Changes in Serum Zinc Levels Associated with Giardiasis and Dietary Zinc Intake in Mice

**Luis Quihui-Cota · Rosa Olivia Méndez Estrada · Humberto Astiazarán-García ·
Gloria Guadalupe Morales-Figueroa · Mario Jesús Moreno-Reyes ·
Denisse Cuadras-Romo · Rafael Canett-Romero**

Abstract The association of giardiasis with the malabsorption of zinc remains controversial. This study investigated changes in serum zinc levels in *Giardia*-infected mice subjected to different dietary zinc regimens. Thirty-five mice (strain C_3H/H_eJ) were randomly categorized into two groups. The first group was inoculated with 5×10^6 *Giardia* trophozoites ($n=18$), and the second group remained *Giardia* free ($n=17$). Each group (*Giardia* infected and *Giardia* free) was randomly classified into three subgroups and given low (9 mg Zn/kg), normal (33 mg Zn/kg), and high levels (288 mg Zn/kg) of dietary zinc over a 2-week period for acclimation. Fourteen days post-*Giardia* infection, all of the mice were euthanized and blood samples were collected. The number of trophozoites was quantified (hematocytometer), and serum zinc levels were determined via atomic absorption spectrophotometry. Significant increases in the median weights were only found in the *Giardia*-free mice ($p<0.05$). A higher final median weight was found in the *Giardia*-free group when compared with that of the *Giardia*-infected group given low dietary zinc ($p=0.013$). In the *Giardia*-infected group with low dietary zinc, the geometric mean of trophozoites was $3,498\pm101$ (SE) per milliliter. The *Giardia*-infected group had lower serum zinc levels than did the *Giardia*-free group with the high dietary zinc regimens ($p<0.05$). Our results are consistent with studies among human populations, but further studies are required to elucidate the actual mechanism governing the zinc–giardiasis interaction.

Keywords Dietary zinc · Serum zinc · Giardiasis · Mice · Zinc deficiency

L. Quihui-Cota (✉) · G. G. Morales-Figueroa
Department of Public Nutrition and Health, Coordination of Nutrition, Centro de Investigación en Alimentación y Desarrollo, A.C. Carretera al Ejido La Victoria Km 0.6,
Hermosillo, Sonora, Apartado Postal 1735, CP 83304, Mexico
e-mail: lquihui@ciad.mx

R. O. Méndez Estrada · H. Astiazarán-García
Department of Nutrition and Metabolism, Coordination of Nutrition, Centro de Investigación en Alimentación y Desarrollo, A.C. Carretera al Ejido La Victoria Km 0.6,
Hermosillo, Sonora, Apartado Postal 1735, CP 83304, Mexico

M. J. Moreno-Reyes · D. Cuadras-Romo · R. Canett-Romero
Department of Research and Postgraduate Studies on Food, Universidad de Sonora,
Building 5-P, Rosales and Blvd. Luis Encinas,
Hermosillo, Sonora, AP 1658, CP 83000, Mexico

Introduction

Giardiasis is the result of infection with *Giardia duodenalis* and has become a worldwide public health problem. In 2001 [1], there were a billion cases of giardiasis estimated globally, and in 2004 [2], global prevalence of the infection had reached approximately 30%. The current worldwide prevalence of giardiasis among children under 10 years of age may range from 15% to 20% [2]. Zinc deficiency is another rising public health problem; its global prevalence was estimated to be 31% in 2004, while rates ranged from 4% up to 73% in developing countries [3]. Zinc deficiency occurs not only in developing countries but also in more advanced countries due to inadequate eating habits or medical conditions, such as hepatic or renal failure, diabetes mellitus, or human immunodeficiency virus infection [4, 5]. Zinc deficiency has been associated with growth retardation, neurosensory changes, impaired cognitive abilities, compromised immune functions, and even death [6–8]. Although a high frequency of bacterial, viral, and fungal infections occur in zinc-deficient humans [6], studies of the association between parasitic infection and zinc levels in human or animal hosts have not often been attempted. In 1993 [9], giardiasis was first

reported as a risk factor for zinc malabsorption in children. Other authors have also reported this risk [10–12], but the link remains uncertain. One study failed to show the association between giardiasis and zinc malabsorption in children [13], and other studies demonstrated that the association between giardiasis and malabsorption of other nutrients, different to zinc, in mice and children remained unclear [14, 15].

