The relationship between haematological measurements and hookworm intensity in children of two rural Bolivian communities

Correlazioni tra rilievi ematologici e intensità dell'infezione da anchilostomi in bambini di due comunità rurali boliviane

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SUMMARY **Objectives** The iron status of 2- to 9-year-old children living in two rural Bolivian communities (Mora and Zanja Honda) was evaluated and analyzed to determine the relationship with hookworm infection.
Methods Iron status was assessed by measuring haemoglobin (Hb), serum ferritin (SF), serum iron (SI), and total transferrin levels (TS). A coproparasitological examination was also performed.
Results Haemoglobin, serum ferritin, serum iron, and transferrin saturation mean levels were within the range of normal values. The total prevalence of anaemia was 23.4%. Of the 184 subjects examined in the two communities, 66 (35.9%) harboured hookworm. Faecal cultures of 43 positive specimens produced 754 larvae of *Necator*. The distribution of eggs per gram of faeces (epg) was mainly between 0 and 2000 epg, and only few subjects presented heavier parasite loads. In Mora there was a significant negative correlation between both serum ferritin and transferrin saturation levels and hookworm intensity. In both communities negative correlations, although not statistically significant, were also observed between the other haematological measurements and hookworm intensity.
Conclusions Hookworm infection seems to be an important contributory cause of iron-deficiency anaemia in these two communities.

KEY WORDS: hookworm, iron deficiency, anaemia, Bolivia

RIASSUNTO ***Obiettivi*** *Valutare il bilancio ematico del ferro in bambini di età compresa tra 2 e 9 anni di due comunità rurali boliviane e metterlo in relazione all'infezione da anchilostomi.*
Metodi *Sono stato determinati i livelli ematici di emoglobina (Hb), ferritina (SF), sideremia (SI) e transferrina totale (TS). Inoltre è stato eseguito un esame coproparassitologico.*
Risultati *I livelli medi di emoglobino, ferritina, sideremia e transferrina sono risultati nella norma. La prevalenza totale di anemia è risultata pari al 23,4%. Dei 184 soggetti esaminati nelle 2 comunità 66 (35,9%) sono risultati infestati da anchilostomi. Le colture fecali di 43 campioni positivi hanno prodotto 754 larve di* Necator. *La distribuzione di uova per grammo di feci (epg) era nello maggior parte dei casi compresa tra 0 e 2000 epg, e solo pochi soggetti presentavano cariche parassitarie più elevate. A Mora è stata riscontrata uno significativa correlazione negativa tra livelli di ferritina e saturazione transferrinica e intensità dell'infezione da anchilostomi. In entrambe le comunità sono state osservate correlazioni negative, anche se non statisticamente significative, tra gli altri parametri ematologici e l'intensità dell'infezione da anchilostomi.*
Conclusioni *L'anchilostomiasi sembra essere un'importante concausa nel determinismo dell'anemia sideropenica in queste due comunità.*

PAROLE CHIAVE: anchilostomiasi, carenza marziale, anemia, Bolivia

Introduction

Anaemia is defined as the reduction in haemoglobin concentration below levels considered normal for age, sex, and pregnancy status. A storage iron depletion occurs before blood haemoglobin (Hb) concentrations fall below the normal values. A deteriorating iron status can be monitored by the measurement of serum ferritin (SF), transferrin saturation (TS), and free erythrocyte protoporphyrin (FEP)(1). Low SF levels not accompanied by any other abnormal parameter of iron status indicate storage iron depletion before the onset of latent or manifest iron deficiency. Decreased ferritin and TS with increased FEP detect latent iron deficiency.
Abnormal iron deficiency indices combined with a significant fall in circulating haemoglobin levels identify iron deficiency anaemia.
Hookworm infection is one of the most important causes of iron loss in the tropics due to blood loss occurring during the parasite feeding activity on the gastrointestinal mucosa (2). Progress to iron deficiency is dependent on several factors including the species of hookworm involved, the intensity and duration of infection, the dietary intake of biosavailable iron, and the state of iron stores in the body (3).
A survey carried out in three communities of the Cordillera Province, Santa Cruz Department, South-East Bolivia showed a hookworm infection prevalence of 28.6% (4).
In the present study the iron status of children living in two rural communities of the same area was evaluated and analyzed to determine the relationship with hookworm infection.
The study was conducted with the agreement of the Bolivian National Department of Epidemiology of the Ministry of Social Welfare and Public Health, during an evaluation of the deworming campaign (5).

