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Intestinal parasitism and nutritional status among indigenous children from the Argentinian Atlantic Forest: Determinants of enteroparasites infections in minority populations

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ABSTRACT

Objective: Intestinal parasitoses, especially in the less favored populations of tropical and subtropical areas, are a scourge of high impact in public health. We conducted a cross-sectional survey to investigate the prevalence of helminths and protozoa pathogens, malnutrition, and their determinants in children from indigenous Mbyá Guaraní villages of Iguazú, in the subtropical Atlantic Forest of Argentina.

Methods: Parasitological assessment was performed using a combination of flotation, sedimentation, and centrifugation techniques, as well as temporal and permanent stains. Nutritional assessment was based on nutritional indicators derived from anthropometric measurements. Statistical analysis of socio-demographic determinants was assessed by Generalized Linear Mixed Models at individual, household, and village levels.

Results: A total of 303 children from 140 families from Fortin Mbororé and Yriapú Jungle villages participated, and 87.8% of them resulted positive to at least one parasite. Multiparasitism reached 70% and children with up to six different parasites were detected. Thirteen genera were identified, of which eight were pathogenic. The most frequent soil-transmitted helminths were hookworms and *Strongyloides stercoralis* with 60.7 and 41.9%, respectively. *Enterobius vermicularis* was detected in 28.4% of children. *Giardia duodenalis* was the main protozoan and reached the 33.3%. The prevalence of stunting and underweight were 38.9% and 6.9%, whereas for overweight and obesity were 28.1% and 12.9%, respectively. An association was observed between stunting in older children and the presence of parasites, multiparasitism, and giardiasis. Individual conditions and habits were important determinants for most of the parasitoses.

Conclusions: We evidenced that the community is affected by the double burden of malnutrition and parasitoses. To face this alarming situation, public policies are needed to improve sanitation, hygiene education access, community deworming programs, and quality nutrition on a regular basis of intercultural approaches.

1. Introduction

Intestinal parasitic infections (IPIs), especially in vulnerable populations of tropical and subtropical areas, are of global public health concern (Hotez, 2014; WHO, 2012). Some of the most important causal agents of these infections are soil-transmitted helminths (STH) and the protozoan parasite *G. duodenalis* which are considered neglected tropical diseases (NTDs) (Hotez et al., 2008). Assessment of their impact over some neglected communities such as indigenous villages of Latin America has provided mounting evidence of the severity of such impact (Gracey and King, 2009; Montenegro and Stephens, 2006; Navone et al., 2006; Taranto et al., 2003; Toledo et al., 2009; Zonta et al., 2010). In particular, the co-existence of IPIs and malnutrition among disadvantaged communities in developing countries has been well documented. Intestinal parasites can cause malabsorption, intestinal syndrome, iron and vitamins deficiencies, among other effects, provoking a deleterious effect on the nutritional status (Gamboa et al., 2011; Humphrey, 2009; Stephenson et al., 2000; Zonta et al., 2011). In

