JIDC | THE JOURNAL OF INFECTION IN DEVELOPING COUNTRIES

Original Article

Cryptosporidiosis: a neglected infection and its association with nutritional status in schoolchildren in northwestern Mexico

Luis Quihui-Cota, Carmen M Lugo-Flores, Jose A Ponce-Martinez, Gloria G Morales-Figueroa

Departmento de Nutrición Pública y Salud, Coordinación de Nutrición, Centro de Investigación en Alimentación y Desarrollo, Hermosillo Sonora, México

Abstract

Introduction: Undernutrition is a recognized public health problem in Mexican children and cryptosporidiosis is probably a factor contributing to this problem. However, it remains a neglected and poorly attended infection in Mexico. This study aimed to determine the prevalence of *Cryptosporidium parvum* and to establish its association with the nutritional status in schoolchildren of northwestern Mexico.

Methodology: A total of 405 schoolchildren between 6 and 13 years of age were included in this study. Weight-for-age (W/A), height-for-age (H/A) and body mass index-for-age (BMI/A) Z scores were calculated. The Faust technique was used to detect intestinal parasites in stool samples and *C. parvum* coproantigen was detected by enzyme-linked immunosorbent assay (ELISA).

Results: The overall prevalence of *C. parvum* was 28.4%. Some of the study children were *C. parvum* free (n = 86) and some were *C. parvum* infected (n = 77). The prevalences of risk of undernutrition found in both groups of children for H/A, W/A, and BMI/A Z scores were 18%, 21% and 28%, respectively. Weight, ZW/A, and ZH/A were significantly higher in the *C. parvum*-free group compared to the *C. parvum*-infected group ($p \leq 0.05$). The children with cryptosporidiosis were 2.7 times more likely to be at risk of undernutrition by W/A Z score and 2.9 times more likely to be at risk of undernutrition by ZH/A than were the *C. parvum*-free children.

Conclusions: Cryptosporidiosis may be a contributing factor to childhood undernutrition in northwestern Mexico. The proper authorities must implement control and prevention measurements in Mexico and other developing countries.

Key words: *C. parvum*; undernutrition; schoolchildren; Mexico.

J Infect Dev Ctries 2015; 9(8):878-883. doi:10.3855/jidc.6751

(Received 16 February 2015 – Accepted 30 March 2015)

Introduction

Cryptosporidiosis is a world public health problem, especially affecting immunocompromised persons, such as children and the elderly. More than 20 species of *Cryptosporidium* are recognized, but 90% of human cryptosporidiosis is attributed to *Cryptosporidium parvum* and *Cryptosporidiun hominis* [1]. Its transmission has been associated with contaminated drinking water and food, low socioeconomic status and overcrowding conditions [2]. *C. parvum* has a cosmopolitan distribution and its prevalence can range from 0.1% to 31.5% in developing countries [3]. Human cryptosporidiosis can be accompanied by abdominal pain, fever, vomiting, malabsorption and diarrhea [4] that may lead to undernutrition, as found in children in West Africa and South America [5] and in Bangladesh [6]. Undernutrition is a well-recognized worldwide health problem, and some years ago, it was found to be responsible of 54% of deaths in children in developing countries [7]. In 2011, the prevalence of moderate and severe underweight in preschool children in South Asia, West and Central Africa, sub-Saharan Africa, and Latin America and the Caribbean were estimated to be 33%, 23%, 21% and 3%, respectively. In addition, the prevalences of stunting and wasting, respectively, in the same regions were 39% and 16%, 39% and 12%, 40% and 9%, and 12% and 2%, respectively [8]. It was estimated that most of global undernutrition was associated with impaired intestinal absorptive function resulting from multiple and repeated enteric infections [9], which is critical in regions where children are mildly nourished [5]. In Mexico, it was estimated that, in 2012, 302, 279 (2.8%) children under five years of age were underweight, 1,467,757 (13.6%) were stunted and 171,982 (1.6%) showed emaciation. The prevalence of low height/age was 9%, 11.4% and 19.2% in north, center, and south Mexican regions, respectively [10]. However, human cryptosporidiosis remains neglected in Mexico, and some information is provided only in limited publications. In 2006, a study found

cryptosporidiosis in 41% of 100 children under one year of age in south Mexico [11], while in 2010, 16% of 100 patients with diarrhea had cryptosporidiosis in north Mexico [12]. Based on this, we investigated the prevalence of *C. parvum* and its association with the nutritional status of schoolchildren in northwestern Mexico.

