

**TITLE OF ARTICLE**

**Nutritional hematic parameters and presence of parasitic infection in children from an urban environment in La Plata, Argentina.**

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**Abstract**

The objective was to evaluate the levels of hematic parameters of nutrition and frequency of parasitic infections in children from La Plata, province of Buenos Aires, in the central region of Argentina. Hemoglobin, calcium and magnesium levels and presence of antitoxocara antibodies and intestinal parasites were determined. Parents were informed about the study and they were requested to give their informed consent and to be present at the moment of blood extraction. Protocols developed were approved by the Universidad Nacional de La Plata. Anemia occurred in 19.2% of 183 children. In the group 3- to 5-year-old children it was more frequent that among the 6- to 12-year-olds ( $p= 0.0031$ ). Low

calcium and magnesium levels occurred in 71.6% and 9.9% of the subjects, respectively. 15.9% of the children were seropositive for toxocariasis and 74.4% registered intestinal parasitosis. Children 6 to 12 years old were more infected than younger children, 80.0% and 64.7% ( $p= 0.0243$ ). Some authors relate parasitic infections with nutritional disorders in infected children. However, other authors report controversy over this connection. In our study, no relationship between parasite-infected children and presence of anemia and/or reduced levels of calcium in blood was observed, which strengthened the dietary origin of the nutritional situation of schoolchildren.

**Keywords:** nutrition, parasitosis, schoolchildren, Argentina.

### **Introduction**

In the last three decades, many developing countries have experienced a significant decrease in childhood mortality. However, issues such as malnutrition, anemia and parasitic infection, affecting the children's physical and intellectual development still persist (Culha & Sagun, 2007).

Nutrition is a basic, universal right, and no effort has been enough in any country to secure adequate access to the whole world population. Disturbances in nutrition and micronutrient deficiency are public health issues in developing countries due to the negative impact they have on health, education, economy, since they mainly affect children. Generally, the lowest social strata have little access to healthcare services; undergo unsanitary conditions and a high morbidity rate, which compromises the correct biological exploitation of food and affecting physical growth, nutritional status and body build (Bolzan et al., 2005).

Malnutrition impacts areas of the brain involved in cognitive functions, memory and locomotor skills. This situation implies increased mortality risk, physical and intellectual deficit, fewer school years and decreased-income adults, perpetuating the problem generation to generation (Prendergast & Humphrey, 2014). Furthermore, there is an increased prevalence of chronic infections slowly damaging health in these populations.

The Program for the Control of Intestinal Parasitoses and Nutrition (PROCOPIN) is a program conducted by the Universidad Nacional de La Plata professionals and students. The purpose of this program is to improve health conditions of schoolchildren in small vulnerable communities in the province of Buenos Aires, Argentina, South America.

The program is developed in four stages: 1. Evaluation of the nutritional and parasitic condition of children; 2. Therapeutic intervention in children with nutritional disorders and/or parasites; 3. Educational intervention to avoid their return to the diseased state; 4. Post-intervention control.

In this article we present data obtained in the first stage of the program regarding the levels of hematic parameters of nutrition and frequency of parasitic infections in children from an urban environment in La Plata, Argentina.

### **Methodology**

The study was conducted among 3 to 12 year-old children from a peripheral neighborhood in La Plata, capital of the province of Buenos Aires, Argentina (-34°55'16" S -57°57'16" W) during 2014. Most of these children live in precarious houses where they are overcrowded, lack drinking water supply and sewer systems, as referred by school authorities and professionals at the Healthcare Unit in the area. The study started with

conferences held at the schools where the children's parents and guardians were interviewed in person and demographic, socio-cultural and environmental data were recorded through a structured and closed survey. In order to evaluate hematic parameters in nutrition, hemoglobin, calcium and magnesium concentrations were determined. So as to determine parasitic infection, the presence of serum anti-Toxocara antibodies was evaluated and a parasitological study to detect intestinal parasites was implemented.

For the hematologic study, following an explanation of the procedure to parents/guardians and children and after obtaining informed consent, 5 mL of blood from a peripheral vein were extracted. The sample was portioned in two tubes, one with EDTAK<sub>3</sub> (ethylenediaminetetraacetic acid, tripotassium salt) and the other with no anticoagulant. The whole blood samples were preserved at 4°C and analyzed within the same day. The serum was separated within 2 hours of sample being collected and preserved at -20°C until processed.