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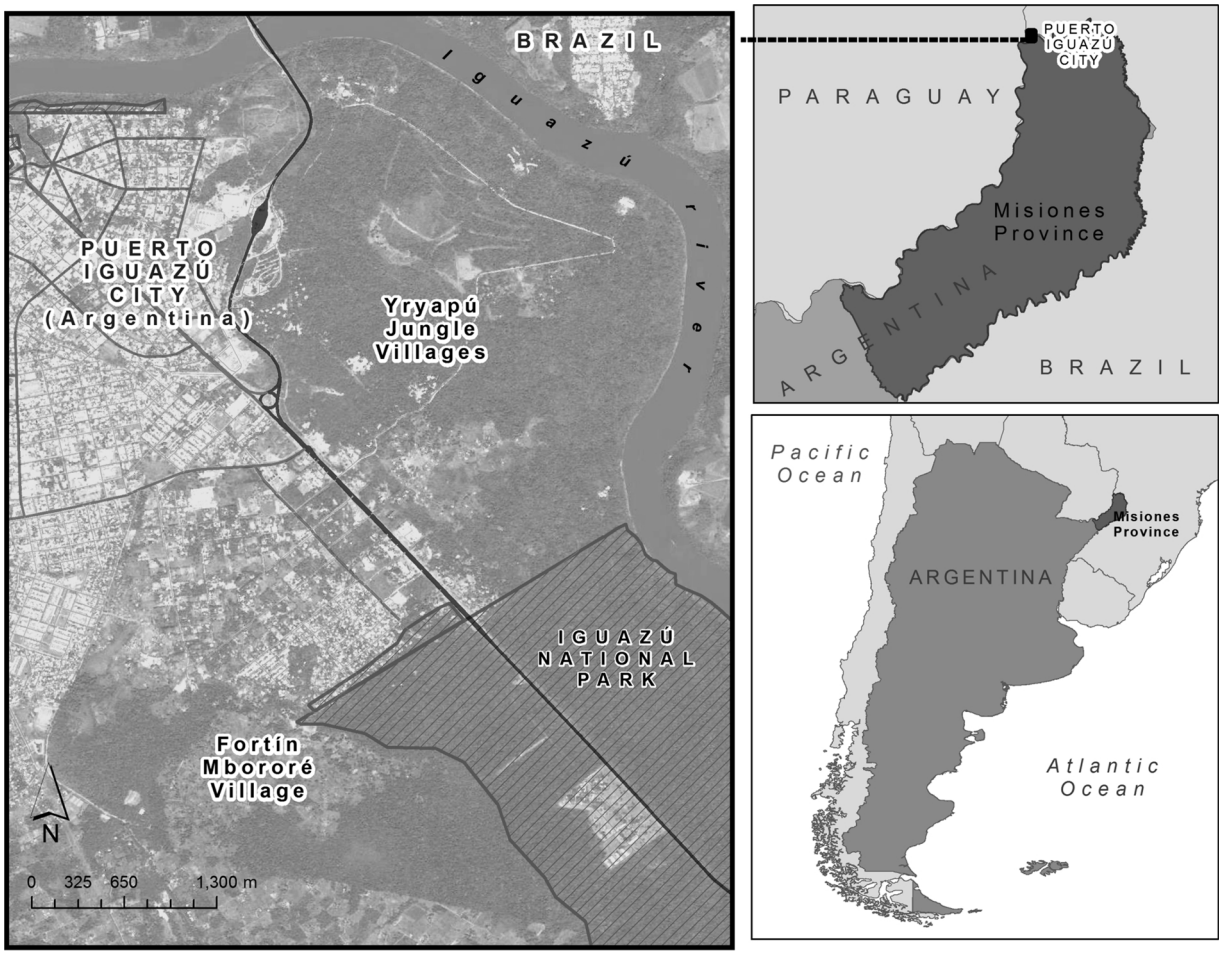

Fig. 1. Study area. Location of the Mbyá-Guaraní villages included in the survey and the Puerto Iguazú City at the tri-border area of Argentina, Brazil and Paraguay.

addition, environmental degradation, forced migrations, precarious housing, socioeconomic difficulties, substandard hygiene and sanitation, lack or limited access to health care, cultural habits, and discrimination define a complex scenario over indigenous communities where children health and development are constantly threatened (Brandelli et al., 2012; Escobar-Pardo et al., 2010; Hotez, 2014; King et al., 2009; Montenegro and Stephens, 2006).

At the northeastern subtropical Argentine region in Misiones province, the indigenous ethnic group Mbyá-Guaraní constitutes almost half of the indigenous population of the province and most live in rural areas (INDEC, 2010). The Mbyá-Guaraní is an ancient indigenous ethnicity of Amazonian roots. Their ancestors arrived from Paraguay and Brazil, and the current population migrates between these countries. Although few, some studies concerning infectious diseases, including IPIs (Delfino et al., 2012; Eirin et al., 2017; Navone et al., 2006; Tonon et al., 2004; Zonta et al., 2010), and nutritional aspects concerning Mbyá-Guaraní communities have been reported (Hirsch et al., 2015; Orden and Oyhenart, 2006). However, most Amerindians have undergone a rapid socioeconomic transition, which has affected strongly their lifestyles (Hotez, 2014; Montenegro and Stephens, 2006). Consequently, the establishment of baseline data is urgently needed.