In Mexico, the prevalence of *G. duodenalis* was estimated at 32% in 1994 [15] and is currently the most important protozoan parasite associated with intestinal infection in northwestern Mexico [16]. Three studies in Mexico have shown evidence of zinc deficiency in women and children, and only one of these studies showed low levels of dietary zinc consumption in 19% to 24% of schoolchildren [17–19].

We hypothesized that giardiasis may be a risk factor for zinc deficiency regardless of dietary zinc intake. This study investigated this issue using the mouse as an experimental model.

Materials and Methods

Animals

Mice that were aged 5–7 weeks (18–30 g) were obtained from the Animal Bioterio Center of the University of Sonora in northwest Mexico to be used in this study. The strain of mice used was C_3H/H_eJ, which is known to be a strain that is susceptible to *G. duodenalis* infection [20]. Care and use of the animals were in accordance with the guidelines of the institutional ethical committee [21].

Mice Groups

The mouse cages were sanitized with sodium 10% hypochloride. The temperature in the mouse rooms was maintained between 20°C and 26°C (mean 22±1°C) and humidity between 40% and 70%. There were 10 to 14 air room changes per hour and 12–12-h light/dark cycles. The mice were housed in sanitized stainless steel racks. Biohazard bags (VWR autoclavable bags, 1.5 mil 14220-010, 2005) were used for autoclaving infectious biological materials at the end of the study (urine, fecal material, and carcasses).

Measurements of Weights of the Mice and Their Food

The weights of the prepared diets were measured using a precision (±0.001 kg) electronic balance OHAUS [Model CD-11 Indicator (01010056DB), Florham Park, New Jersey, USA, 1999]. The mice's weights were measured using a precision (±0.01 g) electronic balance OHAUS [(7124331499) Pine Brook, New Jersey, USA, 2003] at baseline and at the time of euthanization.

Preparation of the Diets and Dietary Zinc Content

The standard basal diet (Harlan Teklad Rodent Diet, WI 8604) was comprised of casein (200 g/kg), cornstarch (653 g/kg), corn oil (50 g/kg), vitamin mixture (10 g/kg), mineral mixture (35 g/kg), fiber (50 g/kg), coline (2 g/kg), and zinc. The zinc was added to meet the zinc requirements for the mice groups. The total amount of the study basal diet was estimated using the following calculations: mice number per group × daily food consumption × study days (6 mice×12 g×29 days=2.1 kg per group) and multiplying 6 mice groups×2.1 kg, calculating 12.6 kg as the total amount. Next, this amount was separated into three portions, mixing each of them with varying zinc concentrations by adding 0, 0.03, and 0.25 g of zinc gluconate per kilogram of basal diet to obtain the low, normal, and high dietary zinc contents, respectively. Dietary zinc concentration was validated by atomic absorption spectrophotometry (Varian-Spectra AA-20) the method 968.08 of the AOAC [22] under qualified technical supervision. One milligram of each dietary regimen, mixed with 5 mL of HNO_3 and deionized water, was digested using a microwave (MDS 2000. Falcon, Mexico). A certified sample of bovine liver was used to validate this method. A total of 1.4 mL of HCl and deionized water was added to the digested samples. The concentrations of the final solutions were measured at 213.9 nm using a hollow cathode zinc lamp. A calibration curve build up with 0.5, 1.0 and 2.0 ppm of zinc solutions was used to estimate zinc concentrations. The actual zinc concentrations of the low, normal, and high dietary zinc regimens were 9 (original content of the basal diet), 33, and 288 mg/kg, respectively.

