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Cryptosporidium parvum and its association to risk of malnutrition in school children of Northwest Mexico: A brief report

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High prevalence of malnutrition and cryptosporidiosis has been found in Mexican children, although Mexican studies on cryptosporidiosis are limited. The objective was to determine the prevalence of *Cryptosporidium parvum* and to establish its association with the nutritional status in school children of Northwestern Mexico. A total of 321 school children of 6 to 13 years old participated in the study. Weight-for-age, height-for-age, and body mass index for age (BMI/A) Z scores were calculated. Enzyme linked immuno-absorbent assay (ELISA) was used to detect fecal antigens of *C. parvum*. The overall prevalence of *C. parvum* was 39.3%. *C. parvum*-infected children were more likely to be at risk of malnutrition than *C. parvum*-free children. Cryptosporidiosis may be a risk factor for children malnutrition in Northwestern Mexico.

Key words: *Cryptosporidium parvum*, malnutrition, school children, Mexico.

INTRODUCTION

Cryptosporidiosis is a world public health problem, especially affecting immunocompromised persons, children and elderly. Twenty species of *Cryptosporidium* are recognized nowadays, but infection in human is attributed frequently to 8 of those species (*Cryptosporidium hominis, Cryptosporidium parvum, Cryptosporidium andersoni, Cryptosporidium felis, Cryptosporidum canis, Crytosporidium suis, Cryptosporidium muris, Cryptosporidium meleagridis*) (Hadfield et al., 2011). Its transmission has been associated with contaminated drinking water and food, low socioeconomic status and overcrowding conditions (Karanis et al., 2007). This infection can be accompanied by diarrhea, abdominal pain, fever, vomiting and malabsorption in man (Chalmers and Davies, 2010) that may explain its association with low weight and height in children younger than one year in West Africa and South America (Checkley et al., 1997). *C. parvum* has a cosmopolitan distribution and its prevalence can range from 0.1 to 31.5% in developing countries (Karanis et al., 2007). Malnutrition is also a well-recognized worldwide health problem and many years ago it was directly responsible for 54% of deaths in children of developing countries (WHO, 2005). In 2011, the prevalences of moderate and severe underweight in preschool-children of South Asia, West and Central Africa, Sub-Saharan

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Africa, and Latin America and the Caribbean were 33, 23, 21 and 3%, respectively. In addition, the prevalences of moderate and severe stunting and wasting in the same regions were 39 and 16, 39 and 12, 40 and 9, and 12 and 2%, respectively (UNICEF, 2011). In Mexico, the prevalences for stunting in preschool-children were 26.9, 21.5 and 15.5% in 1988, 1999 and 2006, respectively; and for wasting 6.2, 2.1 and 2.0%, respectively in the same years (Gonzalez-de Cossio et al., 2009). Now, it is well known that malnutrition results from different risk factors, but most of global malnutrition is associated with impaired intestinal absorptive function resulting from multiple and repeated enteric infections (Guerrant et al., 2008). This is critical in regions where children are mildly nourished even when the enteric infections are asymptomatic (Checkley et al., 1997). Mexican studies about cryptosporidiosis in children are limited. Some years ago, the only local study published a prevalence of 23% of cryptosporidiosis in 100 children (0 to 5 years) in Northwestern Mexico (Gómez et al., 1996). In 2006, cryptosporidiosis was found in 41% of 100 children under a year of age in Mexico City (Sanchez-Vega et al., 2006) and in 2010, *C. parvum* was found in 16% of 100 patients with diarrhea in Ciudad Juárez (Alvarado-Samarrón and Olivas-Enriquez, 2010). However, the current overall prevalence of *C. parvum* in the Mexican population remains unknown. On the other hand, in agreement to the National Survey in 2006, the national prevalences of low height-for-age and low weight-for-age (<-2 Z Scores) were 15.5 and 2% in children, respectively. The prevalence of low height-for-age in Northern Mexico was 8.3%, and it was around 8.0% in both the urban and rural populations of the same region (SS, 2006). However, when a child at risk of malnutrition (from -2 to <-1 Z Scores) is exposed to the cumulative effects from repetitive re-infections, the negative effects can become irreversible in the first three years of life. Based on these findings, this study investigated the prevalence of *C. parvum* and its association with nutritional status in school children of Northwestern Mexico.