The relation among the factors that influence parasite infections remains one of the most fundamental questions in this field of study, being its comprehension at different levels and cultures a critical element in the rational development of control strategies (Halpenny et al., 2013; Karagiannis-Voules et al., 2015; Pullan et al., 2008). The degree to which Mbyá-Guaraní children are acquainted with IPIs remains largely unknown. Thus, in this work we aimed to evaluate the prevalence of intestinal parasites and multi-parasitism among Mbyá-Guaraní children that live in a tri-border area in the Atlantic rainforest of Puerto Iguazú, Argentina. Through a cross-sectional survey, we also examined individual factors as malnutrition, and family and household conditions that individually or combined can be determinants of the persistence of IPIs in these vulnerable communities.

2. Material and methods

2.1. Ethical considerations

This study was approved by the Bioethical Committee of Dr. Madariaga Hospital and Research Ethical Committee of Misiones, Argentina. We followed the Declaration of Helsinki and national regulations concerning personal data protection (national law No. 25.326), Indigenous Policy, and Support to Indigenous Communities (national laws No. 23.302 and 24.071). We held village meetings, and handed over detailed explanations of the aims, procedures, potential risks, and benefits of the study to village authorities and local health personnel. Informed written consent (signature) was obtained from each child's mother. We offered treatment to those children detected positive to any parasite infection following standard clinical practice according to the National Ministry of Health.

2.2. Study area and population

Misiones province is part of the Atlantic Forest Eco region (Olson and Dinerstein, 1998). The region is characterized by a subtropical climate with no dry season. The predominant soil type is lateritic of deep red color (Bertonatti and Corcuera, 2000). The population of this

region is one of the most impoverished and indigent in the country (INDEC, 2016). At its northwest point is located Puerto Iguazú city (25° 35'52″S and 54° 34′ 55″W) bordering with Brazil and Paraguay. The city is the main touristic point of the province and one of the most important in the country since it embraces one of the New Natural World Wonders, the Iguazú Falls at the Iguazú National Park. Mbyá-Guaraní villages located close to the city can be grouped into ´The Yriapú Jungle´ villages (YJV) with 95 families at the north and the Mbyá-Guaraní community Fortin Mbororé (FMV) with 290 families at the southeast (Fig. 1). Only after the "cacique" (the maximum political authority) of each village provided the authorization, a hundred and seventy families comprising 44.15% of the targeted population participated voluntarily in the study. A total of 390 children were invited to participate. Among them, 82.3% (303) provided proper stool samples, complete information on the socio-demographic questionnaires, and signed informed consent. Among this cohort, 167 children were from YJV and 136 from FMV. The communities have access to Public Health Care Centers (PHCC) one located at YJV and the other one at FMV, both PHCC are under-equipped rural dispensaries and, provide elementary medical assistance less than three times a week. The employment situation of the families is precarious, since the economic income of these villages comes from the sale of handicrafts, sporadic farming works, and mainly from national assistance programs. The housing situation is highly precarious because most houses are built with canes and straw, in some cases complemented with tarpaulins and cardboard.

2.3. Study design

This study involves a cross-sectional parasitological, anthropometric, and nutritional status appraisal of Mbyá-Guaraní communities based on a household survey. It was conducted from March 2017 to December of 2017. Families with children under 15 years of age neither with gastrointestinal symptomatology nor under parasitological treatment were invited to participate. Houses were selected by simple random sampling method and were visited by the interdisciplinary and intercultural team. During the household visit, a disposable collection kit was provided to the mother for each child. Each kit contained a tray, spoon, gloves, gauzes, a jar of preservative solution for collecting stool and a jar with formaldehyde 5% for gauzes collection. Mothers were instructed to follow a serial collection method with SAF (Sodium acetate-acetic acid-formalin) as fixative solution, every other day during a week in order to collect a total of three feces samples. For the diagnosis of *Enterobius vermicularis* mothers were instructed to use the gauze provided to collect an anal swabbing every other day during a week, totalizing three samples. Oral and written bilingual (Spanish/Mbyá-Guaraní) explanations about samples collection as well as illustrated instructions were provided whit the collection kit. All these materials were labeled with the child's name and an identification code. Field staffs were trained in proper hygienic and bio-safety measures. Stool samples were kept in ice-boxes during transportation and at 4 °C in the lab until their analyses.

