNS-2006, respectively. Weighted and adjusted values were 62.5% and 40.0% for NHS-2000, and 63.7 and 43.1% for NHNS-2006. Coastal states and children presented the largest increases between surveys, while the center of the country showed a decrease. An apparently higher prevalence of *T. gondii* infection was observed in both surveys compared to that performed in 1987, while a geographical re-distribution was found from 2000 to 2006, with a positive correlation between temperature and frequency deltas in 21 states where prevalence increased.

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1. Introduction

Toxoplasmosis is a common parasitic disease caused by *Toxoplasma gondii*, a remarkably successful parasite found

in many species throughout the world.¹ This infection has a significant impact from the public health perspective, mainly due to severe complications in immunosuppressed subjects and the risk of congenital infection, which can lead to abortion, spontaneous stillbirth or clinical manifestations such as severe neurological abnormalities and ocular lesions.^{2–4} Moreover, it is now known that *T. gondii* may cause behavioral and psychiatric disorders.⁵

Toxoplasmosis is a health concern worldwide: in 2000 it was estimated that one third of the human population might be parasitized, but its prevalence varies from 12 to 90% in different parts of the world.^{2,6,7} Although urbanization, alimentary habits and serological techniques

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employed may explain some differences, environmental conditions play a major role. Sporulation and survival of *T. gondii* oocysts are favored in warm-humid climates, like coastal zones of warm regions, where young children are more often infected, due to higher exposure to the environmental forms, the oocysts.^{4,8–14} In this regard, it has been suggested that climatic change could increase the frequency of several infectious diseases, including toxoplasmosis, worldwide.^{15,16}

Mexico is a country with heterogeneous socio-demographical and geographical conditions, and a high ratio of internal and external migration. In 1987 the first National Seroepidemiological Survey was carried out to study different diseases, including toxoplasmosis. Using immunofluorescence for IgG antibodies as a screening test, a nationwide prevalence of 19.5% and 32.0% was found using 1:128 and 1:16 serum titer cut off points, respectively. Higher frequency was found in humid/warm than in dry or cold places. Infection was not related to gender, and the prevalence in children was higher in the coastal zones. Furthermore, in the urban population toxoplasmosis was relatively high, which confirmed the relevance of the domestic cat in *T. gondii* transmission.¹⁷

Since the late 1980s, dramatic demographic changes have occurred in Mexico as in many parts of the world, including alimentary habits, access to health services, urbanization, and migration within and outside the country. Also, climatic changes have occurred, especially regarding humidity and temperature.¹⁸ Since *T. gondii* transmission largely depends on these aspects, we decided to make use of two subsequent nationwide serological surveys conducted in 2000 and 2006 in Mexico to study the prevalence of human toxoplasmosis, including its geographical distribution and variations due to age or gender.^{19,20}

2. Materials and methods

2.1. Study design

The work was performed using serum samples and data from the National Health Survey 2000 (NHS-2000) and the National Health and Nutrition Survey 2006 (NHNS-2006). Briefly, NHS-2000 was a probabilistic household-based survey, which included users of health services. Information was gathered through direct interviews with adult informants.¹⁹ The second was designed to determine the nutritional status of the Mexican population, based on national probabilistic survey of rural and urban strata in different regions of the country.²⁰ Unfortunately, the serum bank from the 1987 National Sero-Survey (NSS-1987) designed to analyse various diseases detectable in serum, was no longer available when we performed this study. Thus, we used data taken from the study by Velasco-Castrejón et al. for comparison.¹⁷ All three surveys were coordinated by the Mexican Ministry of Health with designs based on polystage (primary [state/municipality] secondary [household] and tertiary [individuals]) units, stratified (metropolitan and/or urban vs rural) and household sampling. The detailed information on survey designs and overall results have been specifically published.^{19–21}