Hemoglobin (Hb) concentration was determined by cyanmethemoglobin method (Hemocian B, Laboratorio Brizuela, Argentina). Serum calcium (Ca) and magnesium (Mg) concentrations were estimated by Atomic Absorption Spectroscopy (GBC 902 model), with oxidizing-type air/acetylene flame, at 324.7 nm, by using the technique described by Piper and Higgins (1967). Serological determination of anti-Toxocara antibodies was performed by applying the Toxocara Microwell Serum ELISA kit (IVD Research, Inc., Carlsbad, USA).

The limit value to define anemia was 11.5 g/dL, as established by the Sociedad Argentina de Pediatría -Argentine Pediatrics Society- (Donato et al, 2009). Anemia severity was evaluated according to World Health Organization differential values. [http://www.who.int/vmnis/indicators/haemoglobin\\_es.pdf](http://www.who.int/vmnis/indicators/haemoglobin_es.pdf).

Eosinophilia was considered to exist when values exceeded 4% <http://www.nlm.nih.gov/medlineplus/spanish/ency/article/003657.htm>. Reference values for minerals in the blood were: Ca (8.5-11.5 mg/dL) y Mg (1.6-3 mg/dL) (Henry JB, 2005).

For the parasitologic study, a serial copro-parasitological analysis and serial anal scraping were carried out. Instructions for sample-taking were imparted orally and in writing to parents/guardians. For the serial copro-parasitological analysis, a daily collection for 5 days of a portion of stools in a container with preservative was indicated. For the anal scraping, each parent/guardian had to dab a folded piece of gauze previously soaked in water around the margins of the child's anus every morning after waking up, for 5 days, and put the gauze pieces in a second container with preservative. Stools were processed by the modified Telemann technique (Pezzani et al, 2009) and the obtained pellets were observed under optical microscope (three smears per tube). The serial anal scraping samples were processed by cutting and homogenizing the gauze pieces with the same preservative in the container. After transferring the whole contents to a centrifuge tube, it was concentrated by centrifugation at 1000 xg for 5 minutes. Finally, three smears per tube were observed under the optical microscope.

Ethical Aspects: parents/guardians were informed orally and in detail about the study in group meetings held at the school. They were requested to give their consent in writing and to be present at the moment of blood extraction. The children whose parents/guardians gave consent were informed about the study and the blood-extraction procedure using age-adjusted vocabulary, and their consent to participate was requested. Protocols developed were approved by the Universidad Nacional de La Plata, School of Medical Sciences Ethics Committee. Personal information remained confidential and was obtained in accordance

with the Declaration of Helsinki (1964), the Nuremberg Code (1947) and National Act #25,326. Approval of school and municipal authorities in the district was also obtained. For the statistical analysis, anemia frequencies were estimated, total and specific parasitoses and variables were registered. The possible associations were analyzed using the Chi-Square Test and Fisher's Exact Test. In the associations that turned out to be significant ( $p \leq 0.05$ ), the odds ratio (OR) and 95% confidence interval (CI) were estimated. Statistical analysis was performed through InfoStat software, 2014 version. URL <http://www.infostat.com.ar>

## Results

The study was performed on 239 children and 183 of them (76.6%) successfully completed all analysis. Of these, 60% were males. Age and sex group distributions are shown in Figure 1.

Mean values and deviations from the evaluated hematology parameters were: Hb 12.2 g/dL (0.82), Ca 8.2 mg/dL (1.25) and Mg 1.9 mg/dL (0.30). Anemia occurred in 19.2% of 183 children. Only two kids showed moderate anemia; the remaining children had mild anemia. In the group of 3- to 5-year-old children it was more frequent (30.9%) than among the 6- to 12-year-old (12.2%), with significant differences being found between both proportions ( $p = 0.0031$ ). Females under 6 years old showed a frequency of 36.4% (8/22) compared to older girls (11.8%, 6/51) ( $p = 0.0006$ ), a difference also seen among males 28.3% (13/46) versus 12.5% (8/64) ( $p = 0.0497$ ).

Low calcium levels occurred in 71.6% of the children, with similar distribution in both males and females and with no age-group differences. On the other hand, low magnesium levels were detected only in 9.9% of the subjects. No significant differences were found regarding sex and age.