2.4. Socio-demographic data

A pre-tested questionnaire was applied to mothers of each indigenous family. To ensure reliable information, mothers were interviewed by local health workers in their mother tongue. Through a sequence of questions, demographic variables (personal and family, e.g. age, sex, family composition, educational level, parents' labor situation), and household features variables (e.g. house building construction, unsatisfied basic needs –UBN (a multidimensional national index that evaluate whether a household has privations in terms of school attendance, quality of household, overcrowding, sanitary conditions and subsistence capacity), water supply, excreta disposal, waste disposal) were recorded (Table 1). Customs, play, and hygiene habits were addressed (e.g. play with soil, wearing shoes, hand washing). Information referred to previous parasitic diseases and symptoms as well as previous treatments were also recorded to risk evaluation.

2.5. Anthropometrics assessment

Child nutritional status was assessed by anthropometric measurement. Weight (kg) and height (cm) measurements were collected using standard calibrated instruments. On the basis of the available individual-level data for each record (age, gender, weight, and height at each visit), Z-scores were calculated for weight for age (WAZ), length/height for age (HAZ) and body mass for age (BMIZ) using the sex-specific WHO Child Growth Standards through WHO Anthro and Anthro Plus software (WHO, 1995, 2006). We defined the prevalence of stunting (for HAZ) and of underweight (for WAZ) as the proportion of children with a Z-score below −2. We defined the prevalence of overnutrition (for BMIZ), as the proportion of children with a Z-score between +1 and +2 for overweight and above +2 for obesity (Table 1).

2.6. Parasitological examinations

Copro-parasitological status of each child was assessed by direct smear with Lugol solution, sugar's flotation, and modified Telemann´s (formalin-ether centrifugation) techniques (WHO, 1991). Anal swab gauzes for *E. vermicularis* were subjected to centrifugation at 1500 rpm, 10 min and the pellet was observed under light microscopy. Additionally, Kinyoun (Henriksen and Pohlenz, 1981) was performed to each sample in order to evidence *Criptosporidium parvum*, and trichromic staining (Mora et al., 2008) for *Giardia duodenalis* and *Entamoeba histolytica/dispar/moshkovskii*. In any case, a sample was classified as positive if there was an egg, larva, trophozoite, or oo/cyst count of one or more on any slide or technique. Microscopic analysis of each technique was conducted in duplicate by two experienced microscopists. As quality control, the entire negative slides and a random 10% of the positive samples were re-examined by a senior laboratory researcher.

2.7. Statistical analyses

We selected the presence of intestinal parasites and multiparasitism (i.e. the number of species of parasites detected) as the main response variables of our analyses. We also summarized as response variables the presence/absence of the most prevalent groups or parasites: STH, *E. vermicularis* and *Giardia duodenalis*. The variables characterizing children's conditions, habits, and their local environment (family, village; Table 1) were included in the analysis as explanatory variables (fixed effects) in generalized linear mixed-effects models (GLMM), including the household as a random effect to consider the dependence of the data and nested factors (children belonging to each household) (Zuur et al., 2009). The number of children per family was considered as a weight argument in these models to balance the potential bias in household level factors caused by families with a high number of children (Zuur et al., 2009). The presence of parasites or group of parasites were modeled as binary response variables with a logit-link function, and the multiparasitism (count data) was modeled using a Poisson error distribution (Zuur et al., 2009).

Firstly, univariate analyses were performed to explore and describe the general associations among responses and independent variables (Magalhães et al., 2011). In a second step, the significant independent variables were analyzed by groups (see groups in Table 1) in multivariate GLMM to discern the most important factors conditioning the persistence of each parasite. The models obtained for each group of variables were compared and combined hierarchically using the Akaike Information Criterion corrected for small samples (AICc) to identify the best fit models containing only the most important and uncorrelated variables of each group (Burnham and Anderson, 2002; Zuur et al.,

Table 1
Socio-demographic variables analysed at individual, household and village level.