Patients, materials and methods

Study design and population

Fieldwork was carried out during the dry season (July-August 1990) in two rural communities, Mora and Zanja Honda, situated in the Cordillera Province, respectively about 100, and 85 km south of Santa Cruz de la Sierra.
The two localities lie in the foothills of the Andes at an altitude of about 450 meters. The hygienic and sanitary conditions in both communities are very poor.
The population (544 people in Mora, and 224 in Zanja Honda) lives in huts without any hygienic-sanitary equipment or services. Indiscriminate defaecation outdoors is normal, as is also the habit of walking barefoot.
Subsistance activities consist mainly of agriculture and animal breeding. Diet is mainly based on maize, rice, beans, and potatoes.
A meeting with the inhabitants of the two communities was organized to explain the purpose of the study, and its procedures.The study subjects consisted of all available, and apparently healthy, children two- to nine-years old (134, of whom 91 females and 93 males).
The study population of Mora included 119 subjects, while the Zanja Honda sample population consisted of 65 children.
Faecal sample, fingerprick and venous blood sample were taken from each subject.
At the time of stool and blood collection, each child received anthelmintic treatment, in agreement with the Ministry of Health Program of Parasitic Disease Control. None of the subjects had previously received deworming treatment or iron supplements.

Haematology and parasitology

Haemoglobin concentration was assessed from fingerprick blood samples using a portable haemoglobinometer (Chema 1, SEAC, Italy), which was recharged and calibrated each day before use. Anaemia was diagnosed for Hb levels below 11 g/dL in children aged 2 to 6 years, and below 12 g/dL in children aged 6 to 9 years (6).
Venous blood samples were centrifuged and the serum recovered. Serum samples were aliquoted, frozen at –20°C, and transported to Italy in dry ice; samples were still frozen on arrival in Florence.
Iron status was assessed by measuring serum ferritin, serum iron, and total transferrin levels. Serum ferritin was assessed by an immunometric assay (IMX, Abbott, Chicago, Illinois), serum iron by a ferrozine method (Eris, Merck, Germany), and total transferrin by a nephelometric metod (BNA, Behring, Germany). Iron deficiency was considered for either TS below 15% and/or SF below 12 ng/mL (7,8).
A single normally-passed stool sample from each child was collected in a labelled plastic vial with 10% formalin (Para-Pak, Meridian Diagnostics, Inc., Cincinnati, Ohio) and then processed according to a modified ether-formalin sedimentation technique (Para-Pak Macro-Con System, Meridian Diagnostics, Inc., Cincinnati, Ohio).
As part of the study design for efficacy evaluation of the deparasitization program, 50 mg of a stool sample were also examined in the field using the Kato thick smear technique to determine the egg counts (9). Faecal samples found positive for hookworm were cultured in order to differentiate hookworm species.

Statistical analysis

Data were analyzed by the Chi-square test, multiple linear regression, and analysis of variance for unbalanced design. Data on hookworm intensity and other variables (age, haemoglobin, serum ferritin, transferrin saturation, and serum iron) were logarithmically transformed before analysis. An adjustment procedure for missing values was done by imputing the mean of the recorded values to missing data (10).

Results

The mean levels of indicators of iron status according to age and localities are shown in table 1. Haemoglobin, serum ferritin, serum iron, and transferrin saturation mean levels were within the range of normal values. No differences were observed between sexes for Hb, SI and TS mean levels, while SF mean level was significantly lower in males than in females (P=0.02), when the populations of the two communities were considered globally. The 2- to 5-year-old children living in Mora showed the significantly lowest haemoglobin mean level in the examined groups (P=0.0008 when compared with children of the same age living in Zanja Honda, and P=0.0003 compared with older children in Mora). In the same community also the other mean values (SI, TS, and SF) tended to increase with increasing age. On the contrary, in Zanja Honda only SI and TS showed this trend, while haemoglobin and SF mean values tended to decrease with increasing age.