Study Design

Thirty-five mice were randomly categorized into *Giardia*-infected (n=18) and *Giardia*-free (n=18) groups. Next, each group was randomly classified into three subgroups to receive low, normal, and high dietary zinc regimens [2, 22]. All of the mouse groups were acclimated to their corresponding experimental diets daily ad libitum for 2 weeks prior to the start of the study. Fresh ultrapure water purified with Milli-Q system (Millipore Corp., Billerica, MA, USA) was also offered ad libitum daily throughout the study. On day 0, the groups to be infected were inoculated with *G. duodenalis* trophozoites by oral via. All of the mice were maintained on their diets by allowing them to feed ad libitum. On day 14 postinfection, all of the mice were euthanized to obtain the study samples. Blood samples were drawn before the euthanization process. The maximum load of *Giardia* trophozoites has been found between 9 and 11 days after oral infection in the intestinal tissue of normally nourished NMRI mice [23].

Giardia Inoculation and Confirmation of Infection

G. duodenalis trophozoites (Portland strain) were axenically cultured in a TYI-S-33 medium supplemented with 7.0 mL of 10% bovine serum (Bovine Adult FERUM, SIGMA B2771) using a Purifier Class II Biosafety Cabinet (Delta Series, LABCONCO). For experimental inoculation, actively growing trophozoites (48–72-h-old cultures) were harvested by being chilled in ice for 20 min. Trophozoites were washed with PBS at pH 7.2 (GIBCO) by 10-min centrifugation ($800 \times g$) at 4°C and were resuspended in 500 μL of PBS. Dilutions were prepared with PBS (1:15) and 0.4% trypan blue (1:2) (Sigma, Co.) to obtain a suspension of 5×10^6 trophozoites per 200 μL. Before inoculation, a 6- to 9-h fasting period with no water restraint was required to guarantee infection. Using oral administration, the trophozoites were inoculated directly to the duodenum using a syringe fitted with a cannula needle to prevent tissue damage. Separately, two additional inoculated mice consuming low dietary zinc were also sacrificed on day 14 postinfection to confirm qualitatively but not quantitatively the trophozoites in the duodenal tissue.

Euthanization Process

Fourteen days following Giardia inoculation and sustained zinc dietary regimens, all of the mice were anesthetized with chloroform by inhalation. Each mouse was restrained on a styrofoam bed using pins. Abdominal asepsis with alcohol was performed, and sterile surgical equipment was used to dissect the abdominal and thoracic sections of each mouse to obtain the intestinal and blood samples.

Counting Trophozoites

A 5-cm section of the duodenum was removed and longitudinally dissected in the Giardia-free and the Giardia-infected groups. This section was transferred into 5-mL screw cap tubes with 3 mL sterile PBS at 4°C and shaken at 500 rpm for 30 min using a mechanical rotor SOLBAT series 1-86 to release the trophozoites from the tissue. Supernatant was transferred into 1-mL sterile conic tubes and centrifuged at $800 \times g$ at 4°C for 10 min. The supernatant was discarded and 0.5 μL of sediment was transferred to a Neubauer chamber. The total number of trophozoites (sum of trophozoites of the four of 1-mm^2 squares) was multiplied by 10 mm to estimate the number of trophozoites per milliliter.

Zinc Determination in Serum Samples

One-mL syringes (Plastipak) were used to withdraw blood samples by intracardial puncture. After extraction, the whole blood was centrifuged at $800 \times g$ for 10 min and serum (500–600 μL) was separated and transferred into milliliter sterile cryogenic heparinized conic tubes and stored at −20°C until analysis. Hemolyzed samples were rejected [24]. The determination of the serum zinc levels was developed according to D´Haese et al., [25]. Measurements were carried out at 213.9 nm using a hollow cathode zinc lamp with a coefficient of variation of 2.6% and a recovery of 97%. The cutoff point for zinc deficiency in mice was defined as less than 4.65 ± 0.532 μmol/L [26].