Group of variables	Variables	Type and description
Individual		
Children	Sex	Binary [a]. Male vs. female children.
	Age	Continuous [a]. Children age in years.
Nutritional conditions	Stunted	Binary [b]. Height for age Z-score (HAZ) < −2 SD.
	Underweight	Binary [b]. Weight for age Z-score (WAZ) < −2 SD.
	Overweight	Binary [b]. Body mass for age Z-score (BMIZ) > 1 SD & < 2 SD.
	Obese	Binary [b]. Body mass for age Z-score (BMIZ) > 2 SD.
Exposure	Previous deworming treatment	Binary [a]. Children who received at some point of their life antiparasitic treatment.
Habits	Hand washing	Binary [a]. Child with hand washing habit.
	Wearing shoes	Binary [a]. Child with the habit of wearing shoes.
	Playing with soil	Binary [a]. Child with the habit of playing with soil.
	Fingernail eating	Binary [a]. Child with the habit of fingernail eating.
	Thumb sucking	Binary [a]. Child with the habit of thumb sucking.
Symptoms	Anal itching	Binary [a]. Child whose mother observed anal itching at previous parasitic disease.
	Grinding teeth	Binary [a]. Child whose mother observed grinding teeth at previous parasitic disease.
	Stomach-ache	Binary [a]. Child whose mother observed stomach-ache at previous parasitic disease.
	Diarrhoea	Binary [a]. Child whose mother observed diarrhoea at previous parasitic disease.
	Any symptom	Binary [a]. Child whose mother referred any of the above-mentioned symptoms in previous parasitic disease.
Household		
House	Economic status (low vs. very low)	Binary [a]. Variable created considering parents' employment condition/incomes.
	Housing poor condition	Binary [a]. Variable created considering house building materials and unsatisfied basic needs.
Yard	Poor peridomiciliary hygiene	Binary [a]. Presence of trash around households.
	Farm animals	Binary [a]. Presence of farm animals in the yard.
WASH	Drink water from springs (vs. well-pumped)	Binary [a]. Origin of drinking water.
	Excreta disposal in open space (vs. latrine)	Binary [a]. Excreta disposal.
	Littering (vs. trash burning)	Binary [a]. Waste disposal.
Family	Large family	Binary [a]. Families with more than 3 children.
	Single mother	Binary [a]. Single mother as head of household.
	Mother literacy	Binary [a]. Mother with primary school education completed.
	Overcrowding	Binary [a]. More than three children per bedroom in the house.
	Bed-sharing	Binary [a]. More than one child per single bed.
Village area	Fortín Mbororé village (vs. Yriapú villages)	Binary [a]. Mbyá Guaraní village where each child lives.

[a] Data extracted from the pre-tested and structured questionnaire (see Methods).
[b] Data calculated from the children measurements collected by the local authorities at each PHCC. Calculations were done using the sex-specific WHO Child Growth Standards through WHO Anthro and WHO Anthro Plus software.

2009). Statistical analyses were carried out with R software (R Development Core Team, 2016) through library *lme4* (Bates et al., 2015). Best-fitting models were selected on the basis of the AICc through library *MuMIn* (Barton, 2015).

3. Results

3.1. Socio-demographic characterization

Overall, communities' socio-economic situation was poor and housing construction very precarious (Table 2). Wood houses with bare earth soil and tacuara cane, tarpaulins and cardboard roof prevail. The employment situation of the families was precarious subsisting with financial assistance from different national programs. Mothers' educational level was mainly defined by primary education (Table 2). Almost half of the families were large families with more than three children and overcrowding and bed-sharing were common conditions (Table 2). Considering WASH variables (water, sanitation, and hygiene), lack of safe drinking water was observed, being the main sources spring and well-pumped water without any treatment of potabilisation or regular sanitary control. Open-space defecation prevailed as excretal disposal practice, and burning was the most frequent way of waste disposal (Table 2). Low adherence of children to good hygiene habits and practices was reported by most of the mothers, and the presence of farm animals was common (Table 2), although none of them in proper locations such as pens and chicken coops.