MATERIALS AND METHODS

Study site and population

This was a cross-sectional study conducted from September to December 2008 in the state of Sonora (Northwest Mexico). Sonora is bordering to the east with the state of Chihuahua, south to the state of Sinaloa, west to the Gulf of California, and north to the US State of Arizona. Ninety-six percent of Sonora is dry and semi-dry. The average summer temperature is 38°C (June to August) and 5 to 30°C from September to January. The weather in the municipality of Hermosillo is wilderness with cool winters and can reach up to 45°C during the summer season (June, July, and August). Three primary schools in the municipality of Hermosillo were selected based on high rates of gastrointestinal infections in the local population (SS, 2008), low socioeconomic status (Alvarez et al., 2009) defined as a high percentage of parents with no school education, high number of households with no drainage, electricity, drinking water and poor quality of construction materials (INEGI, 2000) around the selected schools. A total of 720 children officially enrolled in the selected primary schools were invited to participate, while plastic containers were distributed for the collection of the stool samples (three per child). Study protocol was explained to the school authorities and parents. Three hundred and twenty one children (44.4%) agreed to participate in this study.

Ethical consideration

Individual informed consent was obtained from parents or guardians of the participating children. Of the 720 children, 352 were unwilling to participate, and 47 did not meet the study criteria (disabled, supplemented or medicated). Both participant and non-participant children were living in the same living conditions around the selected schools. The ethic committee of the Center of Research in Food and Development approved this study. Children infected with intestinal parasites received the proper treatment by the Ministry of Health.

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, Japan) using standardized recommendations. Ages were validated from reliable sources in Mexico (birth certificates and official school records). Weight-for-age, height-for-age, and body mass index for age (BMI/A) Z Scores were calculated using the software Anthro Plus (WHO, 2011). The status of malnutrition risk was defined from -2 to <-1 Z Scores from the median reference values (WHO, 2005) using the nutritional indices of height-for-age (H/A, stunting), weight-for-age (W/A), and BMI/A. The valid Z scores for analysis in this study were those considered the most occurring in our study population and they were as follows: from -5.0 and +5.0 for H/A; -5.0 to +5.0 for W/A; and -4.0 to +4.0 for BMI/A (4 cases for BMI/A, 1 for H/A and 1 for W/A were outside the considered range).

Fecal sample collection and fecal antigen detection by enzyme linked immuno-absorbent assay (ELISA)

Fecal samples were collected and transported to the parasitology laboratory of the Center of Research of Food and Development. Sample was weighted and 1 g homogenized and transferred into cryogenic vials (2 ml) that were 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 µl of a second anti-*C. parvum* solution was added forming a "sandwich" with the *C. parvum* fecal antigen captured by the first antibody. The reaction was visualized by adding a second *C. parvum* antibody bound to a peroxidase conjugate with a chromogen 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 1 M. A yellow color was developed and read using a Model 680 microplate reader from Bio-Rad Laboratories in an absorbance range between 450 and 650 nm. A 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 to 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 (DRG, 2010).

Table 1. Physical characteristics of the *Cryptosporidium*-free and *Cryptosporidum*-infected school children of Northwestern Mexico.

Characteristic	*C. parvum*-free (n)	*C. parvum*-infected (n)	P-value
Age (years)	8.9 [1.4] (187)	10.3 [1.5] (121)	0.0001[c]
Weight (kg)	36.8 (29.9 - 48.4)[a] (177)	29.9 [24.7-39.9][a] (106)	0.001[b]
Height (cm)	133.4 [10.5] (177)	135.9 [10.8] (106)	0.056[c]
W/A (Z-score)	0.2 [1.2] (177)	-1.32 [9.9] (96)	0.044[c]
H/A (Z-score)	-0.22 [1.1] (177)	-1.26 [1.1] (112)	0.001[c]
BMI/A (Z-score)	0.53 [1.4) (178)	0.47 [1,5] (112)	0.729[c]

[a]Median [25 to 75% inter-quantiles]. All other data are presented as mean ± standard deviation [SD]. [b]Kruskal Wallis rank sum test. [c]Two samples independent t-test.

as the percentage of children with *C. parvum* in any of the fecal samples provided. The proportions were compared using the χ^2 test with the corresponding odds ratios, 95% confidence intervals and *P* values (prevalence of infection). The association between the nutritional status and criptosporidiosis was analyzed using multiple logistic regression models. In all models, the dependent variable was the indicator of mild or risk of malnutrition from -2 to <-1 Z scores for H/A, W/A and BMI/A as defined (WHO, 1996); and the *C. parvum* infection (0 to denote absence and 1 presence) considered as the hypothesized independent variable. These 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 were analyzed with the statistical software STATA/SE 12.0 (STATA 1996-2013) with a significance level of P≤0.05.