Statistical Analysis

Data were analyzed using the NCSS 2000 software [27]. Descriptive statistics were expressed as the median ± confidence interval. Wilcoxon signed rank was used to test for the differences between the weights of the same groups at baseline and 14 days postinfection. Mann–Whitney U test was used to test for the differences in the final weights between two independent groups. Nonparametric ANOVA (Kruskal–Wallis) test was used determine whether there were any differences in the weights and serum zinc levels among the three independent groups subjected to the different dietary regimens at baseline and 14 days postinfection. In the case of rejecting the null hypothesis in the analysis of variance, Dunnett's method for multiple comparisons was performed to establish which of the groups differed significantly.

The association between the serum zinc levels and the infection was analyzed by multiple regression analysis using the serum zinc levels and the Giardia infection as dependent and independent variables, respectively, as adjusted by the administered dietary zinc regimen and weight. All analyses were considered significant at $p \leq 0.05$.

Results

At baseline, no difference was found between the median weights of the mice randomly placed into the Giardia-free groups (21.0 g) and those to be infected with Giardia (22.3 g, $p=0.072$). Comparison of the weights at baseline and 14 days postinfection showed a significant increase in the weights of the three Giardia-free groups, while weights remained unchanged in the Giardia-infected groups (Table 1). In addition, a higher final median weight was found in the Giardia-free mice than in the Giardia-infected mice subjected to low dietary zinc regimens (Table 1); however, no differences were found between the final median weights of the Giardia-free groups and the final median weights of the Giardia-infected groups that were maintained on the normal and high dietary zinc regimens (Table 1). In addition, no differences were found between the initial and final median weights of the three Giardia-free groups. The same was found between the initial and final median weights of the three

Table 1 Comparison of weights between the *Giardia*-free and *Giardia*-infected groups before the administration of the experimental diet (baseline) and 14 days postinfection

Dietary zinc levels	*n* per group	Baseline *Giardia*-free[a] weight (CI)	14 days after *Giardia*-free[a] weight (CI)	*p*[b]	*n* per group	Baseline *Giardia*-infected[a] weight (CI)	14 days after *Giardia*-infected[a] weight (CI)	*p*[b]
Low	5[c]	20.9 (18.8–23.1)[d]	26.9 (21.3–28.5)[e]	0.023	6	23.8 (19.5–26.3)[f]	24.4 (20.4–27.4)[g]	0.198
Normal	6	21.4 (18.8–24.3)[h]	25.5 (23.5–27.3)[i]	0.038	6	21.6 (18.4–25.5)[f]	23.3 (20.4–27.5)[j]	0.229
High	6	19.8 (18.7–21.0)[h]	23.8 (19.6–25.6)[i]	0.044	6	21.8 (19.5–24.4)[f]	23.5 (20.8–26.7)[j]	0.133

CI confidence interval

[a] Median

[b] *p*, Wilcoxon signed rank test, significance at $p<0.05$

[c] Mice death during the study course

[d] No different to h ($p=0.319$, Kruskal–Wallis test)

[e] No different to i ($p=0.254$, Kruskal–Wallis test); no difference among f ($p=0.300$, Kruskal–Wallis test); different to g ($p=0.042$, Mann–Whitney *U* test)

[f] $p=0.300$; Kruskal–Wallis test

[g] No different to j ($p=0.559$, Kruskal–Wallis test)

[h] $p=0.319$, Kruskal–Wallis test

[i] No different to j ($p>0.05$, Mann–Whitney *U* test)

[j] $p>0.05$, Mann–Whitney *U* test

Giardia-infected groups that were maintained on the three different dietary zinc intakes (Table 1).

Fourteen days postinfection, the geometric mean of trophozoites was 3,498±101 (SE) per milliliter in the *Giardia*-infected group maintained on low dietary zinc. No trophozoites were detected in the other two *Giardia*-infected groups that were maintained on the normal and high dietary zinc regimens.

