Folate and Vitamin B12 Deficiency Among Nonpregnant Women of Childbearing Age in Guatemala 2009–2010: Prevalence and Identification of Vulnerable Populations

Jorge Rosenthal[1], **Eunice Lopez-Pazos, MOH**[2], **Nicole F. Dowling**[1], **Christine M. Pfeiffer**[3], **Joe Mulinare**[1], **Claudia Vellozzi**[1], **Mindy Zhang**[3], **Donna J Lavoie**[3], **Roberto Molina**[4], **Nicte Ramirez**[2,5], and **Mary-Elizabeth Reeve**[6]

[1]National Center on Birth Defects and Developmental Disabilities, Centers for Disease Control and Prevention

[2]Guatemala Ministerio de Salud Pública y Asistencia Pública

[3]National Center for Environmental Health, Centers for Disease Control and Prevention

[4]Universidad del Valle, Guatemala

[5]Fundación de Alimentos y Nutrición de Centro América y Panamá (FANCAP)

[6]Consultant, Global Maternal and Preconception Health

Abstract

Introduction—Information on folate and vitamin B12 deficiency rates in Guatemala is essential to evaluate the current fortification program. The objectives of this study were to describe the prevalence of folate and vitamin B12 deficiencies among women of childbearing age (WCBA) in Guatemala and to identify vulnerable populations at greater risk for nutrient deficiency.

Methods—A multistage cluster probability study was designed with national and regional representation of nonpregnant WCBA (15–49 years of age). Primary data collection was carried out in 2009–2010. Demographic and health information was collected through face-to-face interviews. Blood samples were collected from 1,473 WCBA for serum and red blood cell (RBC) folate and serum vitamin B12. Biochemical concentrations were normalized using geometric means. Prevalence rate ratios were estimated to assess relative differences among different socioeconomic and cultural groups including ethnicity, age, education level, wealth index and rural versus urban locality.

Results—National prevalence estimates for deficient serum (<10 nanomoles per liter [nmol/L]) and RBC folate (<340 nmol/L) concentrations were 5.1% (95% CI 3.8, 6.4) and 8.9% (95% CI 6.7, 11.7), respectively; for vitamin B12 deficiency (<148 pmol/L) 18.5% (95% CI 15.6, 21.3). Serum and RBC folate deficiency prevalences were higher for rural areas than for urban areas (8.0% vs. 2.0% and 13.5% vs. 3.9%, respectively). The prevalence of RBC folate deficiency

Corresponding Author:, Jorge Rosenthal Ph.D., MC, Epidemiologist, jyr4@cdc.gov, Centers of Disease Control and Prevention, National Center of Birth Defects and Developmental Disabilities, MS-86, Atlanta, GA 30333, Tel: (404)498-3925.

showed wide variation by geographic region (3.2%–24.9%) and by wealth index (4.1%–15.1%). The prevalence of vitamin B12 deficiency also varied among regions (12.3% –26.1%).

Conclusions—In Guatemala, folate deficiency was more prevalent among indigenous rural and urban poor populations. Vitamin B12 deficiency was widespread among WCBA. Our results suggest the ongoing need to monitor existing fortification programs, in particular regarding its reach to vulnerable populations.

Keywords

Prevalence; serum and red blood cell folate deficiency; vitamin B12; deficiency; women of childbearing age; Guatemala

Background

Micronutrient deficiencies and food insecurity can lead to severe, acute, or chronic malnutrition for women and children around the world [1, 2]. Micronutrient deficiencies among women of childbearing age (WCBA) (15–49 years of age) can have adverse effects on birth outcomes, increasing the risk for sequelae such as neural tube defects (NTDs) and slow intellectual and motor development among offspring [3, 4]. Folate and vitamin B12 deficiencies, specifically, have significant public health consequences, including an increased prevalence of NTDs [5, 6] and of cardiovascular diseases [7, 8]. Micronutrient deficiencies and malnutrition historically have been chronic problems in Central America [9].

Fortifying food products has been a principal strategy in Central America to reduce various micronutrient deficiencies, including that of folic acid. In 2003 Guatemala aligned their folic acid fortification levels to the regional levels of 1.80 mg/kg [10, 11], while also selectively fortifying MASECA® corn masa flour [12].

Imhoff-Kunsch et al. (2007) studied the effects of fortification of wheat flour with iron and folic acid using data from the Living Standards Measurement Survey for Guatemala [13]. Data from this survey demonstrated that fortified wheat flour provided 71% and 78% of the estimated average requirements (EARs) for folate for women identified as being nonpoor or living in urban areas, respectively; in contrast, fortified wheat flour provided only 4.7%, 15.5%, and 10.5% of EARs to women identified as extremely poor, living in rural areas, or indigenous, respectively [13]. These data also demonstrated that fortified foods were more available and affordable in urban areas, for the nonpoor, and among those with cultural preferences for specific types of foods. However similar data were not obtained for age, education and wealth index. Nutritional needs differ by age, particularly for WCBA and educational level. Education level is important because it aids in informed decision making about nutrition and diet. Wealth index is important because it can determine differences in population and health indicators between the index levels.

Ours is the first study in Guatemala to provide information on the folate and vitamin B12 status of nonpregnant WCBA, taking into account different demographic and geographic characteristics. The major aim of this study was to assess the overall impact of the Guatemala fortification program by describing national and regional prevalences of folate

and vitamin B12 deficiencies and by identifying vulnerable populations at risk of not benefiting from existing fortification strategies.

Methods

Guatemalan Population

In 2010 the total population of Guatemala was 14,361,666. Women made up slightly more than half of that total and 41% were indigenous, 51% lived below the poverty line, 51% lived in rural areas, and 29% were 15–49 years of age [14].

Study Population and Sample Selection—Our study used the sampling frame from the Encuesta Nacional de Salud Materno Infantil (ENSMI). The ENSMI, conducted in 2008, used a complex, multistage probability design of the civilian, noninstitutionalized Guatemalan population to select 734 sectors, from which 26,000 men, women, and children, stratified by department (province) and urban and rural settings were selected [15]. The ENSMI identified all sampled households with WCBA and children 6–59 months of age. Our study, the Encuesta Nacional de Micronutrientes (ENMICRON), selected a subset (246 sectors) of the household sampling frame surveyed by the ENSMI. Using this subset, we assessed the micronutrient status of a sample of nonpregnant WCBA and children 6–59 months of age to obtain national and regional representation. The minimum sample size required was 150 nonpregnant WCBA in each region in order to detect a minimum difference of 10% prevalence with a relative standard error of 30%, given a survey design effect of 1.5. Primary data collection was conducted by the Ministry of Health in 2009–2010.