RESULTS

The Z scores of 6 school children were outside of the considered data analysis interval of this study and the age of 7 school children was not confirmed. Mean age of the analyzed children (n = 308) was 9.5 years (± 1.6). Participation of girls (55%, n = 169) was higher than boys (45%, n = 139) (z = 2.49, P = 0.013). Due to the low prevalence of undernutrition by <-2 Z Scores for H/A, W/A and BMI/A (3.9, 1.6 and 2.3%, respectively), derived data from undernutrition status was not used in the multiple logistic regression models. On the contrary, the prevalence of risk of malnutrition by ZH/A, ZW/A and ZBMI/A was 11, 15 and 17%, respectively. A total of 705 fecal samples were collected from September to December 2008. 20% (n = 62) and 54% (n = 167) of the children provided 2 and 3 fecal samples, respectively. The overall prevalence of *C. parvum* was 39.3% (n = 121) in this study. No difference was found in the height and ZBMI/A between the *C. parvum*-free and *C. parvum*-infected children. Although the *C. parvum*-infected children were older than the *C. parvum* free children, the latter group showed ZW/A and ZH/A higher than the *C. parvum* infected children (Table 1).

No difference was found in the prevalence of cryptosporidiosis by gender (43% vs. 37%; z = -1.48, P = 0.139). Univariate models (unadjusted) were constructed analyzing the risk of malnutrition with types of community, gender and age and no association was found, because community, gender and age can also influence the nutritional status (data not shown). Later, stepwise analysis was developed to test the association between the nutritional status and infection (Table 2). In this study, the children with cryptosporidiosis were 2.3 times more likely to be at risk of malnutrition by ZW/A and 2.0 times more likely to be at risk of malnutrition by ZBMI/A than the *C. parvum*-free children (Table 2). No association was found between cryptosporidiosis and risk of malnutrition by ZH/A using a similar model (OR = 1.1, P = 0.728).

DISCUSSION

This study investigated the prevalence of *C. parvum* and its association with nutritional status in 308 children (September to December 2008) of 3 public elementary schools belonging to the municipality of Hermosillo in Northwestern Mexico. In this study, the prevalence of undernutrition by ZH/A was lower (3.9%) than that (15.5%) published by the Mexican survey in 2006, although both of them showed a similar prevalence of undernutrition by W/A (1.6% vs. 2%). However, our study population is not representative of the children population of Northern Mexico. On the other hand, it was found that *C. parvum*-infected children showed lower means of ZH/A and ZW/A than the *C. parvum*-free children. This finding is not surprising because in 2006, another cross-sectional study in Haitian children aged 36 months old published that 49 *Cryptosporidium*-infected children showed lower (<-2) ZW/A and ZH/A than 41 *Cryptosporidium*-free children (Kirkpatrick et al., 2006). These authors used stool smears stained with the Ziehl-Nielsen modified acid-fast stain to look for *Cryptoporidium* species. In addition, an unexpected high prevalence of cryptosporidiosis was found in this study. This prevalence was higher than that published by only a study at local level by Gómez et al. (1996) who estimated a prevalence of cryptosporidiosis of 23.2% in 100 children using monoclonal antibodies against *C. parvum* in diarrheal stools. On the other hand, the proportion of children at risk of malnutrition was relatively high in this study. This finding is more relevant when it was found to

Table 2. Unadjusted and adjusted association between the risk of malnutrition and cryptosporidiosis in school children of Northwestern Mexico.

Variable	Univariate analysis Unadjusted OR (±SE)	95%CI	P	Stepwise Adjusted OR (±SE)	95% CI	P
ZW/A (n=271)						
C. parvum-infected	1.7±0.50	1.02-3.1	0.045	2.31±0.76	1.2-4.4	0.011
Age (years)	1.08±0.08	0.74-1.26	0.165	1.28±0.09	1.02-2.32	0.026
ZH/A (n=289)						
C. parvum-infected	1.1±0.32	0.62-1.9	0.728	-	-	-
ZIMC/A (n=290)						
C. parvum-infected	2.03±0.74	1.02-4.17	0.044	2.03±0.74	1,02-4.17	0.049
Age (years)	1.04±0.09	0.85-1.32	0.109	1.46±0.09	0.94-2.34	0.052

OR (SE) = odds ratio (standard error). Significance at P<0.05.

be associated with cryptosporidiosis. Re-infections are serious in regions where children are at risk of malnutrition. Although the results of this study are not representative for the general population of Northwestern Mexico, this revealed that cryptosporidiosis may be a risk factor of malnutrition in school children living in our local conditions. Further studies are recommended to identify the risk factors for a better understanding of the epidemiology of *C. parvum* in the study sites in order to the proper authorities of Northwestern Mexico can establish strategies to prevent this infection.

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