Antitoxocara antibodies were detected in 15.9% of children. Females exceeded males, with 17.8% versus 14.7%, respectively, but it did not turn out to be significant. That was also the case among age groups.

Frequency for intestinal parasitosis was 74.4%; 75.5% among males and 72.6% among females. Schoolchildren of 6 to 12 years old were more infected than younger children, 80.0% and 64.7% respectively ( $p = 0.0243$ ). Specific prevalences are shown in Table 1, which prove the predominant presence of *Enterobius vermicularis*, *Blastocystis hominis*, and *Giardia intestinalis*. Figures 2 and 3 show the specific prevalences by sex and age. Intestinal parasite infection was not related to anemia, hypocalcemia and/or hypomagnesemia.

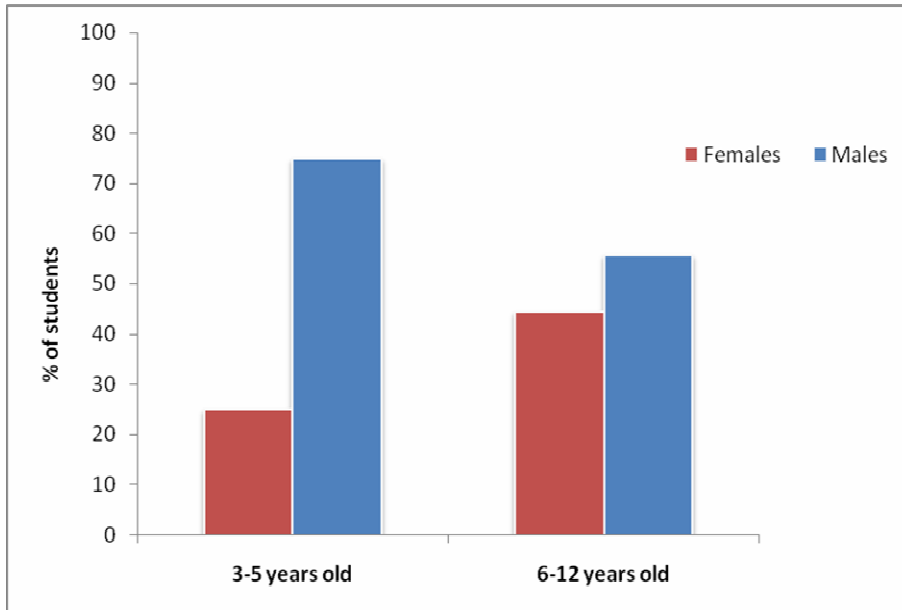


Figure 1: Schoolchildren distribution by age and sex

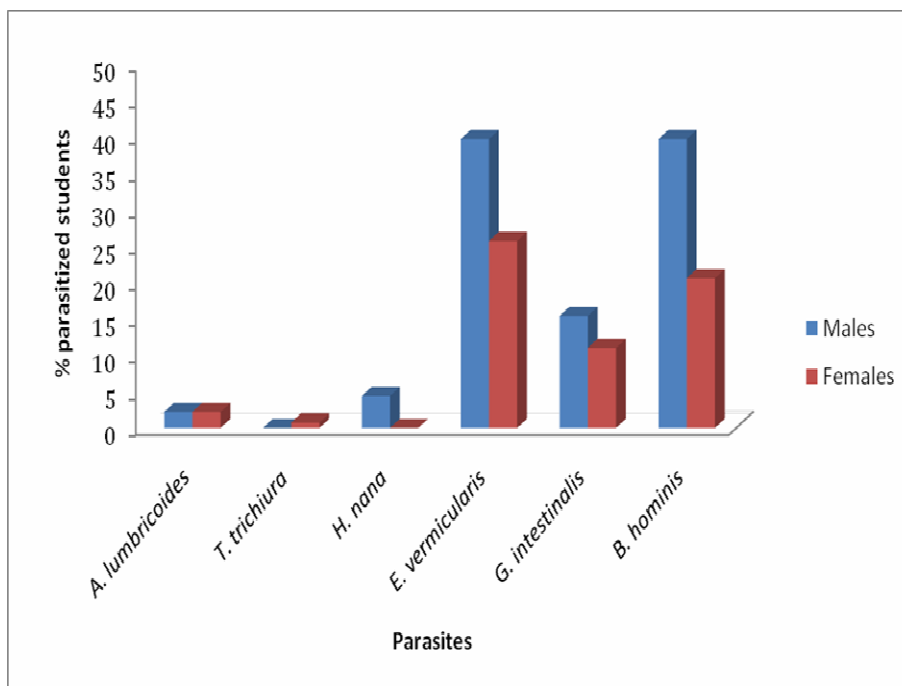


Figure 2: Proportion of parasite species present in schoolchildren by sex.