The selected households were screened to ensure that previously identified nonpregnant WCBA were still in residence. Informed consent was obtained and WCBA were interviewed using a short, standardized questionnaire, after which blood was drawn. The household interviews were conducted in Spanish or Mayan. The ENMICRON used the ENSMI self-reported data on sociodemographic information using the following ENSMI variables: area (rural or urban), ethnicity (indigenous or nonindigenous), educational level (none, primary, and secondary or higher), wealth index (low, middle, or high) and region to identify vulnerable populations. Area was defined by the location of the household. Urban areas included large cities, small cities (population over 50,000) and towns (population over 2,500. All rural areas were locations with populations of <2,500). Participants who reported they were Mayan were categorized as indigenous. Participants who reported as mestizos or White were categorized as nonindigenous. A woman's education level was based on the highest grade or level of education completed. Wealth index was established by information on household characteristics (e.g., building materials, number of rooms, water source, and type of toilet) [16]. Due to regional differences in the distribution of rural vs urban population, indigenous population, wealth and dietary customs (preference for staples such as rice, corn or wheat) we selected *region* as an independent variable.

Biochemical Analyses

Whole blood was collected from nonfasting participants. EDTA and nonanticoagulated whole blood vacutainers were refrigerated and protected from light for 2–3 days prior to being processed at the central laboratory in Guatemala City. Folate has been shown to be stable under such conditions [17]. Serum and EDTA whole blood hemolysate (whole blood diluted 1:11 with 10 g/L ascorbic acid) were stored at −20°C for less than 3 months, and at −80°C for a year until shipment on dry ice to the CDC laboratory. During 2011–2013, the CDC laboratory conducted biochemical analyses for serum and RBC folate concentrations by microbiologic assay (MBA) [18] and for serum vitamin B12 by the Roche[R] electro-chemiluminescence assay on the E-170 instrument platform (Roche Vitamin B12 assay package insert, 2007–8, V4). The mean coefficients of variation for three quality control (QC) pools measured in each assay in duplicate for serum folate (8.4–49.5 nmol/L), whole blood folate (191–401 nmol/L), and serum vitamin B12 (212–395 pmol/L were 8.4%–9.4%, 7.5%–9.4%, and 4.0%–4.4%, respectively.

To assess folate status, we used two biomarkers: serum and RBC folate. The serum folate concentration better reflects recent dietary intake of folate. Because the highest level of serum folate typically occurs approximately 45–90 minutes after eating, serum folate ideally should be measured under fasting conditions. RBC folate concentrations reflect tissue stores and folate intake during the past 120 days and, thus, are less influenced by recent consumption of folate-containing foods. We used established deficiency criteria as cutoff points for the analyses: <10 nmol/L for serum folate (SF) and <340 nmol/L for RBC folate (RBCF) [19]. The cutoff point for identifying vitamin B12 deficiency was <148 pmol/L; for marginal vitamin B12 deficiency, it was defined as 148–221 pmol/L [20]. We used a 5% threshold criterion above which prevalences of low SF, RBCF, and vitamin B12 were indicative of a public health concern [21].

Quality Assurance

Quality assurance procedures were instituted for each phase of the study. Development of survey instruments involved expert review and pretesting in the community. Interviewers and phlebotomists were trained for 3 weeks; training included practice interviews and blood drawing from patients at health centers and in nonstudy households. In the data collection phase, any questionnaire that was incomplete or contained an error was returned to the interviewer and, when appropriate, they went back to the household for correction. Also, a subsample of households was recontacted to ensure that households listed as ineligible were designated correctly and that eligible women were not missed. Trained staff obtained and entered data twice to ensure accuracy and completeness.

Analytical and Statistical Methods

The crude prevalence point estimates and 95% confidence intervals (CIs) for SF and RBCF deficiencies and for vitamin B12 and vitamin B12 marginal deficiencies were calculated. Variances were calculated by procedures that accounted for the complex survey design and for ratio adjustments used to produce the sample weights. Comparisons using the selected variables were done using prevalence risk ratios (PRRs) for serum and RBC folate and vitamin B12 deficiencies. To facilitate interpretations, adjusted prevalence risk ratios were

calculated from the overall logistic regression models using PREDMARG statement in SUDAAN [22, 23]. The adjusted percentage was generated using the logistic regression model to estimate the probability of deficiency status, averaging the distribution of covariates among the entire weighted sample. The reported risk estimates were adjusted for survey design, as well as the covariates listed previously.

Geometric means for SF, RBCF, and vitamin B12 were calculated to normalize the distribution. Differences in the sociodemographic variables were tested using t-tests derived from linear regression models (Proc Regress SUDAAN). We used multiple linear regressions to adjust for the selected variables because the magnitudes of the correlations were modest or weak (0.01–0.35). The Taylor series approximation was used to obtain the standard errors [24]. All analyses were performed using SUDAAN statistical software (version 9.3; Research Triangle Institute, Research Triangle Park, NC).

The Guatemalan Ministry of Health requested that the study protocol be reviewed by the CDC Institutional Review Board (IRB) in Atlanta, Georgia, in the United States. CDC accepted this request, and the protocol was approved by the CDC IRB.

Results

Response Rates and Characteristics of Participants

We identified 1,475 households and data were collected on 1,453 (98.5%) nonpregnant WCBA. Blood samples were obtained from 1,448 eligible women. Table 1 shows the general characteristics of the ENMICRON sample of nonpregnant WCBA in Guatemala: 53.3% lived in rural areas, 61.9% were of nonindigenous ethnicity, 22.2% were 15–19 years of age, 19.1% had no education, and 31.6% were classified in the low wealth tertile. The ENMICRON population reflects the ENSMI data characteristics (data not show).

In Tables 2 through 4, results are presented by type of deficiency (SF and RBCF and vitamin B12 and vitamin B12 marginal deficiencies) first, at the national level; second, based on the variables of area, ethnicity, age, education level, and wealth index; and finally by geographic region within Guatemala. The data are then presented as geometric means of serum and RBC folate and serum vitamin B12 stratified by the selected variables.