The sample size of NHS-2000 was calculated to estimate a theoretical proportion of 5% or higher, regarding any health event, 30% relative precision, 95% confidence level, 30% lack of response and design effect of 3, obtaining a sample size of 47 040 households.¹⁹ In each selected household, one child (0–9 years old), one adolescent (10–19 years old) and one adult (over 19 years old) were selected by randomization. The final sample size was useful to make estimations at national and state level including all 32 which constitute Mexico. The 90 916 persons selected from the sample frame (24 184 [26.6%] children; 21 365 [23.5%] adolescents; 45 276 [49.8%] adults) were interviewed at home and gave a blood sample. To estimate the seroprevalence of *T. gondii*, we calculated a sample size of 3585 taking a theoretical seroprevalence of 30%, 95% confidence level and specific relative precision of 5%.^{17,22,23} A random selection was carried out and then selected serum samples were weighted taking into account the sampling structure of NHS-2000. Finally, we studied 3599 samples from NHS-2000.

NHNS-2006 sample size was calculated to estimate a theoretical proportion of 8.1%, relative precision of 25%, confidence level of 95%, lack of response 20% and design effect of 1.7, obtaining a sample size of 46 800 households.²⁰ In each selected household, one child (0–9 years old), one adolescent (10–19 years old) and one adult (over 19 years old) were selected by randomization. The final sample size was also useful to make estimations at national and state levels. To estimate of *T. gondii* seroprevalence we calculated a sample size of 3585 observations, also taking a nationwide seroprevalence of 30%, 95% confidence level and specific relative precision of 5%. A random selection was carried out and selected serum samples were weighted taking into account the sampling structure of NHNS-2006. Finally, we selected 3824 samples but we could only find 2916 samples in the NHNS-2006 serum bank established at the National Institute of Public Health. This number was sufficient, given the prevalence found (from 40% to 62%; see below in the Results section).

2.2. Serologic assays

Anti-*T. gondii* IgG antibodies were searched by ELISA and Western blot according to previously reported methods.^{24,25} Methods were previously standardized and validated for the Mexican population of all regions of the country, three age groups and both genders, basically by contrasting them to a reference standard built up with results of three techniques (H. Caballero-Ortega et al., 2012 submitted). Sensitivity values of 98.4% and 87.8% and specificities of 65.4% and 94.0% were obtained for ELISA and Western blot, respectively. From this validation the fixed part of the cutoff point, i.e., three SDs of the absorbance from normally distributed negative samples was also obtained.

For ELISA, polystyrene plates (Maxisorb, Nunc, Roskilde, Denmark) were incubated with 2 µg/mL of the RH strain crude extract of *T. gondii* dissolved in 15 mM carbonate buffer, pH 9.6, overnight at 4 °C. Non-specific binding sites were blocked with 1% bovine serum albumin in PBS-0.05% tween 20 (PBS-T). Plates were washed five times

with PBS-T using an automated BIO-RAD, Hercules, CA, USA 1575 ImmunoWash machine. The human samples were diluted 1:500 and developed with a goat anti-human IgG-peroxidase conjugate diluted 1:5000 (Sigma Aldrich Corp., St Louis, MO, USA, product A8419). The reaction was developed with O-phenyldiamine-H₂O₂ citrate buffer solution. Absorbance was measured at 490 nm on a Turner Biosystems 9300-010 micro-ELISA autoreader using the Modulus™ Microplate Reader. Cut off was set as the mean of one low, one medium and one high negative control in duplicate included in each plate, plus the constant value obtained from validation (H. Caballero-Ortega et al., 2012 submitted). The absorbance value of each serum was divided by the cut-off point to establish the reactivity index (RI). Serum samples with RI ≥ 1.1 were considered positive. Those having a RI 0.9 to 1.09 were tested by Western blot to confirm result.

Western blot was carried out according to a method reported by others.²⁴ In brief, 15×10^6 parasites/gel were electrophoresed under reducing conditions and transferred onto nitrocellulose membranes. Strips were cut and each strip was incubated with individual serum diluted 1:200 and then with anti-human IgG-peroxidase conjugate 1:2500 and the standard 4-chloro-1-naphthol-H₂O₂ chromogen/substrate solution for reaction development. Reaction was stopped by washing with distilled water.