Methodology

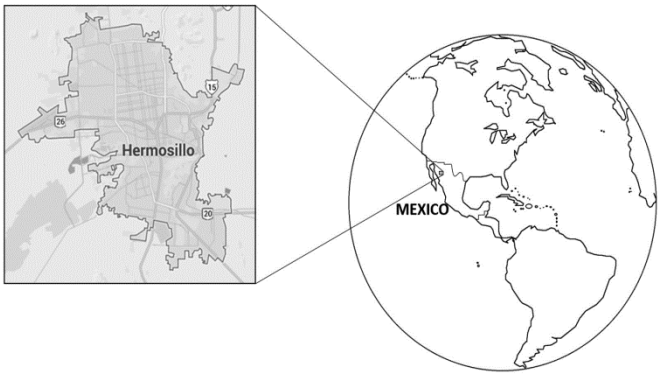
Study site and population

This was a cross-sectional study conducted from September 2008 to September 2009 in the state of Sonora (northwestern Mexico). Sonora borders the state of Chihuahua to the east, the state of Sinaloa to the south, the Gulf of California to the west, and the American state of Arizona to the north (Figure 1). Ninety-six percent of Sonora is dry and semi-dry. The average summer temperature is 38°C (June–August), and the temperature ranges from 5°C to 30°C from September to January. Four primary schools in the municipality of Hermosillo were selected based on high rates of gastrointestinal infections in the local population [13] and low socioeconomic status [14] defined as a high percentage of parents with no secondary education, and a high number of households with no drainage, no electricity, no safe drinking water and poor quality of construction materials [15] around the selected schools. A total of 883 children officially enrolled in the selected primary schools were invited to participate. Plastic containers were distributed for the collection of stool samples (three per child). Study protocol was explained to the school authorities and parents. A total of 405 schoolchildren (45.8%) agreed to participate in this study, and they were categorized into either the *C. parvum*-free group (*i.e.*, free of intestinal parasites or showing only non-pathogenic intestinal parasites such as *Entamoeba coli*, *Endolimax nana*, *I. butschlii*, and *Chilomastixmesnili*), or the *C. parvum*-infected group (only with *C. parvum* alone or plus no pathogen intestinal parasites as already previously indicated).

Ethical consideration

Individual informed consent was obtained from the parents or guardians of the participating children. Of the 883 invited children, 478 were unwilling to participate for various reasons. Both participating and non-participating children lived in the same living conditions around the selected schools. The ethics committee of the Centro de Investigación en Alimentación y Desarrollo approved this study.

Figure 1. General location of the study site (municipality of Hermosillo) in northwestern México



Children infected with intestinal parasites received the proper antiparasitic treatment by a qualified physician.

Anthropometric measurements

Standing height was measured using a stadiometer (Holtain Ltd., Dyfed, UK) with 2.05 ± 0.0005 m capacity, and weight was measured to the nearest 50 g using a digital electronic scale (AND FV-150 KA1, A&D Co. Ltd., Toshima-ku, Tokyo, Japan) following the standardized recommendations. Ages were validated from reliable official school records. Weight-for-age (W/A), height-for-age (H/A), and body mass index-for age (BMI/A) Z scores were calculated using Anthro Plus versio 1.0.4 [16] and Anthro [17] software. Undernutrition risk was defined from -2 to < -1 Z scores and moderate and severe undernutrition from < -2 Z scores, considering the median reference values [7] of H/A (stunting), W/A, and BMI/A. The valid Z scores for analysis in this study were those considered the most frequently occurring in the study population: -5.0 and +5.0 for H/A, -5.0 to +5.0 for W/A, and between -4 and +4 for BMI/A. Five cases for BMI/A, three for H/A, and two for W/A were outside the above-mentioned ranges.