Serum and RBC Folate Deficiency Prevalences at the National Level and by Area, Ethnicity, Age, Education Level, and Wealth Index

The national prevalence estimates of folate deficiency based on SF and RBCF concentrations were 5.1% (95% CI, 3.8, 6.4) and 8.9% (95% CI, 6.7, 11.7), respectively. The prevalence of folate deficiency based on SF varied by area, ethnicity, age, education level, and wealth index (Table 2). We found a higher overall prevalence in rural areas than in urban areas (8.0% vs. 2.0%), and the adjusted PRR reached significance (2.4 [95% CI, 1.1, 4.1]). Also, women in the low wealth index had a higher prevalence of serum folate deficiency than women in the middle and high wealth indices (9.7% vs. 5.4% and 0.8 %, respectively). The adjusted PRRs for women in the low and middle wealth indices were significantly higher than that for women in the high wealth index (5.4 [95% CI 1.7, 16.6]

and 4.3 [95% CI 1.3, 13.9]), respectively). The strongest predictors of SF deficiency were residing in a rural area and being in the low and middle wealth indices.

The prevalence estimates of RBCF deficiency also varied across most variables and were similar to those for SF deficiency (Table 2). We found a higher prevalence of deficiencies in rural areas than in urban areas (13.5% vs. 3.9%), with a significant adjusted PRR (2.7 [95% CI 1.03, 7.1]).

Vitamin B12 and Vitamin B12 Marginal Deficiencies at the National Level and by Area, Ethnicity, Age, Education Level, and Wealth Index

The national prevalence estimates of vitamin B12 and vitamin B12 marginal deficiencies were 18.5% (95% CI 15.6, 21.3) and 30.2% (95% CI 27.3, 33.1), respectively (Table 3). The prevalence of B12 deficiency varied across all variables; however, the only adjusted PRRs reaching statistical significance were for women in the low or middle wealth index vs. those in the high wealth index (2.8 [95% CI 1.7, 4.8] and 1.6 [95% CI 1.02, 2.7], respectively). The prevalence of vitamin B12 marginal deficiency also varied; the only adjusted PRRs reaching statistical significance were for women 35–49 vs 15–19 years of age (0.7 [95% CI 0.5, 0.9]) and in the low vs high wealth index (1.7 [95% CI 1.3, 2.3]).

Prevalence of Serum and RBC Folate Deficiencies and Vitamin B12 and Vitamin B12 Marginal Deficiencies by Region

To identify vulnerable women within Guatemala, we compared study results by region (Table 4). The results revealed differences in the prevalences of SF and RBCF deficiencies ranging from 1.4–8.9, and 3.5–24.9, respectively, although only one area (Norte) showed a significantly higher adjusted PRR compared with that of the reference area (Metropolitana).

The prevalences of vitamin B12 deficiency ranged from 12.3% (95% CI 8.9, 16.7) in the Sur-Occidente to 26.1% (95% CI 19.5, 34.0) in the Sur-Oriente (Table 4). The prevalences of vitamin B12 marginal deficiency ranged from 21.5% (95% CI 12.6, 34.2) in the Petén to 37.8% (95% CI 29.5, 46.9) in the Nor-Oriente. However, no significant differences were observed when each region was compared with the referent region (Metropolitana).

Geometric Means of Serum and RBC Folate and Vitamin B12 by the Selected Variables

The national geometric means and their distributions for SF and RBCF and vitamin B12 were 30 nmol/L (95% CI 29, 31), 725 nmol/L (95% CI 710, 738), and 341 pmol/L (95% CI 332, 349), respectively (Table 5, Figure 1). We found that rural areas had significantly lower geometric means of SF than urban areas ($p < 0.001$) as well as significantly lower mean concentrations among women in the lowest education levels and wealth indices. Similarly, for RBCF concentrations, we found significantly lower geometric means among women in rural areas; likewise, we found lower geometric means among women in the low wealth index than among women in the middle and high wealth indices. Geometric means of vitamin B12 were significantly higher for indigenous than for nonindigenous women and were significantly lower for women 15–19 years of age than for women 20–34 and 35–49 years of age. In addition, there were significantly lower mean vitamin B12 concentrations

among women in the low wealth index than among women in the middle and high wealth indices.

Geometric Means of Serum and RBC Folate and Vitamin B12 by Region

Geometric means for SF, RBCF and vitamin B12 showed large variations across regions (Table 5). The Metropolitana region showed significantly higher SF and RBCF concentrations than several other regions. However, for vitamin B12 concentrations, the Metropolitana region showed significantly lower concentrations than the Sur-Occidente region.

Discussion

This is the first national and regional survey from Guatemala that provides information about SF and RBCF and vitamin B12 concentrations among nonpregnant WCBA. We demonstrated that prevalences estimates >5% for SF, RBCF and vitamin B12 deficiencies among this population should raise public health concern [21]. Based on our analyses, we potentially identified two vulnerable groups of WCBA in Guatemala: (1) women who were poor, especially younger women, residing in both urban and rural areas and (2) women of indigenous ethnicity.

- Poor urban women probably enjoyed proximity to fortified foods, but encountered barriers to access because of cost. Poor rural women had both limited access and availability. Food preferences and nutritional needs also might have varied by the age of the women.

- Indigenous women resided primarily in rural areas and were poor; they also likely retained many traditional food preferences. Based on the higher serum vitamin B12 concentrations we identified among this population, their overall intake of meat might have been somewhat higher (than that of other group) most likely because they raised domesticated food animals or due to genetic factors.

These findings can be used to inform future fortification strategies and ensure that fortified staples reach vulnerable populations in Guatemala. These include (1) increasing the types of foods fortified (e.g., corn flour, sugar, and rice); (2) reducing the cost of fortified foods; (3) improving nutritional education, including culturally appropriate outreach to distinct target audiences (taking into consideration cultural factors such as dietary preferences); (4) implementing a national and regional monitoring program or programs to identify high-risk groups; and (5) implementing supplement programs for high-risk groups who have limited access to fortified staples.

Ideally, assessment of the effects of flour fortification on blood concentrations of SF and RBCF should include the analysis of data from the target populations before and after food fortification. In our case, prefortification data were not available. Postfortification data were available for other countries, so we compared our findings with those of other countries in the region and with those of the United States; we selected studies reporting results based on microbiologic assay (MBA) or MBA-equivalent data.