2.3. Data analysis and statistics

The crude prevalence values obtained for the NHS-2000 and NHNS-2006 were weighted by multiplying the survey's expansion factor (i.e., the quantity of individuals from the population that is represented by one person selected from a sample) and the probability of being included in the subsample, taking into account the non-response rate and the differential response by gender and age, as it was done for sample size calculation. Since the sensitivity and specificity of the ELISA used are known, all frequency values were adjusted from the weighted prevalence, using the Rogan-Gladen estimator (P_{RG}):²⁶

$$P_{RG} = \frac{AP + Sp - 1}{Se + Sp - 1}$$

where: AP= Apparent prevalence; Sp= Specificity; Se= Sensitivity.

Temperature and precipitation data for Mexico were obtained from the National Meteorological Service and the National Water Commission.¹⁸ Prevalence deltas per state were compared between 2000 and 2006, and were related to changes in precipitation or temperature between 1999 and 2005, considering one year sufficient to affect *T. gondii* transmission.

Statistical analysis was performed using SPSS 18.0 (SPSS Inc., Chicago, IL, USA). Toxoplasmosis frequencies were compared among regions, age groups and genders using χ^2 as statistical test.²³ Changes (deltas) in prevalence were estimated by subtracting the crude values reported by Velasco-Castrejón et al., from those of 2000, adjusted to minimize differences.¹⁷ Deltas were also obtained comparing crude, weighted and adjusted prevalence values between 2000 and 2006. The delta distributions of the

central, coastal and northern regions were statistically compared by Kruskal-Wallis, followed by Mann-Whitney-U test. Correlations between changes in temperature or precipitation and of prevalence values were assessed by linear regression (r , 95% CI). A $p \leq 0.05$ was considered statistically significant.

3. Results

Crude prevalence was 60.1% (95% CI 58.5–61.7%) for NHS-2000 and 62.6% (95% CI 60.3–63.9%) for NHNS-2006 ($p=0.057$). The weighted and adjusted prevalence values were 62.5% (95% CI 59.7–65.2%) and 40.0% (95% CI 38.4–41.6%) for NHS-2000, and 63.7 (95% CI 60.4–67.1%) and 43.1% (95% CI 41.3–44.9) for NHNS-2006. Thus, a slight increase in national prevalence of toxoplasmosis seemed to occur between 2000 and 2006. Geographical distribution changed more evidently: it increased in all coastal and most northern states (7 out of 10), while it decreased in 8 out of 10 central states (Figures 1 and 2). A decrease in humidity occurred between 2000 and 2005 in most states, but no global correlation with toxoplasmosis frequency was found ($r < 0.01$).

A change in age related frequency was observed, with a significant increase among children in 2006 as compared to 2000 (Table 1). This was exclusively observed in the northern and coastal regions, with no significant variation in the center of the country. Toxoplasmosis frequency decreased in the center between 2000 and 2006, regardless of age, although the delta was significant in adolescents and adults only (Figure 2 and Table 1). The opposite was observed for the coastal and northern zones, with statistical significance for all age groups in the former, and in children in the north ($p=0.026$). No difference between genders was found.

Changes in temperature and precipitation between the end of the 1990s and 2005 were heterogeneous among states, with a global change of $+0.6 \pm 1.2^\circ\text{C}$ (range -4.5 to 2.9°C) and -10.6 ± 206.7 mm (range -390 to 647 mm) respectively. No positive or negative relation was found between the two climatic aspects, neither between precipitation nor prevalence delta, but a significant positive correlation was observed between temperature and prevalence in the 21 states where the latter increased ($r=0.489$, $p=0.029$; Figure 3).