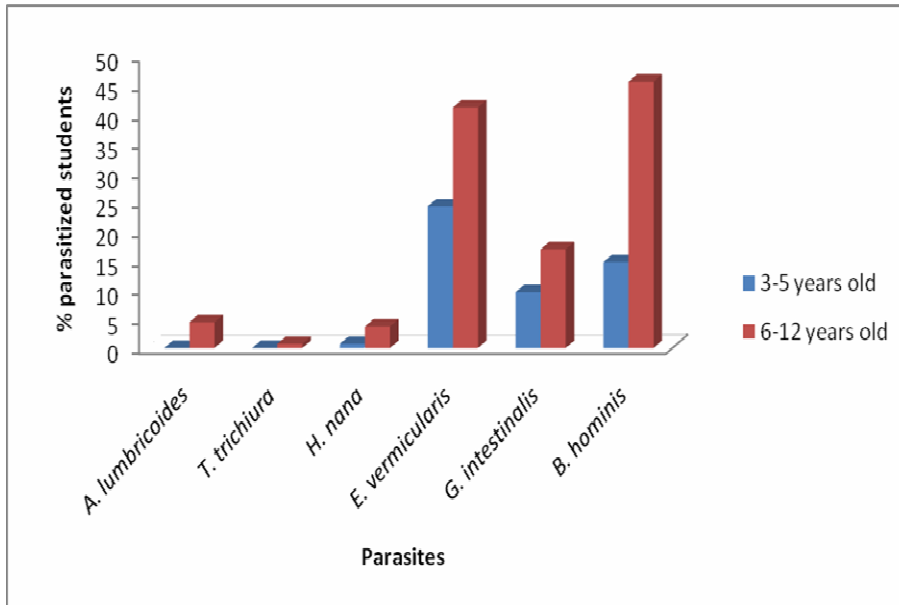


Figure 3: Proportion of intestinal parasite species present in schoolchildren by age group.

**Discussion**

Among the working team’s previous reports, Orden et al. (2014) studied the relationship between the nutritional state and intestinal parasitosis in children from suburban and rural environments in two districts in Argentina. The results suggested an association between intestinal parasitosis and growth disorders only among suburban children. In this study, the association between hematic parameters of nutrition and the presence of intestinal parasites and toxocarasis in urban schoolchildren from a low social stratum in Argentina was evaluated.

In our country, data provided by the National Nutrition and Health Survey (Duran et al., 2009) show a prevalence of anemia of 8.9% in 2 to 5 year-old children. The percentage found in this study (30.9%) in 3 to 5 year-old children exceeds the national average for the age group studied, and approaches the one reported in Rosario, Argentina -24 to 42 month-old children- (Christensen et al., 2013), Peru -children under 5 years of age- (Sobrinho et al., 2014), and Palestine -4 to 5-year-old- (Sirdah et al., 2014). The global prevalence of anemia (19.2%) in our study resembles the one found in 945 Brazilian children over 2 years old that showed a frequency of 21.8% (Albuquerque et al., 2014).

The anemic children of this study attended school, since their anemia was mild, corresponding to a chronic-type anemia and showing no relation to the condition of “being parasited”, which would result from an inadequate diet. To Winocur et al. (2004) and Jukes M. (2005), this kind of anemia produces intelligence-quotient and physical development disorders that will affect the person’s whole life. In a child’s rapid-growth period, occurring in the first four years of life, there is a greater risk of iron deficiency-related anemia since children probably do not consume enough iron-containing food in those years, and that condition supports the difference found in our study of a higher prevalence of anemia in the 3 to 5 year-old group.