Guatemala's overall SF deficiency rate (5.1%) was higher than that reported from the postfortification period in the Dominican Republic (3.1%) [25] and Nicaragua (2.3%) [26]. Postfortification prevalence of RBCF deficiency in our study (8.9%) was slightly higher than that reported by the Dominican Republic (7.3%) [25], and much higher than that reported by Nicaragua (1.1%) [26]. Also, our RBCF deficiency prevalence rates were higher than rates reported for the U.S. postfortification period (1988–2010) among WCBA [27, 28], but the folate concentrations observed were somewhat higher than those reported for the U.S. prefortification period [27], from which we deduced a positive effect of the Guatemalan fortification program.

The observed differences in the prevalences of blood folate deficiency or blood folate concentration levels, or both, between Guatemala and other Central American countries and the United States might be explained by differences in population characteristics; differential access to fortified staples among population groups; and, possibly most importantly, because most of these other countries fortified multiple staples. In contrast to other countries, in Guatemala maize is the main staple and the majority of households consume maize flour in the form of masa made at home or obtained from small mills. While there is some voluntary fortification of masa flour, it might not have been accessible or available to the entire population. Populations who resided in urban areas and who were better educated and wealthier most likely had access to fortified flour and probably masa products. It remains to be seen whether the Guatemalan food fortification program could improve its effectiveness by fortifying additional food staples. This is especially important because folic acid has been shown to significantly reduce the risk of having a pregnancy affected by an NTD [5, 6].

Our study revealed that two regions in Guatemala differed from the other six regions in the prevalence of folate deficiencies, and that these differences could be explained by different population characteristics (e.g., proportion of rural and indigenous women). The Norte region showed the highest prevalences of serum and RBC folate deficiencies and had a population that was 84% rural and 88% indigenous (data not shown). In contrast, the Metropolitana and Petén regions showed the lowest prevalence of SF and RBCF deficiencies; these regions also tended to be more urban (76% and 52%, respectively) and to have smaller indigenous populations (15% and 22%, respectively).

Our study also found that vitamin B12 and vitamin B12 marginal deficiencies (19% and 30%, respectively) were high among WCBA in Guatemala. A high prevalence of maternal vitamin B12 deficiency is an independent risk factor for NTDs [5, 30]. The results of our study for vitamin B12 deficiency were higher (18.5%) than the overall prevalence reported by Costa Rica (5%) [30], but lower than that reported among women in rural Mexico (28%) [31]. The high prevalence of vitamin B12 deficiency in Guatemala, as in other low or middle income countries, likely was related to an inadequate intake of dietary vitamin B12, due to a diet low in animal products [32, 33]. Contrarily, it was surprising to observe that the indigenous population showed higher geometric means of vitamin B12 levels than the nonindigenous populations. Further research on indigenous diets could assist in explaining these results.

A major strength of our study was that survey data were collected via carefully designed, reliable procedures and a high response rate. Moreover, rigorous protocols were used in carrying out the field work and handling the biological samples, thus minimizing factors that could have compromised measurement of biomarkers. The study population was representative of the Guatemalan population of WCBA and was large enough to provide sufficient power for stable estimates within each of eight regions.

There were, however, several limitations to our analyses. First, ENMICRON was a cross-sectional study and could not be used to determine causation; thus, we were not able to draw conclusions about either the benefits or safety of either folic acid fortification or intake using these data. Second, we were not able to compare our postfortification era estimates with prefortification estimates, however we could compare with neighboring countries that also fortify their food base. Third, we were not able to determine the causes of some of the disparities in prevalence because we did not assess dietary intake. Fourth, the estimation of the wealth index might have had an urban bias because some wealth index assets were not available in many rural areas, and data were not collected to include individual land resources and animals.

Conclusions

The folate fortification program undertaken in Guatemala might have improved serum and RBC folate levels; however, there were significant differences between groups of women based on region of residence and other characteristics. The differences revealed in this study identified vulnerable populations (populations at greater risk of folate and B12 deficiency) within Guatemala, including (1) women who were poor, especially young women, residing in both urban and rural areas, and (3) women of indigenous ethnicity. The greatest need existed where these characteristics co-occurred, as in the Norte region of Guatemala.

Mandatory fortification with folic acid in Guatemala may have benefitted regions with better access to folic acid-fortified products or staples with high folate content, but results were still below what would be expected from a fully effective fortification program (such as those seen in Chile [35,36], Costa Rica [37], and the United States [27]). It remains to be determined whether the Guatemalan food fortification program could improve its effectiveness and reach a greater proportion of the population through fortification of additional staples (e.g., maize flour). Such a strategy could be complemented by consideration of supplementation programs to reach the most vulnerable. Furthermore, the addition of vitamin B12 to the Guatemalan fortification program would have the potential to address high vitamin B12 deficiencies across all WCBA. These strategies would need to be supported by targeted educational outreach.

Our study is the first to provide national, regional, and subpopulation prevalence estimates for folate and vitamin B12 deficiency. As such, it can serve as a reference point to assess changes in these deficiencies over time, which would allow for identification of continuing disparities and provide a means to further refine the program.

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(a) serum folate (nmol/L)