4. Discussion

Toxoplasma gondii causes one of the commonest zoonoses worldwide. This is due to its lack of host-species specificity and its multiple transmission mechanisms: herbivores are infected by consumption of pastures or water contaminated with oocysts, while carnivores may acquire toxoplasmosis by raw meat (or contaminated water) ingestion. Omnivores – like human beings – are exposed to all these risk factors; besides, they might have domestic cats in their houses, which may release oocysts if they are fed with raw meat or are allowed to hunt.²⁷ The prevalence in a certain region largely depends on the main transmission mechanism. In places where raw or undercooked meat is the main risk, prevalence is usually low and exposure starts relatively late in life (i.e., when people start eating

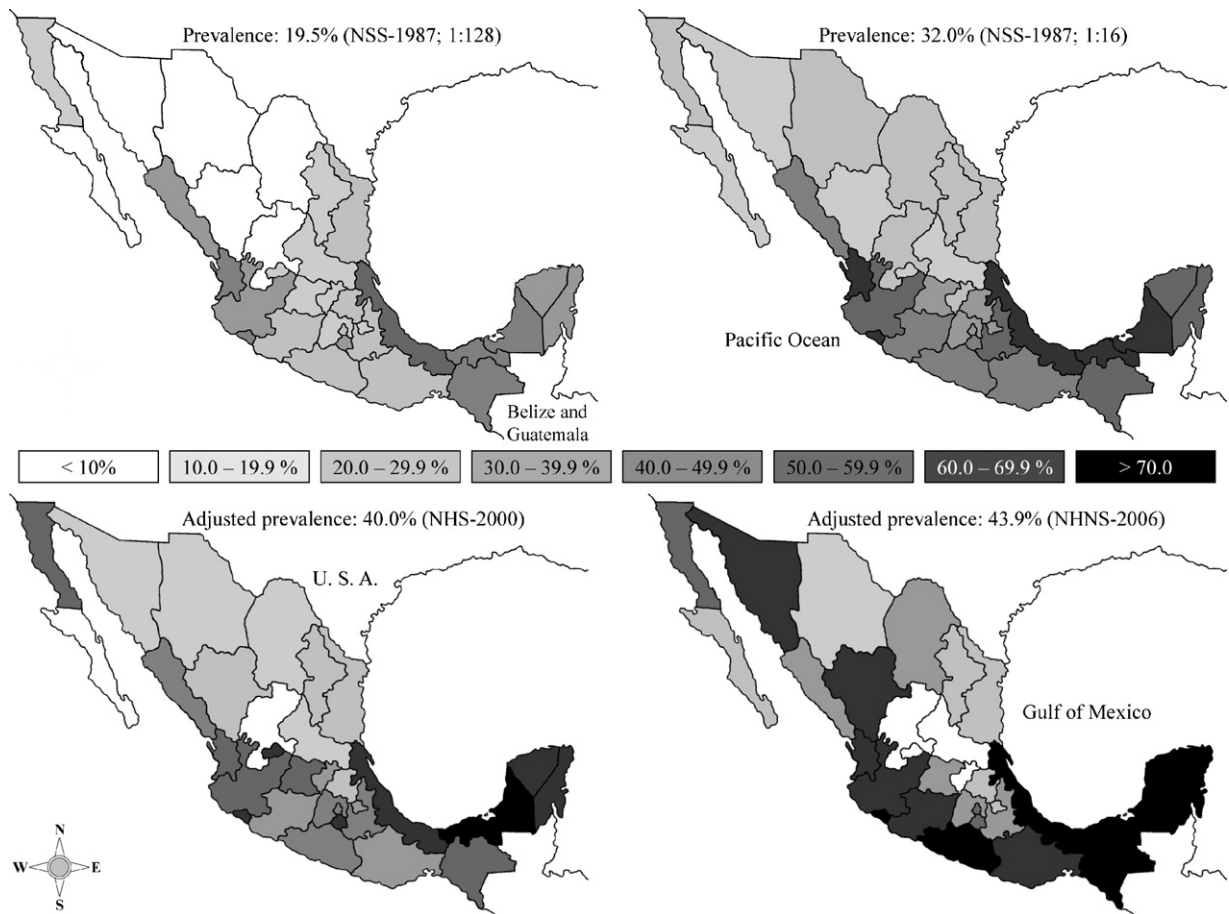


Figure 1. Maps of Mexico showing prevalence ranges per state of anti-*Toxoplasma gondii* antibodies in the National Seroepidemiological Survey of 1987 (NSS-1987; using 1:128 and 1:16 serum dilutions cut offs in IFAT), the National Health Survey 2000 (NHS-2000) and the National Health and Nutrition Survey 2006 (NHNS-2006). Maps of the NSS-1987 were built up from data provided in article by Velasco-Castrejón et al.¹⁷ and thus they are crude. For the NHS-2000 and NHNS-2006 prevalence values were adjusted taking into account the sensitivity and specificity of the ELISA employed. Prevalence ranges are specified by different shades of grey.

meat). Conversely, environmental contamination with oocysts is normally associated with high prevalence and early exposure in life, since children sometimes play with soil and drinking water may be contaminated.^{12–14,27,28} As mentioned before, the climatic change is of major concern because of possible reemergence of infectious diseases, among them vector borne and environmentally related parasitoses, like *T. gondii* infection.