Figure 1 shows the distribution of abnormal values of Hb, SF, and TS according to age and locality. The total prevalence of anaemia was 23.4%. The older children presented higher anaemia prevalence than the younger ones. This general trend was present in Zanja Honda; in Mora, where the total anaemia prevalence (26.9%) was higher than that of Zanja Honda (16.9%) (P=0.12), the 2- to 5-year-old children showed a significantly higher prevalence (29.4%) than the same age group in Zanja Honda (4.9%) (P=0.001). Abnormal SF and TS levels (21.2% and 41.8%, respectively) were distributed in the studied population without significant differences for locality, age and sex, but the general trend showed higher prevalences of low concentrations in younger children.

The findings concerning the prevalence of hookworm infection in the two communities examined are reported in figure 2. Of the 184 subjects examined in the two communities, 66 (35.9%) harboured hookworm. As expected children in the 6-9 years age group showed a significantly higher prevalence (52.0%) than those in the 2-5 years age group (24.8%) (P=0.0001). Females presented a higher, although not significantly, infection rate (40.6%) than males (31.2%). People living in Zanja Honda resulted significantly (P=0.005) more parasitized than those living in Mora, with prevalence rates significantly higher in both age groups. Faecal cultures of 43 positive specimens produced 754 larvae of *Necator*. As far as hookworm intensity is concerned, the distribution of eggs per gram of faeces (epg) was mainly between 0 and 2000 epg (figure 3), and only few subjects presented heavier parasite loads, with a maximum value of 8220 epg. In Mora the arithmetic mean (± standard deviation) of number of eggs eliminated by positive subjects was 770±1534.3 (643.6±980.1 in younger and 830.4±1755.7 in older children), while in Zanja Honda it was 1288.1±1683.4 (1295±2130.3 in younger children and 1281.2±1148.3 in older children) (P=0.0006). The children of Zanja Honda presented a significantly higher parasite load both in the 2-5 (P=0.004) and in the 6-9 years age class (P=0.01). We observed no significant differences in intensity between the sexes or between the two age groups in either locality.

The relationships between haemoglobin and ferritin levels and hookworm intensity, is shown in figure 4. No relation is observed for Hb levels, while ferritin levels tended to decrease with an increase in epg. A negative correlation between SF levels and hookworm intensity was observed when the analysis by multiple regression models was applied, both in Mora (statistically significant; P=0.0001, b=−0.17) and in Zanja Honda (not statistically significant; P=0.096, b=−0.05).

There was also a negative correlation, although not statistically significant, between haemoglobin and hookworm intensity,

LEVELS OF INDICATORS OF IRON ACCORDING TO AGE AND LOCALITY

Locality	Age (years)	Hb (g/dL)*	SI (µg/mL)*	TS (%)*	SF (ng/mL)*
Mora	2-5	11.52±1.60	56.88±24.07	16.24±7.67	23.37±16.44
	6-9	12.48±1.12	61.86±26.73	17.54±8.37	27.63±19.16
Total		11.94±1.49	59.02±25.25	16.80±8.08	25.20±17.71
Z. Honda	2-5	12.44±0.73	63.56±22.73	17.47±6.83	23.74±19.76
	6-9	12.20±1.15	76.54±29.00	20.84±8.17	19.79±6.96
Total		12.35±0.90	68.36±25.39	18.72±7.47	22.28±16.28
Total	2-5	11.96±1.41	59.39±23.69	16.70±7.49	23.51±17.67
	6-9	12.39±1.13	66.56±27.82	18.60±8.39	25.12±16.63
Total		12.08±1.32	62.31±25.63	17.48±7.90	24.17±17.23