The calcium deficit detected adds to the above. In Argentina, many school-age children choose to consume sodas or juice instead of milk, thus adversely affecting the levels of

many nutrients, including calcium. Thus, Vartanian et al. (2007) found associations of the consumption of sodas with increased body weight and less milk drinking, causing deficit of calcium and other nutrients. Calcium plays an essential role in the comprehensive control of membrane permeability, and in the cellular response to stimulation, as well as having an important role in skeletal mineralization, among other functions. In case there is a calcium deficiency, bone growth suffers the most likely impact in two ways: 1) growth may continue but with inadequate mineralization; or 2) growth advances slowly, so that mineral deposition is normal (Gomez Alonso et al., 2004). These children's diets were not registered, but a survey conducted by the Argentines Center of Studies on Child Nutrition found that 85% and 62% of children between 1 and 4 years old showed a deficit ingestion or poor iron and calcium density, respectively <http://www.cesni.org.ar/sistema/archivos/183-comocomenlibro.pdf>. These deficits are very common in low-income populations and occur under sub clinical forms known as "hidden undernourishment".

A high frequency of intestinal parasitosis, close to 74% of children and reaching 80% in the 6 to 12 year-old group, adds to the above situation. The literature published in recent years shows that the prevalence of intestinal parasitosis in the province of Buenos Aires ranges between 23 and 86% (Gamboa et al., 2011; Molina et al., 2011, Pezzani et al., 2012). The most frequently detected parasites were *B. hominis*, *E. vermicularis*, and *G. intestinalis*, matching the results of other authors in Argentina (Bracciaforte et al., 2010; Soriano et al., 2005; Zonta et al., 2007).

Prevalence parasitoses of these children are by direct or indirect transmission, high contagion risk through cysts or already infecting eggs in just produced feces or in perianal margins, and the school environment plays an important epidemiologic multiplying role, as shown in the increased prevalence in the group with longer attendance time.

This high parasite frequency, together with the presence of infection by *Toxocara* spp., may be related to the deficient hygiene conditions of the environment and homes of these urban children, as demonstrated in other studies from our group (Basualdo et al., 2007; Chiodo et al., 2010). Some authors relate parasitic infections with nutritional disorders in infected children (Carvalho-Costa et al., 2007; Wilson et al., 1999). However, other authors report controversy over this connection (Alvarado & Vasquez, 2006; Campbell et al., 2004). Thus, it is unclear whether parasites may influence the nutritional status of the child (Hesham et al., 2004). In our study, no relationship between parasite-infected children and presence of anemia and/or reduced levels of Ca in blood was observed, which strengthened the dietary origin of the nutritional situation of schoolchildren.

En this community, parasitic infections as well as nutritional deficiencies coexist and may influence each other. Poverty, ignorance, low purchasing power and poor standard of living, and their consequences, anemia, hypocalcemia and increased prevalence of parasitic infections, produce together a vicious cycle in these children. Consequences will be severe, since they will impact them in their childhood causing damage to their psychological, intellectual and physical development.

Socio-economic and political alterations improving sanitary and nutritional conditions may break the cycle the same way specific interventions in nutrition, health and related areas do. The strategies to fight nutrient deficiencies should include: accessibility and education to promote diet diversity, preventive and therapeutic supplementation, and food fortification.

The PROCOPIN program develops secondary prevention [www.who.int/whr/2008/08\\_report\\_es.pdf](http://www.who.int/whr/2008/08_report_es.pdf) 154 pp., focusing in diagnosing incipient (no clinical manifestations) disease. It implies the search of disease in "apparently healthy"

schoolchildren as early as possible. It comprises measures in deprived communities with parasite and nutrition-disorders hyperendemic focal points. Secondary prevention, early diagnostics, timely training, and the right treatment are essential to control the disease. This is particularly relevant regarding chronic diseases such as intestinal parasitoses and nutritional disorders.

Table 1. Parasite species detected in 183 Argentinean schoolchildren

Species	N	(%)
<i>Enterobius vermicularis</i>	89	48.6
<i>Blastocystis hominis</i>	82	44.8
<i>Giardia intestinalis</i>	36	19.7
<i>Hymenolepis nana</i>	6	3.3
<i>Ascaris lumbricoides</i>	6	3.3
<i>Trichuris trichiura</i>	1	0.6

### Conclusions

In our study, no relationship between parasite-infected children and presence of anemia and/or reduced levels of calcium in blood was observed, which strengthened the dietary origin of the nutritional situation of schoolchildren.

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