^{15,29,30} Making use of serum banks gathered in two nationwide surveys in 2000 and 2006, and comparing them with the one performed in 1987, we were able to determine if there was a change in global prevalence, as well as in age- and geographical-related distribution of *T. gondii* in Mexico. We found evidence that it was so. Prevalence in 1953–1962 estimated using the dye test, and in 1987 using immunofluorescence, was 30% and up to 32%, respectively.^{17,31} In the present study, higher crude and weighted values were obtained in 2000 and 2006 (60.1 and 62.6%). This could be due to different sensitivities or specificities of the immunoassays employed, but even comparing the prevalence calculated using the IFAT 1:16 dilution cut-off in 1987 with that estimated using adjusted ELISA results of 2000, an increase

at least of 8% is suggested. Thus, it seems there was an increase in *T. gondii* transmission and it might be partially due to climatic changes. Global increases in temperature of 0.4 °C and 0.6 °C occurred between 1987 and 2000, and between 2000 and 2005 in Mexico, respectively; the corresponding increases in global *T. gondii* infection prevalence (8 and 3%) could be partially due to weather change.¹⁸

It has to be emphasized that the 1987, 2000 and 2006 national surveys in Mexico had national and state level representation.^{19–21} There are elements that could have caused some bias in NSS-1987 and in NHNS-2006. In the former, age group distribution among individuals that provided blood samples had an over-representation of men for age ranges 1–4, 5–9 and 10–14 years, and under-representation among groups from 20 to 64.¹⁷ Unfortunately, the authors of that work did not mention the control of non-response rate or the use of an expansion factor in the analysis of their results. Nevertheless, *T. gondii* infection frequency has not been associated to gender, and this was corroborated for NHS-2000 and NHNS-2006. Regarding NHNS-2006 we found that 24.1% of serum samples selected were empty. Further analysis of