(b) red blood cell folate (nmol/L)
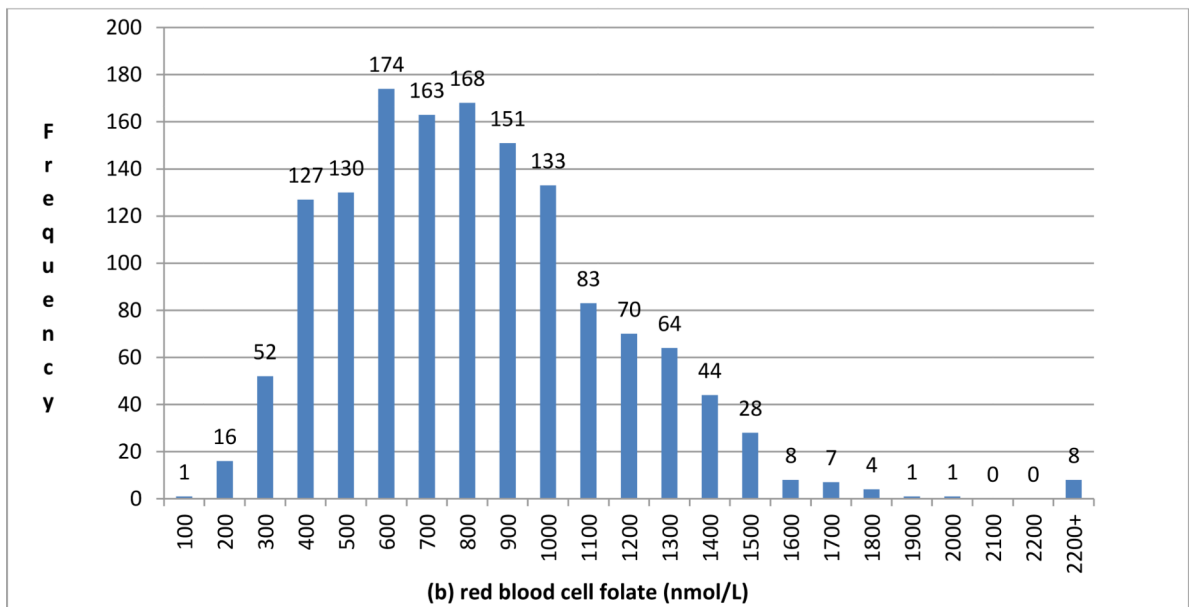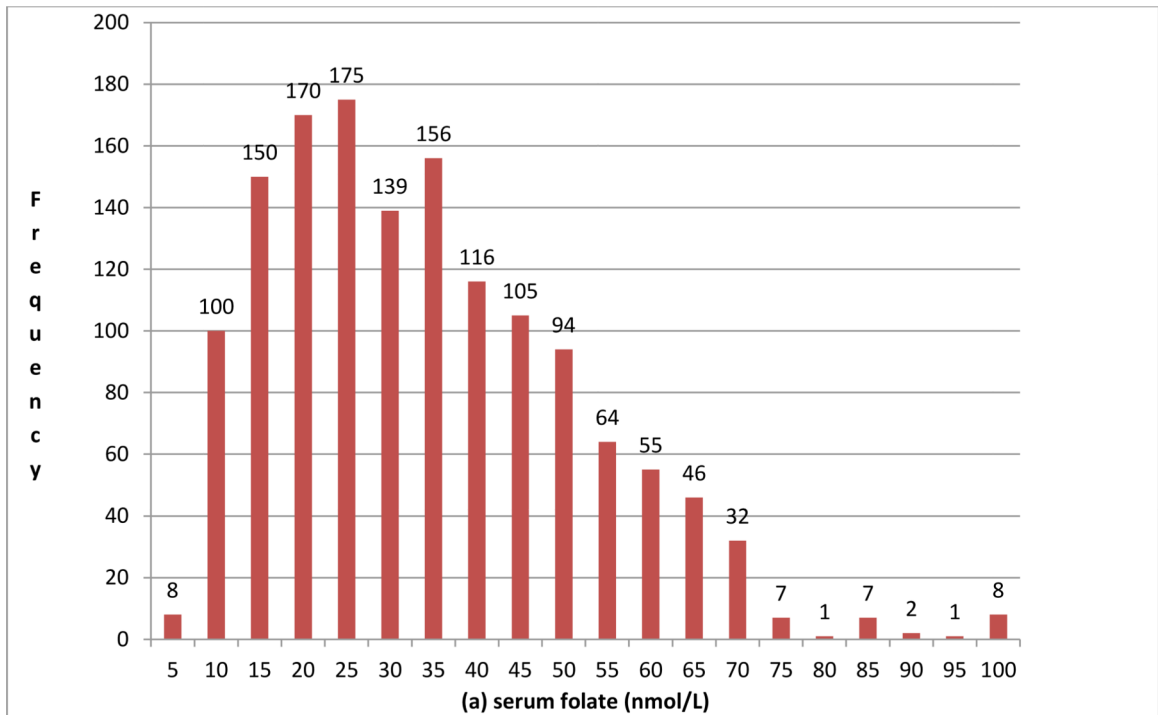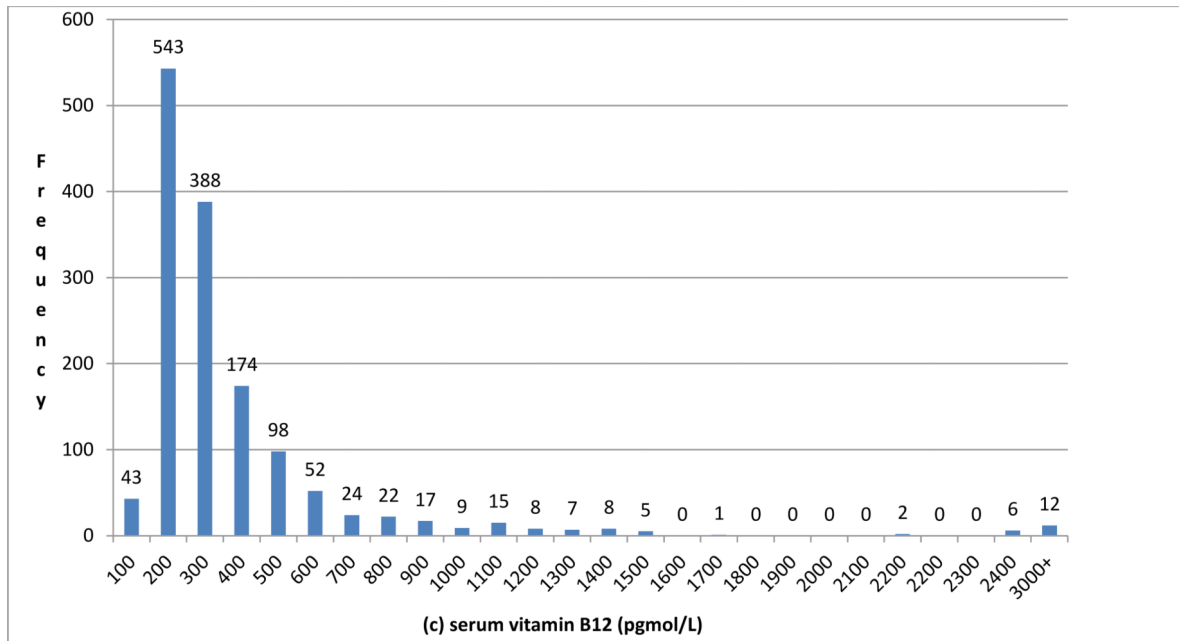
Figure 1.
Percentile distribution of serum and red blood cell folate and vitamin B12 among women of childbearing-age in Guatemala. ENMICRON 2009–2010