3.2. Nutritional status

The nutritional pattern suggested that children were highly affected by stunting and overweight (Table 2). Only 13.2% (n = 40/303) of the children did not present malnutrition conditions. The prevalence of under- and over- nutrition was higher in children under 5 years of age. In this age group, stunting and underweight reached 57% (n = 81/143) and 11% (n = 16/143), whereas in children older than 5 years the prevalence decreased to 24% (n = 37/160) and 3% (n = 5/160), respectively. For overweight and obesity, the prevalence in children under 5 years were 33% (n = 47/143) and 23% (n = 33/143) and both declined to 23% (n = 37/160) and 3% (n = 5/160) in children over 5 years, respectively. The prevalence of children affected simultaneously by stunting and obesity reached to about 8% (Table 2), but most of them were children under 5 years old (24 out of 26 children). Finally, almost 40% of the children were affected by either overweight or obesity (Table 2), and this prevalence rose to 56.3% (n = 90/160) in the under-5-years-of-age group.

3.3. Prevalence of intestinal parasites and multiple infections

A total of 13 different genera of intestinal parasites were identified in this study (Table 3). Hookworms and *Strongyloides stercoralis* were the most prevalent STH with 60.7 and 41.9% respectively, whereas *G. duodenalis* was the most prevalent protozoan parasite with 33.3%. The 87.8% of children resulted positive to a parasite infection representing 266 positive cases out of 303 children. From these, 79.7% (n = 212/266) were cases of multiparasitism, with four children harboring at least six different parasites. Boys were slightly more infected (89%, n = 154/173) than girls (86.1%, n = 112/130).

3.4. Risk factors for intestinal parasite infections in Mbyá Guaraní children

The general exploration of univariate associations between parasite infections and the risk factors are summarized in Table 4 and fully

Table 2
Socio-demographic characteristics of the studied population at individual and household level, expressed by the frequency and percentage of children or families with this characteristic.

Group of variables	Variables & characteristics	Frequency	Percentage (%)
Individual *			
Sex	Female	130	42.9
	Male	173	57.1
Age	Under 5 years old	143	47.2
	From 5 to 15 years old	160	52.8
Nutritional conditions	Stunted (HAZ) [a]	118	38.9
	Underweight (WAZ) [b]	21	6.9
	Overweight (BMIZ) [c]	85	28.1
	Obese (BMIZ) [c]	39	12.9
	Stunted AND obese	24	7.9
	Overweight OR obese	121	39.9
Habits	Hand washing	44	14.5
	Thumb sucking	74	24.4
	Fingernail eating	40	13.2
	Play with soil	244	80.5
	Wearing shoes	44	14.5
Household **			
House	Economic status = low	48	34.3
	Economic status = very low	92	65.7
	Housing poor condition	93	66.4
Yard	Farm animals	95	67.9
WASH	Drink water from spring	19	13.6
	Drink water from well-pumped	121	86.4
	Excreta disposal in latrines	112	80.0
	Excreta disposal in open space	28	20.0
	Waste disposal by littering	11	7.9
	Waste disposal by burning	129	92.1
Family	Large family	71	50.7
	Single mother	20	14.3
	Mother literacy (primary school)	46	32.9
	Unschooled mother	94	67.1
	Overcrowding	116	82.9
	Bed-sharing	76	54.3

* Calculated from the 303 children of the study.
** Calculated from the 140 families of the study.
[a] HAZ: Height for age Z-score. Mean Z-score = −1.80.
[b] WAZ: Weight for age Z-score Mean Z-score = −0.65.
[c] BMIZ: Body mass index for age Z-score Mean Z-score = 0.68.

described with the statistical parameters in Tables S1 and S2 in the Supplementary materials. We found that younger children, especially those under five years of age, were less parasitized for all groups of parasites analyzed with the exception of *Giardia*. Boys showed a stronger association with infection and multiparasitism than girls, especially STH, whereas girls showed a stronger association of infection with *E. vermicularis* than boys. Interestingly, we found an interaction between age and nutritional conditions, where with increasing age stunted children were more likely to be parasitized and multiparasited than younger children. Remarkably, when we inquired on the questionnaires whether children had ever had previous deworming treatment we observed that this variable proved to be important for predicting infection in children with at least one parasite (Table 4).