* Values shown are means ± SD

TABLE 1

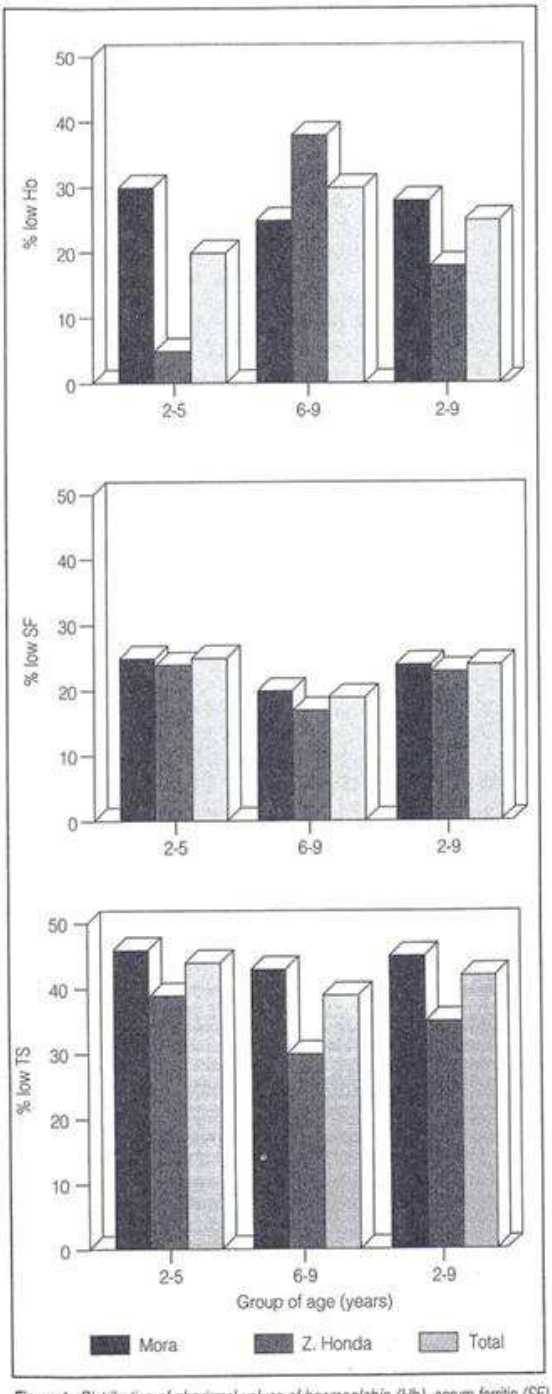

Figure 1 *Distribution of abnormal values of haemoglobin (Hb), serum ferritin (SF) and transferrin saturation (TS) in children living in Mora and Zanja Honda, Bolivia.*

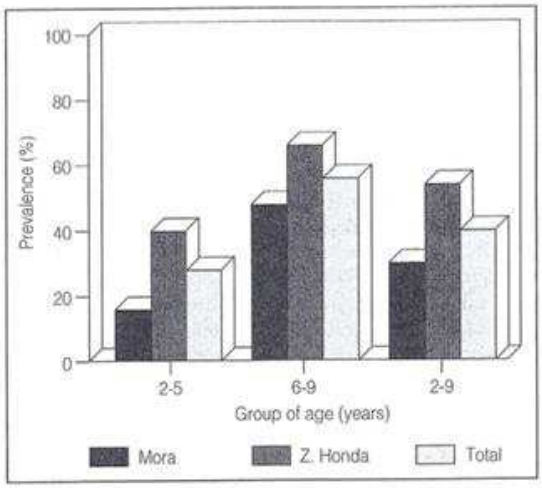

Figure 2 *Prevalence of hookworm infection, according to age and locality.*

both in Mora and Zanja Honda, as well as a negative correlation between ST levels and epg both in Zanja Honda (not statistically significant; P=0.26; b=−0.03), and in Mora (statistically significant; P=0.03; b=−0.05).

Discussion

In this study on the relationship between iron status and hookworm intensity in the 2- to 9-year-old children of two rural Bolivian communities the findings on anaemia are in agreement with the estimated prevalence of anaemia in other Latin American countries (11).