Table 1

Characteristics of non-pregnat women of childbearing age (15–49 years of age), ENMICRON[*] 2009–2010

	ENMICRON			
	N	**N**		
	(unweighted)	**(weighted)**	**Percentage (%)**	**95% CI[†]**
Area				
Rural	773	1,322,949	53.3	45.9, 60.1
Urban	675	1,159,972	46.7	39.4, 54.1
Ethnicity				
Indigeneous	559	945,282	38.1	33.5, 42.7
Non indigeneous	889	1,537,639	61.9	57.3, 66.4
Age (yrs.)				
15 – 19	233	551,918	22.2	19.6, 25.2
20 – 34	525	832,324	33.5	29.3, 37.2
35 – 49	690	1,098,679	44.3	40.2, 48.4
Education (yrs)				
None	314	473,973	19.1	45.0, 53.2
Primary	715	1,219,185	49.1	27.2, 36.8
Secondary or higher	419	789,763	31.8	15.9, 22.7
Wealth index (terciles)				
Low	478	784,571	31.6	24.6, 37..0
Middle	538	860,869	34.7	29.4, 40.3
High	432	837,481	33.7	27.9, 40.0

[*] Encuesta Nacional de Micronutrientes

[†] Confidence interval

Table 2

Prevalence and unadjusted and adjusted prevalence risk ratios (PRR) of serum and red blood cell (RBC) folate deficiencies, ENMICRON, 2009–2010

	N		Unadjusted	Adjusted
	(unweighted)	Prevalence	PRR[‡] (95% CI)	PRR (95% CI)[†]
		(95%CI)		
		Serum Folate	**Deficiency (<10 nmol/L)**	
National	1448	5.1 (3.8, 6.4)		
Area				
Rural	773	8.0 (5.9, 10.7)	4.0 (2.1, 7.7)[***]	2.4 (1.1, 4.1)[***]
Urban	675	2.0 (1.2, 3.5)	Referent	Referent
Ethnicity				
Indigeneous	559	7.1 (5.0, 9.9)	1.8 (1.1, 2.9)[*]	1.2 (0.7, 2.1)
Non-Indigeneous	889	3.9(2.7, 5.5)	Referent	Referent
Age (yrs.)				
15 – 19	233	3.1 (1.4, 6.8)	Referent	Referent
20 – 34	525	4.5 (3.1, 6.6)	1.6 (0.7, 3.8)	1.3 (0.5, 3.0)
35 – 49	690	6.5 (4.6, 9.2)	2.2 (0.9, 5.1)	1.6 (0.7, 4.0)
Education (yrs)				
None	314	10.7 (7.4,15.1)	5.9 (2.3, 15.3)[***]	1.7 (0.6, 4.9)
Primary	715	5.2 (3.7, 7.2)	2.9 (1.2, 7.4)[**]	1.2 (0.5, 3.0)
Secondary or higher	419	1.8 (0.8, 4.2)	Referent	Referent
Wealth index (terciles)				
Low	478	9.7 (7.1,13.2)	11.7 (3.4, 38.5)[***]	5.4 (1.7, 16.6)[***]
Middle	538	5.4 (3.7, 7.9)	6.5 (2.1, 22.7)[***]	4.3(1.3, 13.9)[***]
High	432	0.8 (0.3, 2.6)	Referent	Referent
		RBC Folate	**Deficiency (<340 nmol/L)**	
National	1448	8.9 (6.7, 11.7)		
Area				
Rural	773	13.5 (9.9, 18.1)	3.4 (1.6, 7.1)[**]	2.7 (1.03, 7.1)[*]
Urban	675	3.9 (2.0, 7.7)	Referent	Referent
Ethnicity				
Indigeneous	559	11.4 (7.8, 16.5)	1.6 (0.9, 2.6)	1.2 (0.7, 2.1)
Non-Indigeneous	889	7.3 (4.9, 10.7)	Referent	Referent
Age (yrs.)				
15 – 19	233	8.1 (5.0, 12.8)	Referent	Referent
20 – 34	525	8.4 (5.5, 12.6)	0.9 (0.5, 1.5)	0.8 (0.5, 1.4)
35 – 49	690	9.7 (6.6, 13.9)	1.2 (0.6, 2.1)	1.1 (0.6, 2.1)

	N		Unadjusted	Adjusted
	(unweighted)	**Prevalence**	**PRR[‡] (95% CI)**	**PRR (95% CI)[†]**
		(95%CI)		
Education (yrs)				
None	314	9.0 (6.0, 13.2)	2.2 (1.1, 4.4)**	0.9 (0.4, 2.4)
Primary	715	12.1 (8.5, 17.0)	2.9 (1.5, 5.9)**	1.7 (0.8, 3.6)
Secondary or higher	419	4.1 (2.2, 7.4)	Referent	Referent
Wealth index (terciles)				
Low	478	15.1 (10.2, 21.6)	3.7 (1.6, 8.2)***	1.8 (0.6, 5.0)
Middle	538	8.5 (5.4, 13.1)	2.1 (0.9, 4.5)	1.2 (0.4, 3.4)
High	432	4.1 (2.0, 8.2)	Referent	Referent

[‡] PRR = prevalence risk ratio

[†] Adjusted by area, ethnicity, age, education and wealth index

[*] p-value<0.05

[**] p-value<0.01

[***] p-value<0.001

Table 3

Prevalence and unadjusted and adjusted Prevalence Risk Ratios (PRR) for vitamin B12 deficiency and marginal deficiency, Guatemala, ENMICRON 2009–2010