Fecal sample collection, processing, and analysis

Fecal samples were collected and transported to the parasitology laboratory of the Center of Research of Food and Development and were stored between 5°C and 7°C for 24–72 hours until analysis using the Faust technique [18]. Covered slides with fecal material in a drop of iodine solution were observed using the 10x and 40x objectives for identification of protozoan cysts and helminth eggs. In addition, 1 g of feces were homogenized, transferred into cryogenic vials (2 mL), properly labeled and stored at -20°C until analysis. Samples were allowed to thaw at room temperature (24°C), and 5 mL of anti-*C. parvum* solution was

added to each vial. Content was homogenized and 200 uL of a second anti-*C. parvum* solution was added, forming a "sandwich" with the *C. parvum* fecal antigen bound by the first antibody. The reaction was visualized by adding a second *C. parvum* antibody bound to a peroxidase conjugate with a chromogenic tetramethylbenzidine against the second *C. parvum* antibody. The blue color revealed the presence of *C. parvum* fecal antigen bound to the anti-*C. parvum*, and reaction was stopped using phosphoric acid 1M. A yellow color was developed and read using a model 680 microplate reader from Bio-Rad Laboratories (Hercules, USA) in an absorbance range between 450 nm and 650 nm. Positive and a negative standard references for quality control were included for each run. A positive result was considered when the reading was ≥ 0.150, in agreement with the manufacturer's instructions. The DRG ELISA kit used had a sensitivity of 93% and a specificity of 98% for the diagnosis of *C. parvum* fecal antigens (*Cryptosporidium* Ag Stool, DRG International, Mountainside, USA).

Statistical analysis

Descriptive statistics were used to express the age and anthropometric indicators of the study children as a mean value. The prevalence of cryptosporidiosis was expressed as the percentage of children with *C. parvum* in any of the fecal samples provided. The proportions were compared using the Chi-square test with the corresponding odds ratios, 95% confidence intervals, and pvalues (prevalence of infection). The association between the nutritional status and cryptosporidiosis was analyzed using multiple logistic regression models. In all models, the dependent variable was the indicator of mild undernutrition or risk for undernutrition from -2 to < -1 Z scores for H/A, W/A and BMI/A [19], and the *C. parvum* infection (0 to denote absence and 1 presence) was considered as the hypothesized independent variable. Those variables judged to be possible confounding factors such as community, age and sex (0 = female; 1 = male) were used to construct the stepwise models. Data was analyzed with the statistical software STATA/SE version 12.0 with a significance level of $p \leq 0.05$.

Results

A total of 198 girls (49%) and 207 boys (51%) (z = -0.632, p = 0.527) were included in this study. The average age of the participants (n = 405) was 7.7 years ± 1.3. Thirty-four percent (n = 138) and 58% (n = 235) of the children provided two and three fecal samples, respectively. The overall prevalence of *C. parvum* was 24% (n = 97) in this study. From the 405 schoolchildren, 163 were categorized as 86 *C. parvum*-free (with no intestinal parasites or with only non-pathogen intestinal parasites) and 77 were categorized as *C. parvum*-infected (only with *C. parvum* or *C. parvum* plus non-pathogen intestinal parasites). The rest did not meet these criteria. Eighty percent of the categorized children provided three samples. Due to the low prevalence of data of severe or moderate undernutrition defined by < -2 Z scores for H/A, W/A and BMI/A (3.4%, 2.1% and 2.8% respectively), they were not used in the multiple logistic regression models. Otherwise, the prevalences of risk of undernutrition found in these groups of children for the ZH/A, ZW/A and ZBMI/A were 18%, 21% and 28%, respectively. No difference in nutritional status was found between boys and girls (p> 0.05; data not shown), Also, the weight, ZW/A, and ZH/A were significantly higher in the *C. parvum*-free group compared to the *C. parvum*-infected group (Table 1). No difference was found in the age, height, and BMI/A between the groups (Table 1).

Table 1. Characteristics of the *Cryptosporidium*-free and *Cryptosporidium*-infected groups of schoolchildren in northwestern Mexico

Variable	*C. parvum*-free (n = 86)	*C. parvum*-infected (n = 77)	p value[d]
Age (years)	7.8 (1.4)	8.1 (1.1)	0.133[c]
Weight (kg)[a]	33.4 (28.4–38.1)	25.7 (20.1–30.9)	0.020[b]
Height (cm)	126.4 (6.5)	124.5. (7.8)	0.091[c]
W/A (Z score)	0.16 (1.1)	-0.39 (2.1)	0.035[c]
H/A (Z score)	-0.16 (0.9)	-0.42 (0.7)	0.042[c]
BMI/A (Z score)	0.39 (0.9)[b]	0.44 (0.6)[c]	0.680[c]

[a] Median (25%–75% inter-quantiles); all other data are presented as mean ± standard deviation; [b] Kruskal-Wallis rank sum test; [c] Two samples independent *t* test; [d] Significance at $p \leq 0.05$.