We found that habits were important as predictors of parasitic infections. Children with hand-washing habit showed lower probabilities of being infected by IPIs compared to children without this habit (Table 4). Wearing shoes was also important, especially to reduce the chances of infection with STH and multiparasitism. Playing with soil also proved to be important in determining the presence of parasites and multiparasites. Thumb sucking was associated with the presence of parasites and multiparasitism, and specifically with the presence of *E. vermicularis*. From the signs and symptoms reported by the mothers when we inquired about previous most common gastrointestinal diseases, anal itching and grinding teeth showed a clear relation with *E. vermicularis*, and stomach pains and diarrhea with the presence of *Giardia*.

Table 3
Prevalence of intestinal parasites in asymptomatic Mbyá-Guaraní children from Puerto Iguazú. Relative and total prevalence is depicted by species and group of parasites as well as by type of coinfection detected.

Parasite species or groups	N	Relative prevalence (% from positive cases N = 266)	Total prevalence (% from total cases N = 303)
Total		**100**	**87.8**
Helminths	**239**	**89.8**	**79.0**
Hookworms	184	69.2	60.7
Strongyloides stercoralis	127	47.7	41.9
Enterobius vermicularis	86	32.3	28.4
Hymenolepis nana	81	30.4	26.7
Ascaris lumbricoides	16	6.0	5.3
Protozoans	**153**	**57.5**	**50.5**
Giardia duodenalis	101	38.0	33.3
Entamoeba coli	72	27.1	23.8
Blastocystis hominis	18	6.8	5.9
Chilomastix mesnili	3	1.1	1
Endolimax nana	2	0.7	0.7
Cryptosporidium sp.	2	0.7	0.7
Entamoeba polecki	1	0.4	0.3
Iodamoeba butschilii	1	0.4	0.3
Multiparasitism	**212**	**79.7**	**70.0**
Double	78	37.1	29.3
Triple	76	36.2	28.6
Quadruple	40	19.0	15.0
Quintuple	14	6.7	5.3
Sextuple	4	1.9	1.5

Concerning univariate associations at household and family level, the presence of trash in the peridomiciliary area was associated with increased multiparasitism (Table 4). Regarding WASH, open space defecation was positively associated with an increased presence of STH. At family level, mother literacy was associated with a lower prevalence of STH, while overcrowding was associated with a greater probability of presence of *E. vermicularis*. Observing the prevalence patterns at the study area level, the FMV showed a lower prevalence of STH and higher prevalence of protozoan parasites than YJV.

After exploring the general associations through univariate models, we combined these factors in multivariate models selecting the most important and predictive variables for each response variable (Table 5). In general, the factors at child level were the most important for predicting parasite infections (Table 5). Older children who have been previously dewormed and frequently presented any symptom were more likely to be parasitized, while those with hand-washing and wearing-shoes habits were less likely to become infected. The level of multiparasitism was mainly associated with these same factors, but in turn, being stunted for older children and inhabiting at YJV was important.

Infections with STH were not only strongly associated with age, but also with gender, being higher in boys than in girls (Table 5). Again, hand-washing and shoe-wearing habits clearly reduced their probability of infection, and factors such as mothers' education played a role in reducing STH prevalence.

In this multivariable analysis, infection with *E. vermicularis* also showed that the youngest children were less likely to be affected; girls, however, were the most likely to be infected by this parasite (Table 5). The habits and symptoms reported as frequent affections in children such as thumb sucking, anal itching, grinding teeth evidenced positive association with *E. vermicularis* infection.