Characteristic	N (Unweighted)	Prevalence	Unadjusted PRR (95% CI)	Adjusted PRR (95% CI)[†]
		(%)		
B12 Deficiency (< 148 pmol/L)				
National	1448	18.5 (15.6, 21.3)		
Area				
Rural	675	21.4 (17.7, 25.6)	0.9 (0.8, 1.0)	1.1 (0.8, 1.5)
Urban	773	15.4 (11.7, 20.0)	Referent	Referent
Ethnicity				
Indigenous	559	19.9 (15.7, 24.9)	1.1 (0.8, 1.5)	0.9 (0.7, 1.2)
Non-Indigeneous	889	17.6 (14.4, 21.3)	Referent	Referent
Age (yrs.)				
15 – 19	233	19.3 (14.2, 26.7)	Referent	Referent
20 –34	525	15.5 (12.3, 19.4)	0.8 (0.5, 1.2)	0.7 (0.5, 1.1)
35 – 49	690	20.0 (15.9, 24.9)	1.0 (0.7, 1.5)	0.9 (0.6, 1.4)
Education (yrs)				
None	314	21.3 (15.4, 28.7)	1.6 (1.01, 2.5)[*]	1.0 (0.6, 1.7)
Primary	715	20.9 (17.0, 25.4)	1.6 (1.1, 2.3)[**]	1.2 (0.8, 1.8)
Secondary or higher	419	13.2 (9.5, 18.1)	Referent	Referent
Wealth index (terciles)				
Lowest	478	28.2 (22.7, 34.4)	2.6 (1.7, 3.9)[***]	2.8 (1.7, 4.8)[***]
Middle	538	17.6 13.3, 22.9)	1.6 (1.05, 2.5)[*]	1.6 (1.02, 2.7)[**]
High	432	11.0 (7.6, 15.7)	Referent	Referent
B12 Marginal Deficiency (148 –221 pmol/L)				
National	1448	30.2 (27.3, 33.1)		
Area				
Rural	675	29.9 (26.2, 33.9)	1.0 (0.8, 1.2)	1.0 (0.8, 1.2)
Urban	773	30.4 (26.3, 34.0)	Referent	Referent
Ethnicity				
Indigeneous	559	25.3 (21.2, 29.9)	0.8 (0.6, 0.9)[***]	0.8 (0.6, 1.0)
Non-Indigeneous	889	33.2 (29.5, 37.0)	Referent	Referent
Age (yrs.)				
15 – 19	233	37.4 (29.9, 45.5)	Referent	Referent
20 –34	525	32.8 (27.8, 38.4)	0.8 (0.7, 1.1)	0.9 (0.7, 1.2)
35 – 49	690	24.6 (21.2, 28.3)	0.6 (0.5, 0.8)[***]	0.7 (0.5, 0.9)[***]

Characteristic	N (Unweighted)	Prevalence	Unadjusted PRR (95% CI)	Adjusted PRR (95% CI)[†]
		(%)		
Education (yrs)				
None	314	22.6 (17.9, 27.9)	0.7 (0.6, 0.9)[***]	0.8 (0.5, 1.1)
Primary	715	31.0 (26.8, 35.6)	0.9 (0.8, 1.3)	1.0 (0.8, 1.3)
Secondary or higher	419	33.3 (27.4, 39.7)	Referent	Referent
Wealth index (terciles)				
Lowest	478	30.9 (26.3, 36.0)	1.0 (0.9, 1.5)	1.7 (1.3, 2.3)[***]
Middle	538	28.7 (24.4, 33.4)	0.9 (0.7, 1.3)	1.2 (0.9, 1.5)
High	432	31.1 (26.3, 36.3)	Referent	Referent

[†]Adjusted by area, ethnicity, age, education and wealth index

[*]P-value<0.05

[**]P-value<0.01

[***]P-value<0.001

Table 4

Prevalence and unadjusted and adjusted prevalence risk ratios (PRR) for serum folate and RBC folate deficiency and vitamin B12 deficiency and marginal deficiency by region, ENMICRON 2009–2010

	N		Unadjusted	Adjusted[†]
	(unweighted)	Prevalence (95% CI)	PRR (95% CI)	PRR (95% CI)
Serum Folate Deficiency[**] **(<10 nmol/L)**				
Region				
Metropolitana	141	1.4 (0.3, 6.9)	**Referent**	**Referent**
Norte	107	8.9 (4.9, 15.7)	5.3 (1.0, 29.4)[*]	1.4 (0.2, 11.7)
Nor-Oriente	216	5.6 (2.8, 10.7)	3.2 (0.5, 20.7)	1.4 (0.2, 7.5)
Sur-Oriente	161	7.1 (3.5, 13.7)	4.1 (0.6, 26.8)	2.3 (0.4, 11.9)
Central	219	6.3 (3.1, 12.4)	3.7 (0.6, 23.8)	2.0 (0.3, 11.6)
Sur-Occidente	431	5.9 (3.9, 9.1)	3.4 (0.6, 20.3)[*]	1.7 (0.3, 10.3)
Nor-Occidente	101	5.2 (1.9, 12.7)	2.9 (0.4, 21.4)	0.9 (0.1, 6.9)
Petén	72	2.7 (0.7, 10.2)	1.5 (0.2, 13.8)	0.6 (0.1, 5.2)
RBC Folate Deficiency (<340 nmol/L)				
Region				
Metropolitana	141	3.5 (0.7, 16.3)	**Referent**	**Referent**
Norte	107	24.9 (10.0, 49.8)	8.1 (1.2, 43.4)[*]	2.7 (1.05, 11.9)[**]
Nor-Oriente	216	13.9 (8.8, 21.1)	4.4 (1.03, 25.4)[*]	1.9 (0.4, 8.8)
Sur-Oriente	161	10.5 (6.5, 16.7)	3.2 (0.6, 18.7)	1.6 (0.3, 7.7)
Central	219	10.8 (5.3, 20.7)	3.3 (0.5, 20.8)	1.6 (0.3, 8.2)
Sur-Occidente	431	7.2 (4.6, 10.9)	2.1 (0.4, 12.3)	1.2 (0.2, 5.6)
Nor-Occidente	101	3.2 (0.8, 12.2)	0.9 (0.1, 8.2)	0.4 (0.1, 2.4)
Petén	72	3.4 (1.1, 9.8)	0.9 (0.1, 7.3)	0.5 (0.1, 3.2)
Vitamin B12 Deficiency (<148pmol/L)				
Region				
Metropolitana	141	14.6 (8.2, 24.6)	**Referent**	**Referent**
Norte	107	25.8 (15.5, 39.7)	1.8 (0.8, 3.6)	1.1 (0.5, 2.2)
Nor-Oriente	216	22.8 (16.1, 31.3)	1.6 (0.8, 2.9)	1.1 (0.7, 2.0)
Sur-Oriente	161	26.1 (19.5, 34.0)	1.8 (0.9, 3.3)	1.4 (0.8, 2.3)
Central	219	25.6 (18.5, 34.4)	1.7 (0.9, 3.3)	1.4 (0.8, 2.4)
Sur-Occidente	431	12.3 (8.9, 16.7)	0.8 (0.4, 1.6)	0.6 (0.4, 1.1)
Nor-Occidente	101	18.7 (9.5, 33.6)	1.3 (0.6, 3.0)	0.7 (0.4, 1.6)
Petén	72	14.6 (8.6, 23.7)	1.0 (0.5, 2.1)	0.6 (0.3, 1.2)
Vitamin B12 Marginal Deficiency (148 – 221 pmol/L)				
Region				
Metropolitana	141	35.2 (28.3, 42,7)	**Referent**	**Referent**
Norte	107	33.6 (24.7, 43.8)	1.0 (0.7, 1.4)	1.0 (0.7, 1.5)