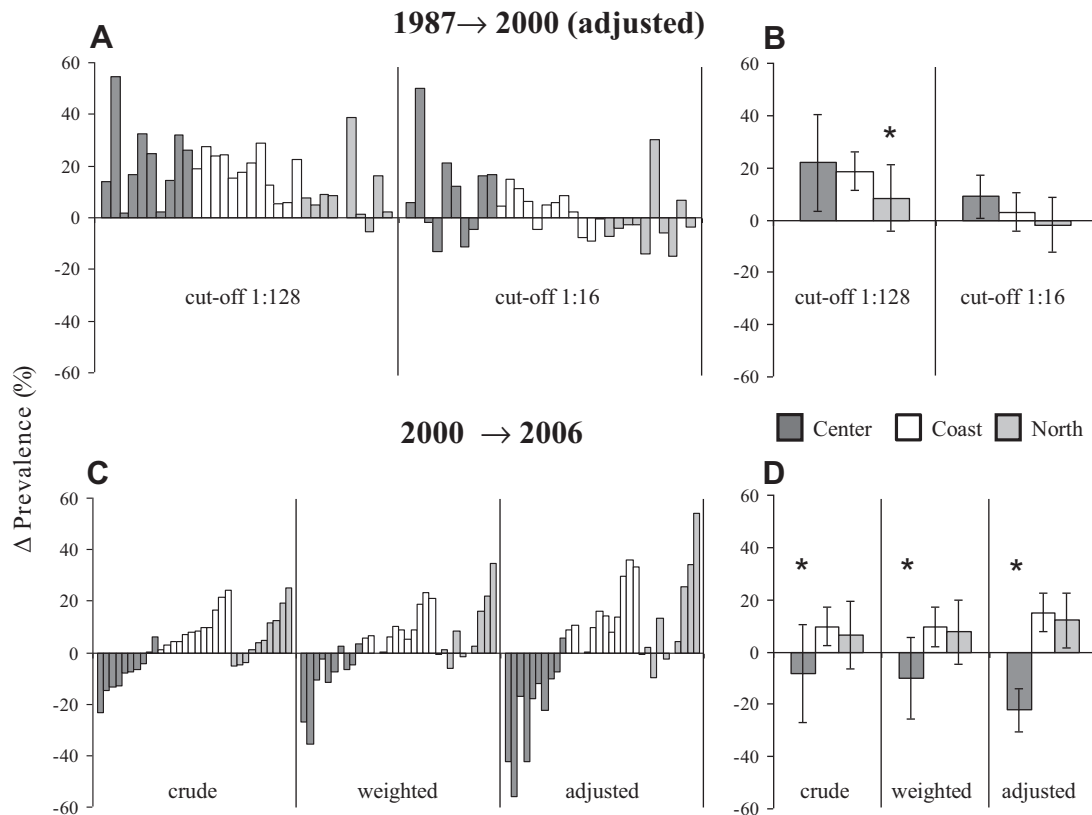


Figure 2. Prevalence deltas from 1987 to 2000 and from 2000 to 2006 for each state (A and C) and for the three regions (B and D). In A and B two cut-off points reported by Velasco-Castrejon et al.¹⁷ were compared to adjusted prevalence in 2000. In C and D the crude, weighted and adjusted prevalence values were used. Asterisks mark statistically different deltas of the northern ($p < 0.04$) and central ($p < 0.005$) regions in the 1987–2000 and 2000–2006 comparisons, respectively.

results showed that samples were especially scarce in the 0–9 years old group. Nevertheless, sample size calculation for both surveys was estimated considering 30% prevalence, and the lowest found was 40% for which a sample of

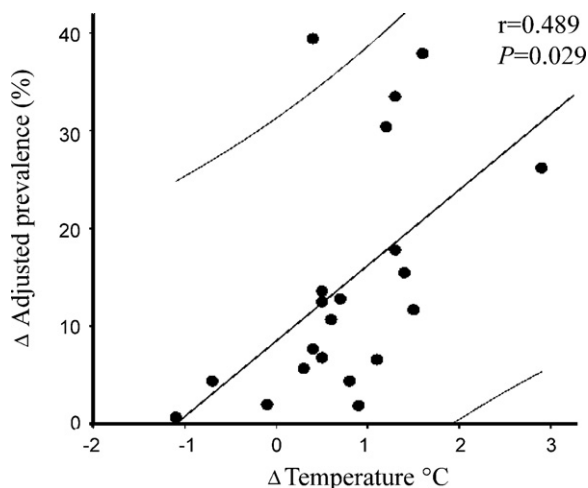


Figure 3. Correlation between temperature and toxoplasmosis prevalence deltas 2000–2006 in the 21 states where an increase in prevalence was observed.

2305 was necessary, i.e., below those individuals analyzed in each survey. Thus, bias could have minimal effect on results. This is further suggested by the fact that the prevalence increase observed in the coastal and northern zones between 2000 and 2006 was similar for all age groups.