The Mann–Whitney *U* test showed a significantly lower median level of serum zinc in the *Giardia*-infected mice than in the *Giardia*-free mice that were maintained on the high dietary zinc regimen (Table 2). However, no difference was found in the serum zinc levels between the *Giardia*-infected and the *Giardia*-free groups maintained on the low and normal dietary zinc regimens (Table 2). The negative impact of *G. duodenalis* on the serum zinc levels was demonstrated by multiple regression analysis ($\beta=-9.29\pm3.46$, $p=0.02$), which was adjusted by the zinc dietary regimens and weight (Table 3). Since the reference in the regression analysis was the *Giardia*-free group, the negative β coefficient value means that the serum zinc level was 9.29 μmol/L lower in the *Giardia*-infected than in the *Giardia*-free mice (Table 3).

Discussion

Over the course of the study, the mice free of giardiasis had higher median weight gains than did the *Giardia*-infected mice regardless of the dietary zinc regimens (Table 1). It is

well recognized that giardiasis can be asymptomatic or associated with acute and chronic diarrhea with or without malabsorption. *Giardia* trophozoites may cause intestinal lesions leading to nutrient malabsorption, which may explain the reduced weight gain in infected humans [28] and/or animals [29]. However, this link remains poorly understood [30]. Thomson et al. [31] found a significant impairment of the weight gain in the *Giardia muris*-infected Swiss albino mice compared to *Giardia*-free controls from 21 to 8 days of follow-up evaluation. However, Scott et al. [30] found no difference in the mean weights between the *Giardia*-infected and the *Giardia*-free mice after 6 to 35 days postinfection. In this study, we found a higher median weight gain in the *Giardia*-free than in the *Giardia*-infected group with low zinc intake 14 days postinfection (Table 1). It is well recognized that zinc deficiency in animals is characterized by reduction in growth, diverse reproductive effects and adverse developmental effects, all of which persist after weaning, as well as reduced immunoresponsiveness [32]. In this case, the low dietary zinc exacerbates apparently the effects of the giardiasis through poor physical development [33]. In addition, no difference was found in the final median weights between the *Giardia*-infected and *Giardia*-free groups with higher consumptions of zinc (Table 1). It is possible that giardiasis and low zinc intakes are exacerbating the growth retardation and that better dietary zinc intakes replace zinc losses caused by malabsorption more effectively, thereby reducing the growth retardation rate. On the other hand, the trophozoites were only detected in the infected

Table 2 Comparison of serum zinc levels between the *Giardia*-free and *Giardia*-infected mice maintained on the three dietary zinc regimens 14 days postinfection

Dietary zinc levels	n per group	*Giardia*-free (μmol/L)[a], $n=17$	n per group	*Giardia*-infected (μmol/L)[a], $n=18$	p value
Low	5	4.1 (3.9–5.2)	6	4.3 (4.0–5.3)	0.268
Normal	6	5.2 (4.4–7.7)	6	4.9 (4.2–4.9)	0.083
High	6	10.2 (6.1–12.5)	6	6.3 (5.6–8.5)	0.020

p = Mann–Whitney U test, significance at $p<0.05$

CI confidence interval

[a] Median

mice that were maintained on low dietary zinc at 14 days postinfection. Low dietary zinc apparently contributed to the longer duration of the *Giardia* infection, while higher levels of dietary zinc intake may have helped to eradicate giardiasis at that time.

Numerous animal and human studies have indicated that zinc deficiency decreases resistance to infectious diseases. Zinc-deficient animals have suppressed immune responses and are more susceptible to a diverse range of infectious agents [34]. Zinc-deficient mice infected with *Trypansoma musculi* harbored three times more parasites than uninfected mice because of a delay in the production of protective antibodies [35]. Further, 40% of moderately zinc-deficient mice succumbed to a normally nonlethal infection of *Plasmodium yoelii* 17XNL malaria [36] because of delayed protection of immunoglobulin G antibodies. In human studies, Junqueira and Queiroz [37] and Al-Mekhafi et al. [38] reported that severely malnourished individuals had more exacerbated cases of giardiasis and a more impaired immune response than those with moderately deficient nutrition. Shukla and Sidhu [39] published that malnourished *Giardia*-infected mice became *Giardia*-free 19 days later (48 days postinoculation) than those normally nourished *Giardia*-infected mice (29 days postinoculation). Finally, in our study,