	N		Unadjusted	Adjusted[†]
	(unweighted)	Prevalence (95% CI)	PRR (95% CI)	PRR (95% CI)
Nor-Oriente	216	37.8 (29.5, 46.9)	1.1 (0.8, 1.5)	1.0 (0.7, 1.4)
Sur-Oriente	161	32.3 (23.0, 43.2)	0.9 (0.6, 1.3)	0.9 (0.6, 1.3)
Central	219	28.9 (24.0, 34.3)	0.8 (0.6, 1.1)	0.8 (0.6, 1.1)
Sur-Occidente	431	28.1 (22.6, 34.2)	0.8 (0.6, 1.1)	0.8 (0.6, .1.2)
Nor-Occidente	101	21.9 (14.2, 32.2)	0.6 (0.4, 0.9)[*]	0.7 (0.4, 1.1)
Petén	72	21.5 (12.6, 34.2)	0.6 (0.3, 1.0)	0.6 (0.3, 0.9)[*]

[†]Adjusted by area, ethnicity, age, education and wealth index

[*]P-value<0.05

[**]P-value<0.01

Table 5

National and regional adjusted geometric means of serum, red blood cell (RBC)and vitamin B12 concentrations by socio-demographic characteristics and regions, ENMICRON 2009–2010

	N	Serum Folate	95% CI	N	RBC Folate	95% CI	N	Vitamin B12	95%CI
	(unweighted)	(nmol/L)		(unweighted)	(nmol/L)		(unweighted)	(pmol/L)	
National	1448	30	29, 31	1448	725	711, 738	1448	341	333, 349
Area									
Rural	773	26	24, 27	766	664	624, 707	767	347	322, 378
Urban	675	33[1]	31, 35	671	763[9]	727, 802	667	334	309, 358
Ethnicity									
Indigeneous	559	29	26, 30	555	702	665, 742	551	369[13]	337, 404
Non-Indigeneous	889	30	28, 32	882	722	689, 756	883	314	297, 332
Age (yrs.) *									
15 – 19	233	30	28, 32	233	681	639, 726	232	303[14,15]	291, 331
20 – 34	525	32	27, 31	520	723	685, 762	520	346	323, 370
35 – 49	690	28	27, 30	689	734	698, 773	682	376	346, 407
Education (yrs) *									
None	314	27[2,3],	27, 29	310	702	653, 755	310	357	349, 372
Primary	713	29[4]	28, 31	710	694	658, 732	708	330	298, 352
Secondary or higher	419	31	29, 33	417	742	699, 787	416	335	301, 349
Wealth index (terciles) *									
Lowest	477	25[5,6],	23, 27	476	624[5,6]	583, 668	473	285[5,6]	257, 315
Middle	538	30[7]	27, 32	532	731	690, 773	534	350	325, 377
High	431	34	31, 37	429	792	738, 851	427	395	357, 437
Region *									
Metropolitan	141	39[8]	36, 43	141	834[10]	774, 898	141	300[16]	271, 332
Norte	107	25	21, 30	107	548[11]	482, 625	107	358	269, 477

	N (unweighted)	Serum Folate (nmol/L)	95% CI	N (unweighted)	RBC Folate (nmol/L)	95% CI	N (unweighted)	Vitamin B12 (pmol/L)	95%CI
Nor-Oriente	216	26	23, 28	216	696	621, 781	216	292[17]	262, 326
Sur-Oriente	161	28	24, 31	161	692[12]	631, 759	161	296[18]	263, 332
Central	219	28	25, 32	219	658	592, 731	219	291[19]	251, 328
Sur-Occidente	431	27	25, 30	431	723	644, 741	431	393	258, 426
Nor-Occidente	101	30	26, 33	101	807	722, 901	101	384	321, 459
Peten	72	24	21, 27	72	745	661, 839	72	372	306, 440

*
all pair-wise comparisons were made

[1] Urban vs. Rural (p<0.001)

[2] Education: none vs. primary (p<0.01)

[3] Education: none vs secondary or more (p<0.001)

[4] Education: primary vs secondary or more (p<0.05)

[5] Wealth Index: Lowest vs Middle (p<0.001)

[6] Wealth Index: Lowest vs High (p<0.001)

[7] Wealth Index: Middle vs High (p<0.05)

[8] Metropolitan compared to all other regions (p<<0.001)

[9] Urban vs. Rural (p<0.05)

[10] Region: Metropolitana vs. Norte; (p<0.001); Nor-Oriente (p<0.001); Central (p<0.05); and Sur-Occidente (p<0.001)

[11] Region:: Norte vs. Nor-Occidente (p<0.001); Peten (<0.05)

[12] Region: Sur-Oriente vs. Nor-Occidente (p<0.01)

[13] Indigeneous vs. Non-indigenous (p<0.001)

[14] Age 15–19 vs. 20–34 (p<0.01)

[15] Age 15–19 vs. 35–49 (p<0.001)

[16] Region: Metropolitana vs. Sur-Ooccidente (p<0.001)

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[17]Region: Nor-Oriente *vs.* Sur-Occidente (p<0.01)

[18]Region: Sur-Oriente *vs.* Sur-Occidente (p<0.001)

[19]Region: Central *vs.* Sur-Occidente (p<0.001)

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