Approximately 95% of the fathers and 45% of the mothers had formal jobs at the time of interview. They represented their family's economic support. Secondary education was completed by 60% and 57% of the fathers and mothers, respectively. Chi-square analysis showed no significant difference between the *C. parvum*-free and *C. parvum*-infected groups in parental employment and education (preparatory school) ($p > 0.05$), or in the quality of walls (cement and brick) ($p > 0.05$) and roof (concrete) ($p > 0.05$) in their homes. Forty-eight percent and 51% of the *C. parvum*-free and *C. parvum*-infected children, respectively, resided in households with no drainage ($p > 0.05$). Although the percentage of treated drinking water (defined as boiled water or water subjected to cartridge or membrane filtration) used and family income tended to be higher in the *C. parvum*-free group ($p = 0.130$ and $p = 0.090$, respectively) and the overcrowding conditions tended to be higher (> 5 people per room in a household) in the *C. parvum*-infected group ($p = 0.090$), both were no different between the groups. Because age, sex, type of community, and socioeconomic conditions may influence nutritional status [20], the nutritional status–cryptosporidiosis relationship was determined after adjusting for those all the significant related variables by univariate and stepwise analysis. Table 2 shows the mentioned association adjusted for those variables with a p value ≤ 0.20 (univariate analysis), and also the nutritional status–cryptosporidiosis association adjusted only for the significant variables (stepwise). The children with cryptosporidiosis were 2.7 times more likely to be at risk of undernutrition by ZW/A and 2.9 times more likely to be at risk of undernutrition by ZH/A than the *C. parvum*-free children (Table 2). No association was found between cryptosporidiosis and risk of undernutrition by ZBMI/A.

Discussion

This study investigated the prevalence of *C. parvum* and its association with nutritional status in children, between September 2008 and September 2009 from four public elementary schools belonging to the municipality of Hermosillo in northwestern Mexico. The schoolchildren (n = 405) showed a high prevalence of *C. parvum* (24%). Otherwise, the prevalence of undernutrition by ZH/A in this study was lower (3.4%) than the national prevalence (13.6%) published by the Mexican survey in 2012, which estimated a prevalence of undernutrition for ZW/A similar to this study (2.1% *vs*. 2.8%). In addition, the Mexican survey also estimated a prevalence of undernutrition for ZH/A of 8.9% in

Table 2. Unadjusted and adjusted association between the risk of undernutrition and cryptosporidiosis in schoolchildren in northwestern Mexico

Variable	Univariate analysisUnadjusted OR (± SE)	95% CI	p value[C]	StepwiseAdjusted OR (± SE)	95% CI	p value[c]
ZW/A (n =163)						
Cryptosporidiosis	2.1. ± 0.42	1.72–3.5	0.037	2.73 ± 0.81	1.4–3.9	0.031
Age (years)	1.11 ± 0.06	0.85–1.39	0.168	1.47 ± 0.12	1.01–2.27	0.035
Unemployed parents[a]	1.43 ± 0.15	0.45–1.56	0.182	1.31 ± 0.15	0.83–2.42	0.169
Parents with no preparatory school	1.21 ± 0.17	0.32–1.62	0.195	1.61 ± 0.17	0.5–2.71	0.152
Untreated water consumption[b]	1.09 ± 0.10	0.65–1.27	0.176	1.39 ± 0.08	0.95–2.12	0.173
ZH/A (n = 163)						
Cryptosporidiosis	2.56. ± 0.82	1.48–4.23	0.040	2.98 ± 0.34	1.32–3.89	0.036
Unemployed parents[a]	1.26 ± 0–0.06	0.34–1.83	0.195	1.54 ± 0.10	0.89–2.79	0.210
Parents with no preparatory school	1.21 ± 0.17	0.32–1.82	0.135	1.61 ± 0.17	0.5–2.71	0.152
Untreated water consumption[b]	1.13 ± 0.09	0.83–1.44	0.115	1.87 ± 0.19	1.03–2.44	0.063
ZBMI/A (n = 163)						
Cryptosporidiosis	1.4 ± 0.27	0.45–1.9	0.347	-	-	-