The variation in age-related distribution of *T. gondii* infection with significant increase in children supports the effect of weather at national and regional levels. Most of those states flanked by the mountain ranges 'Sierra Madre Oriental', 'Sierra Madre Occidental' and 'Sierra Madre del Sur' (the plateau region) presented a prevalence decrease between 2000 and 2006, independently of temperature or precipitation modifications. In the other regions (21 States) the prevalence augmented with a significant relation to temperature change. It is believed that climate has changed and reliable models predict even more increases in mean global temperature. Global warming is threatening the health of humans and animals, partly because infectious diseases disperse through water, food or vectors. Among those which are disseminating more widely are cholera and other diarrheas, malaria, dengue, yellow fever and other encephalitogenic viruses.^{15,29,30} It has been postulated that the environmental changes observed during the last years might favor *T. gondii* dissemination in north-western Europe, along with anthropogenic factors, like migration or feeding habit changes.¹⁶

Table 1
Adjusted seroprevalence of anti-*Toxoplasma gondii* antibodies in Mexico: analysis of the 2000 and 2006 National Health Surveys according to region and age

Age	2000			2006				
	Center Positive/no. (% adjusted prevalence)	Coast	North	Total	Center Positive/no. (% adjusted prevalence)	Coast	North	Total
Children <10 y	168/341 (23.0)	224/411 (24.9) ^a	161/340 (20.0) ^b	553/1,092 (25.1) ^c	122/269 (16.8)	186/286 (47.7) ^a	118/205 (36.0) ^b	426/760 (33.6) ^c
Adolescents 10–19 y	227/385 (38.2) ^d	312/466 (50.7) ^e	218/394 (32.5)	757/1245 (41.1)	162/359 (16.5) ^d	298/402 (61.9) ^e	208/351 (38.6)	668/1112 (39.9)
Adults >19 y	272/394 (54.0) ^f	387/476 (73.2) ^g	195/392 (23.7)	854/1262 (51.8)	214/363 (38.2) ^f	329/359 (89.4) ^g	174/322 (30.5)	717/1044 (53.4)
Total	667/1120 (39.1) ^h	923/1353 (52.7) ⁱ	574/1126 (25.7) ^j	2164/3599 (40.0)	498/991 (24.5) ^h	813/1047 (67.5) ⁱ	500/878 (35.0) ^j	1811/2916 (43.1)

Adjusted prevalence was calculated considering 98.4% sensitivity and 65.4% specificity of the ELISA (H. Caballero-Ortega et al. unpublished data); ^a p=0.005; ^b p=0.026; ^c p<0.0001; ^d p=0.021; ^e p=0.004; ^f p<0.0001; ^g p<0.0001; ^h p<0.0001; ⁱ p<0.0001; ^j p=0.009 χ^2 test.

Toxoplasmosis is an unrecognized health problem in several parts of the world, because its frequency is largely unknown and is thought to cause no problems to immunocompetent hosts. Nevertheless, it is distributed worldwide, it can provoke behavioral and psychiatric disorders in 'asymptomatic' adults and is an important obstetric and pediatric issue.^{5,6,9–12} The Ministry of Health of Mexico has made an effort to analyze the global health status of the Mexican population several times by making nationwide seroepidemiological surveys.^{19–21} We used the surveys to analyze the changes in *T. gondii* prevalence in the last three decades, and conclude that it increased between the late 1980s and 2000; also, its distribution changed in a relatively short period, between 2000 and 2006, polarizing the nation in hyperendemic (coastal) and hypoendemic (central) zones. This information should be taken into account for implementation of toxoplasmosis control programs.

Authors' contributions: HCO, FJUS, CJCG and DC designed the study protocol; CLCG carried out the random selection of serum samples; CCP, JAVV, HLP, ICS and LBOA were involved in performing ELISA and Western blot experiments. All authors worked on analysis and interpretation of the data. HCO drafted the manuscript and all authors read and approved the final manuscript. DC is guarantor of the paper.

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