a median value of serum zinc levels was higher in the *Giardia*-free mice than in the *Giardia*-infected mice consuming high dietary zinc regimen 14 days postinfection (Table 2). However, no differences were found in the serum zinc levels between the *Giardia*-infected and the *Giardia*-free groups consuming low and normal dietary zinc regimens. The serum zinc levels probably were negatively impacted by both the giardiasis and poor and normal zinc intakes in the *Giardia*-infected and the *Giardia*-free groups, respectively (Table 2). Alternatively, a high dietary zinc consumption is not only probably reducing the growth retardation but is also contributing to the better serum zinc levels in the *Giardia*-free group in comparison to the *Giardia*-infected group (Tables 2 and 3). How zinc metabolism is compromised by *G. duodenalis* is not well known, but it is likely that zinc deficiency, as assessed by serum analysis, is not a sensible method for measuring the redistribution of zinc from serum to other tissues [40], the reduction of zinc transporter proteins, nor the rise in the production of metallothionein during the course of giardiasis infection [41].

Although the association between malabsorption and giardiasis is well documented, little is known about the giardiasis–zinc interaction both in humans and animals. A recent follow-up study in schoolchildren [42] found significantly higher serum zinc values in *Giardia*-infected children than in *Giardia*-free children 6 months after treatment, even adjusting for dietary zinc intake. Other studies from Turkey [9, 12] showed that *Giardia*-infected groups of children ranging from 2 to 14 years old had lower mean serum zinc levels than the *Giardia*-free control group (10.3 vs. 22.2 μmol/L and 16.7 vs. 20.8 μmol/L, respectively). Another study from Turkey [4] published similar findings in 20 *Giardia*-infected children from ages 3 months to 14 years. In contrast, a Spanish study [13] found no change in the mean serum zinc levels before (14.1 μmol/L) and 3 months after treatment (14.1 μmol/L) in 25 *Giardia*-infected children, ages 6 to 9 years old. Unfortunately, these studies did not include dietary zinc records. In reference to this current study, the following limitations can be identified: (a) the low number of mice

Table 3 Adjusted association between serum zinc levels and giardiasis in 35 mice maintained on low, normal, and high dietary zinc regimens

Variable	$\beta \pm$ SE	p value
Group		
Giardia free	−9.285±3.46	0.0119
Giardia infected		
Normal Zn diet		
Low Zn diet	−2.975±4.204	0.4846
High Zn diet	24.928±4.761	0.001
Weight	−0.287±0.785	0.5797
Intercept	44.642±20.888	0.0409

Multiple regression model adjusting for dietary regimens and weight

analyzed, (b) no dietary zinc intakes recorded per mouse, and (c) the failure to include an infected group at baseline to estimate the serum zinc levels changes before and after infection, which would have ensured an important step in the interpretation of the mechanism of giardiasis–zinc interaction. However, results from this study show that giardiasis may be a risk factor for zinc deficiency in mice regardless of the dietary intakes. Further studies should be developed to elucidate the possible alterations in zinc metabolism observed over the course of giardiasis in mice. Such a study would help to redesign parasitic control strategies, micronutrient supplementation, and food fortification programs in order to improve the quality of life of human populations vulnerable to parasitic infections.

References

1. Upcroft P, Upcroft JA (2001) Drug targets and mechanisms of resistance in the anaerobic protozoa. Clin Microbiol Rev 14:150–164

2. Mukherjee S, Pennardt A, Sheridan BJ, Hökelek M, Fennelly G, Johnston MH (2011) Giardiasis. In:Medscape Reference. http://emedicine.medscape.com/article/176718-overview. Accessed 15 May 2011

3. Caulfield LE, Black RE (2004) Zinc deficiency. In: Ezzati M, Lopez AD, Rodgers A, Murray CJL (eds) Comparative quantification of health risks. Global and regional burden of disease attributable to selected major risk factors. WHO, Geneva, p 257–279

4. Karakas Z, Demirel N, Tarakcioglu M, Mete N (2001) Serum zinc and copper levels in southeastern Turkish children with giardiasis or amebiasis. Biol Trace Elem Res 84:11–18