The highest anaemia rates were observed in older children

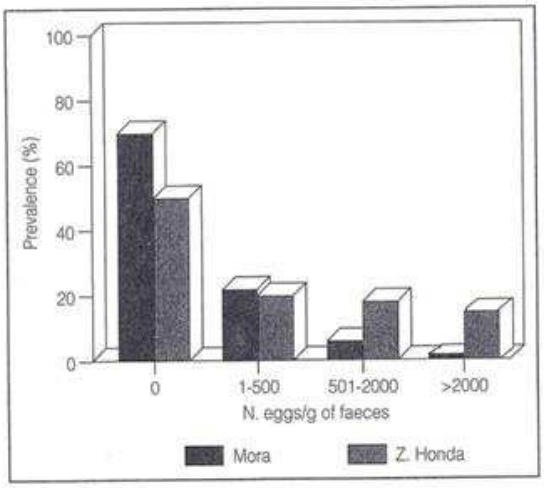

Figure 3 *Hookworm intensity classes, according to locality.*

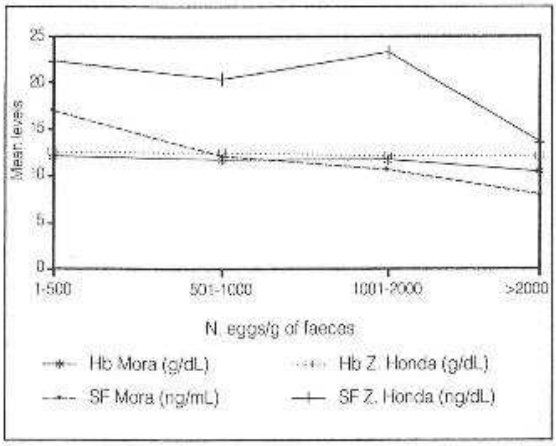

Figure 4. *Relationship between haemoglobin or serum ferritin levels and hookworm intensity in Mora and Zanja Honda.*

living in Zanja Honda and in younger children living in Mora. The parameters of iron status of the population surveyed were within the range of normal values, with some differences between the two age groups. Younger children, particularly those living in Mora, showed a worse iron status that could be explained by their predisposition to develop iron deficiency because of their smaller reserves.

In some cases, subjects with TS below 15% presented normal SF levels. This discrepancy may be related to coexistent interfering conditions such as chronic inflammation, or minor infections, known to produce an increase in SF levels irrespective of iron status (12,13).

Iron deficiency seems to be the predominant cause of anaemia in the population surveyed, and the findings emphasize the importance of combined use of two or more laboratory tests for the assessment of iron status and the diagnosis of iron deficiency anaemia.

When haematological measurements were related to intestinal parasitological status for hookworm, there were negative relationships between hookworm intensity and haemoglobin, serum ferritin and transferrin saturation, that could be explained by storage iron depletion as a consequence of intestinal bleeding due to hookworm infection. However, this negative correlation was significant only in Mora. The absence of a statistically significant correlation between haematological and parasitological parameters in Zanja Honda, where significantly higher parasite prevalence and intensity have been observed, is striking. Nevertheless this finding could be explained by the low infection intensities found in our population, and by the species involved. In fact, *Necator*, probably the sole species responsible for hookworm infection in these communities, as evidenced by faecal cultures of positive specimens, causes an intestinal blood loss five times lower than does *A. duodenale*. Moreover, the evidence of lower iron deficiency rates in the children of Zanja Honda, in contrast with their worse parasitological status, can be explained by their better capability to supply the blood loss due to hookworm infection. The population of Mora includes a group of recent settlers from the Highlands who have a poorer dietary intake of bioavailable iron.

This study presents certain limitations in the approach to evaluate the relationship between iron status and hookworm infection. One is the small size of our sample; the inclusion of older age groups at risk for iron deficiency due to hookworm infection would probably have modified the statistical data. Another limitation is the utilization of egg production as an indirect estimate of hookworm burden. Egg counts are known to be an imperfect measure because of density-dependent fecundity of hookworms, day to day variations in egg output, and sampling heterogeneity (14). However, although there is no solid evidence for a causal relationship, hookworm infection is undoubtedly an important contributory cause of iron-deficiency anaemia in the population of these two rural Bolivian communities.

The low intensity of hookworm infection in the child population studied certainly contributes to the difficulties in assessment of the relationship between hookworm infection and iron deficiency. It is sure that poor dietary intake of bioavailable iron contributes to produce iron deficiency, however, the possibility of other factors as causes of iron-deficiency anaemia cannot be excluded. The communities studied lie in an endemic area for Chagas disease, and the well known blood-sucking action of triatomine bugs must be taken in relation to chronic blood loss (15). On the other hand, malaria seems not to be an important cause of iron-deficiency anaemia in this area, where only low density *P. vivax* infections are present (16).

Considering the difficulties in changing dietary patterns in such populations, one must emphasize the importance of the Bolivian Ministry of Social Welfare and Public Health deparasitization program. Iron supplementation is also advisable in this population with marginal iron intake.

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