OR (SE): odds ratio (standard error); CI: confidence interval; [a] No occupational activity at the time of interview; [b] No boiled water or water not subjected to cartridge or membrane filtration; [c] Significance at $p \leq 0.05$

northern Mexico but did not provide information about ZBMI/A. On the other hand, the *C. parvum*-infected schoolchildren showed lower means of ZH/A and ZW/A than did the *C. parvum*-free children. In 2006, another cross-sectional study in Haitian children 36 months of age found that 49 *C. parvum*-infected children showed lower (< -2) ZW/A and ZH/A than did 41 *C. parvum*-free children [21]. Some years before *Molbak et al*. [22] found that *Cryptosporidium* infection was associated with the highest weight loss for children (both boys and girls) who contracted cryptosporidiosis before reaching one year of age. In addition, the proportion of children at risk for undernutrition was relatively high in this study. Although risk of undernutrition is not an immediate life-threatening condition, frequent reinfections are serious health-deteriorating conditions in regions where children are at risk of undernutrition. Although the sample size in this study was not representative of the general population of northwestern Mexico, *C. parvum* was found to be a risk of undernutrition in the study children. Finally, like undernutrition, cryptosporidiosis is the result of multiple factors, but outbreaks have been highly associated with unsafe drinking water worldwide [23]. It is important to mention that in 1994, the American federal government published the Information Collection Rule, which considered *Cryptosporidium* to be a primary drinking water contaminant [24]. In Mexico, there is a law governing public systems for drinking water operators, whose standard includes determining total fecal coliforms and the presence of thermo-tolerant organisms such as genera *Citrobacter* and *Enterobacter*[25]. This study suggests that, in addition to monitoring bacteria in drinking water as dictated by Mexican law, analysis of *Cryptosporidium* should also be considered in determining the magnitude of the problem of this infection in Mexico.

Conclusions

Complementary studies should be developed to determine the sources of *Cryptosporidium* in the study area and to promote the dissemination of these results at all levels of government. This will provide support, updates, and expansion of health communication efforts among the proper authorities to prevent and control cryptosporidiosis in Mexico.

Acknowledgements

The authors thank Julián Esparza Romero for his advisement in the statistical analysis of this work. They also appreciate the support granted by the Consejo Nacional de Ciencia y Tecnología, San Jose Hospital, the Ministry of Health of the State of Sonora, and the Centro de Investigación en Alimentación y Desarrollo, A.C.

References

1. Morgan-Ryan UM, Fall A, Ward LA, Hijjawi N, Sulaiman I, Fayer R, Thompson RC, Olson M, Lal A, Xiao L (2002) Cryptosporidium hominis n. sp. (Apicomplexa: Cryptosporidiidae) from Homo sapiens. J Eukaryot Microbiol 49: 433-440.
2. Molloy SF, Tanner CJ, Kirwan P, Asaolu SO, Smith HV, Nichols RA, Connelly L, Holland CV (2011) Sporadic Cryptosporidium infection in Nigerian children: risk factors with species identification. Epidemiol Infect 139: 946-954.
3. Karanis P, Kourenti C, Smith H (2007) Waterborne transmission of protozoan parasites: a worldwide review of outbreaks and lessons learnt. J Water Health 5: 1-38.
4. Chalmers RM, Davies AP (2010) Minireview: clinical cryptosporidiosis. Exp Parasitol 124: 138-146.
5. Checkley W, Gilman RH, Epstein LD, Suarez M, Diaz JF, Cabrera L, Black RE, Sterling CR (1997) Asymptomatic and symptomatic cryptosporidiosis: their acute effect on weight gain in Peruvian children. Am J Epidemiol 145: 156-163.
6. Mondal D, Haque R, Sack RB, Kirkpatrick BD, Petri WA, Jr. (2009) Attribution of malnutrition to cause-specific diarrheal illness: evidence from a prospective study of preschool children in Mirpur, Dhaka, Bangladesh. Am J Trop Med Hyg 80: 824-826.
7. WHO (2005) Child Growth Standards: Methods and development. World Health Organization. Available: http://www.who.int/childgrowth/standards/technical_report/en/. Accessed February 20, 2011.
8. UNICEF (2011) Monitoring the situation of children and women. United Nations Children's Fund. Available: http://www.childinfo.org/. Accessed February 15, 2011.
9. Guerrant RL, Oria RB, Moore SR, Oria MO, Lima AA (2008) Malnutrition as an enteric infectious disease with long-term effects on child development. Nutr Rev 66: 487-505.
10. Gutierrez JP, Rivera-Dommarco J, Shamah-Levy T, Villalpando-Hernandez S, Franco A, Cuevas-Nasu L, Romero-Martinez M, Hernandez-Avila M (2012) Health and Nutrition National Survey. National Results. Cuernavaca, Mexico: Instituto Nacional de Salud Publica http://ensanut.insp.mx/informes/ENSANUT2012ResultadosNacionales.pdf.
11. Sanchez-Vega JT, Tay-Zavala J, Aguilar-Chiu A, Ruiz-Sanchez D, Malagon F, Rodriguez-Covarrubias JA, Ordonez-Martinez J, Calderon-Romero L (2006) Cryptosporidiosis and other intestinal protozoan infections in children less than one year of age in Mexico City. Am J Trop Med Hyg 75: 1095-1098.
12. Alvarado-Samarrón DB, Olivas-Enriquez E, Identificación de Cryptosporidium parvum, Cyclospora cayetanensis y otros parásitos intestinales en pacientes con diarrea., Ciencia en la frontera: revista de ciencia y tecnología de la UACJ 2010, p. 15.
13. Secretarìa de Salud (2008) Annual record of new cases of diseases in the State of Sonora. Hermosillo, Sonora, Mexico: Secretaria de Salud, Departamento de Estadistica y Evaluacion. Direccion de Planeacion y Desarrollo. Available:

http://www.epidemiologia.salud.gob.mx/dgae/boletin/indice-2008.html.
14. Alvarez G, Lara F, Harlow SD, Denman C (2009) Infant mortality and urban marginalization: a spatial analysis of their relationship in a medium-sized city in northwest Mexico. Rev Panam Salud Publica 26: 31-38.
15. Instituto Nacional de Estadística y Geografía (2000) Población de México. Available. http://cuentame.inegi.org.mx/temperatura. Accessed January 10, 2011.
16. WHO (2009) Anthro Plus for personal computers. Geneva: WHO.
17. WHO (2010) Anthro for personal computers. Geneva: WHO.
18. Markell EK, Voge's M (2006) Medical parasitology, 9th edition.United States of America: W.B. Saunders Company. 480
19. WHO (2008) Training Course on Child Growth Assessment. C Interpreting Growth Indicators. Geneva: WHO Available: http://www.who.int/childgrowth/training/module_c_interpreting_indicators.pdf. January 2015
20. Zaini MZ, Lim CT, Low WY, Harun F (2005) Factors affecting nutritional status of Malaysian primary school children. Asia Pac J Public Health 17: 71-80.
21. Kirkpatrick BD, Huston CD, Wagner D, Noel F, Rouzier P, Pape JW, Bois G, Larsson CJ, Alston WK, Tenney K, Powden C, O'Neill JP, Sears CL (2006) Serum mannose-binding lectin deficiency is associated with cryptosporidiosis in young Haitian children. Clin Infect Dis 43: 289-294.
22. Molbak K, Andersen M, Aaby P, Hojlyng N, Jakobsen M, Sodemann M, da Silva AP (1997) Cryptosporidium infection in infancy as a cause of malnutrition: a community study from Guinea-Bissau, west Africa. Am J Clin Nutr 65: 149-152.
23. United States Environmental Protection Agency (2001) Cryptosporidium: Drinking Water Health Advisory. Washington, D.C.: USEPA. Available: http://water.epa.gov/action/advisories/drinking/upload/2009_02_03_criteria_humanhealth_microbial_cryptoha.pdf. Accessed on May 2014
24. United States Environmental Protection Agency (2015)Water: Regulatory Information. United States Enviroment Protection Agency. Available: http://water.epa.gov/lawsregs/rulesregs/. Accessed 23 March 2015.
25. Secretaria de Salud (2000) Surveillance and evaluation of the control of water quality for human consumption, distributed by public supply systems. Secretaria de Salud. NOM-179-SSA1-1998. Available: http://www.salud.gob.mx/unidades/cdi/nom/179ssa18.html Accessed on January 30, 2015.

Corresponding author
Luis Quihui Cota
Departmento de Nutrición Pública y Salud, Coordinación de Nutrición, Centro de Investigación en Alimentación y Desarrollo
Carretera a La Victoria Km 0.6
Hermosillo Sonora, México
C.P. 83304, México
Phone: +52 (662) 289-24-00 Ext 301
Fax:+52 (662) 280 0094
Email: lquihui@ciad.mx

Conflict of interests: No conflict of interests is declared.