5. King JC, Hambidge KM, Westcott JL, Kern DL, Marshall G (1994) Daily variation in plasma zinc concentrations in women fed meals at six-hour intervals. J Nutr 124:508–516

6. Nash L, Iwata T, Fernandes G, Good RA, Incefy GS (1979) Effect of zinc deficiency on autologous rosette-forming cells. Cell Immunol 48:238–243

7. Rosado JL (1998) Deficiencia de zinc y sus implicaciones funcionales. Salud Publica Mex 40:181–188

8. Scott ME, Koski KG (2000) Zinc deficiency impairs immune responses against parasitic nematode infections at intestinal and systemic sites. J Nutr 130:14125–14205

9. Jendryczko A, Sodowska H, Drózdz M (1993) Zinc deficiency in children infected with *Giardia lamblia*. Wiad Lek 46:32–35

10. Abou-Shady O, El Raziky MS, Zaki MM, Mohamed RK (2011) Impact of *Giardia lamblia* on growth, serum levels of zinc, copper, and iron in Egyptian children. Biol Trace Elem Res 140:1–6

11. Demirci M, Delibas N, Altuntas I, Oktem F, Yönden Z (2003) Serum iron, zinc and copper levels and lipid peroxidation in children with chronic giardiasis. J Health Popul Nutr 21:72–75

12. Ertan P, Yereli K, Kurt O, Balcioğlu IC, Onağ A (2002) Serological levels of zinc, copper and iron elements among *Giardia lamblia* infected children in Turkey. Pediatr Int 44:286–288

13. Olivares JL, Fernández R, Fleta J, Rodríguez G, Clavel A (2003) Serum mineral levels in children with intestinal parasitic infection. Dig Dis 21:258–261

14. Cheeramakara C, Nontprasert A, Siripanth C, Tanomsak W, Chularerk U, Sucharit P, Areekul S (2004) The hematological status, plasma vitamin B12 and folic acid levels, and intestinal pathology in rats infected with *Giardia lamblia*. Southeast Asian J Trop Med Public Health 35:811–816

15. Tay J, Ruíz A, Schenone Fernández H, Robert L, Sánchez Vega JT, Uribarren T, Becerril MA, Romero-Cabello R (1994) Frequency of intestinal protozoosis in the Mexican Republic. Bol Chil Parasitol 49:9–15

16. INEGI (2010) Anuario Estadistico. Sistema Estatal de Salud, Hermosillo

17. Hunt IF, Murphy NJ, Martner-Hewes PM, Faraji B, Swendseid ME, Reynolds RD, Sanchez A, Mejia A (1987) Zinc, vitamin B-6, and other nutrients in pregnant women attending prenatal clinics in Mexico. Am J Clin Nutr 46:563–569

18. Rivera J, Shamah Levy T, Villalpando Hernández S, González de Cossio T, Hernández Prado B, Sepulveda J (1999) Encuesta Nacional de Nutrición. Estado nutricio de Niños y Mujeres en México. Instituto Nacional de Salud Pública, Cuernavaca

19. Rosado JL, Bourges H, Saint-Martin B (1995) Vitamin and mineral deficiency in Mexico. A critical review of the state of the art. I. Mineral deficiency. Salud Publica Mex 37:130–139

20. Ojeda DC, Granados López AJ (2003) Evaluación de la respuesta inmune humoral sistémica durante la infección por *Giardia lamblia* en un modelo murino. In Departamento de Ciencias Quimico Biológicas Universidad de Sonora Hermosillo, Sonora, México

21. Subcommittee on Laboratory Animal Nutrition Committee on Animal Nutrition, Board on Agriculture, National Research Council (1995) Nutrient requirements of laboratory animals. National Academy Press, Washington, D. C.

22. AOAC (1995) Official methods of analysis of the Association of Official Analytical Chemists. In: Cunniff P (ed) AOAC International. Arlington, VA

23. Khanna R, Joshi K, Kum K, Malik AK, Vinayak VK (1990) An ultrastructural analysis of changes in surface architecture of intestinal mucosa following *Giardia lamblia* infection in mice. Gastroenterol Jpn 25:649–658

24. Herrero Huerta E, Vigil Rodriguez A (2003) Metodología recomendada para la medición del contenido de zinc en especímenes biológicos. Sociedad Española de Bioquímica Clínica y Patología Molecular 22:13–18

25. D'Haese PC, Lamberts LV, Vanheule AO, De Broe ME (1992) Direct determination of zinc in serum by Zeeman atomic absorption spectrometry with a graphite furnace. Clin Chem 38:2439–2443

26. Verbanac D, Milin C, Domitrović R, Giacometti J, Pantović R, Ciganj Z (1997) Determination of standard zinc values in the intact tissues of mice by ICP spectrometry. Biol Trace Elem Res 57:91–96

27. Hintze J (2001) NCSS and PASS. NumberCruncher Statistical Systems. Kaysville

28. Celiksöz A, Aciöz M, Değerli S, Cinar Z, Elaldi N, Erandaç M (2005) Effects of giardiasis on school success, weight and height indices of primary school children in Turkey. Pediatr Int 47:567–571

29. Aloisio F, Filippini G, Antenucci P, Lepri E, Pezzotti G, Cacciò SM, Pozio E (2006) Severe weight loss in lambs infected with *Giardia duodenalis* assemblage B. Vet Parasitol 142:154–158

30. Scott KG, Logan MR, Klammer GM, Teoh DA, Buret AG (2000) Jejunal brush border microvillous alterations in *Giardia muris-*

infected mice: role of T lymphocytes and interleukin-6. Infect Immun 68:3412–3418

31. Roberts-Thomson IC, Stevens DP, Mahmoud AA, Warren KS (1976) Giardiasis in the mouse: an animal model. Gastroenterology 71:57–61

32. WHO (2001) Zinc: environmental health criteria 221. WHO, Geneva

33. Hamaguchi K, Ike K, Yamazaki Y, Morita T, Imai S (2011) Influence of zinc deficiency to the mice infected with *Babesia microti*. J Vet Med Sci 73:263–267

34. Shankar AH, Prasad AS (1998) Zinc and immune function: the biological basis of altered resistance to infection. Am J Clin Nutr 68:447S–463S

35. Lee CM, Humphrey PA, Aboko-Cole GF (1983) Interaction of nutrition and infection: effect of zinc deficiency on resistance to *Trypanosoma musculi*. Int J Biochem 15:841–847

36. Shankar AH, Kumar N, Scott AL (1995) Zinc-deficiency exacerbates experimental malaria infection in mice. FASEB J 9:A4269, abs

37. Muniz-Junqueira MI, Queiroz EF (2002) Relationship between protein-energy malnutrition, vitamin A, and parasitoses in living in Brasília. Rev Soc Bras Med Trop 35:133–141

38. Al-Mekhlafi MS, Azlin M, Nor Aini U, Shaik A, Sa'iah A, Fatmah MS, Ismail MG, Ahmad Firdaus MS, Aisah MY, Rozlida AR, Norhayati M (2005) Giardiasis as a predictor of childhood malnutrition in Orang Asli children in Malaysia. Trans R Soc Trop Med Hyg 99:686–691

39. Shukla G, Sidhu RK (2011) *Lactobacillus casei* as a probiotic in malnourished *Giardia lamblia*-infected mice: a biochemical and histopathological study. Can J Microbiol 57:127–135

40. Quihui L, Morales GG, Méndez RO, Leyva JG, Esparza J, Valencia ME (2010) Could giardiasis be a risk factor for low zinc status in schoolchildren from northwestern Mexico? A cross-sectional study with longitudinal follow-up. BMC Public Health 10:85

41. Cousins RJ, Leinart AS (1988) Tissue-specific regulation of zinc metabolism and metallothionein genes by interleukin 1. FASEB J 2:2884–2890

42. Gabay C, Kushner I (1999) Acute-phase proteins and other systemic responses to inflammation. N Engl J Med 340:1376