G. duodenalis showed an inverse pattern to the other parasites, reducing infection probabilities as age increases (Table 5), but children with chronic nutritional problems (stunted) were more likely to become infected as they grow. Beyond these conditions, hand washing habits showed to be important in reducing the likelihood of infection from this

Table 4
Summary of the univariate associations found between parasite infections and the independent variables, evaluated in different groups and levels thought univariate GLMMs * (the complete statistical details of each association are described in Tables S1 and S2). The sign indicates if the association is positive (+) or negative (-), and the number of signs indicates the strength of the association (one sign $p < 0.05$, two signs $p < 0.01$, and three signs $p < 0.001$, *n/e* not evaluated). GLMMs incorporated the families as a random effect.

Group of variables	Variables	Parasite infection	Multiparasitism	Infection with STH	Infection with *E. vermicularis*	Infection with *G. duodenalis*
Individual level						
Children	Male children (vs. females)	(+ +)		(+ + +)	(–)	
	Age	(+ + +)	(+ + +)	(+ + +)	(+ + +)	(-)
Nutritional conditions	Stunted	(—)	(—)	(—)		
	Overweight or obese		(—)	(—)	(—)	
Interaction of age with nutritional status	Stunted when age increases	(+)	(+ + +)			(+ + +)
	Overweight or obese when age increases			(-)		(+)
Exposure	Previous deworming treatment	(+ + +)			(-)	
Habits	Hand washing	(—)	(—)	(—)	(—)	(–)
	Wearing shoes	(—)	(—)	(—)		
	Playing with soil	(+ + +)			(+ + +)	(+ + +)
	Thumb sucking	(+)			(+ + +)	
Symptoms	Anal itching	*n/e*	*n/e*	*n/e*	(+ + +)	*n/e*
	Grinding teeth	*n/e*	*n/e*	*n/e*	(+ + +)	*n/e*
	Stomach-ache	*n/e*	*n/e*	*n/e*	*n/e*	(+ + +)
	Diarrhoea	*n/e*	*n/e*	*n/e*	*n/e*	(+)
	Any symptom	(+ + +)	(+)	*n/e*	(+ + +)	*n/e*
Household level						
House	Economic status				(–)	(+)
	Housing poor condition				(+ + +)	
Yard	Poor peridomiciliary hygiene		(+ +)		(+ + +)	
	Excreta disposal in open space (vs. latrine)			(+)		
	Mother literacy			(–)		
	Overcrowding				(+ +)	
Village level						
Village	Fortín Mbororé village (vs. Yriapú villages)		(–)	(–)		(+)

*Statistical analyses were developed using R software version 3.4.3 through library *lme4*.

parasite, while playing with soil and thumb sucking increased these odds. Stomach-ache was the main symptom associated with the probabilities of *Giardia* infection.

4. Discussion

The study evaluated Mbyá-Guaraní children in the three-border area of Argentina, Brazil and Paraguay, offering evidence that the community is seriously affected by the double burden of malnutrition and parasitoses, which stands out strongly in the disadvantaged populations (CEPAL, 2016; CEPAL and UNICEF, 2010). Young Mbyá-Guaraní children of Iguazú have risk of stunting nearly four times higher than the provincial values (Nunez et al., 2016), exceeding 50% of the cases and in accordance with reported values for Mbyá children from the central area of the province (Zonta et al., 2010). Stunting in early life impairs growth and has adverse functional consequences on children's cognitive, motor, and language development. In the long term it also affects nations in terms of work capacity and productivity (Onis et al., 2013). At the other extreme, malnutrition leading to overweight/obesity is conceived as a long-term consequence of stunted growth and development in disadvantaged populations (WHO, 2009, 2010). This situation is detected at vulnerable indigenous communities from all over the world at an accelerated pace (CEPAL, 2016; Valeggia et al., 2010). In addition to this, we report a very high frequency of IPIs and multiparasitism in these Mbyá-Guaraní children. Taking all this into account, children from Mbyá-Guaraní villages of the Argentinean Atlantic Forest face an alarming situation which strongly conditions their current well-being and their development into healthy adults.

Available data on Amerindians of neighboring countries such as Brazil (Brandelli et al., 2012) and Paraguay (Echague et al., 2015) reports elevated prevalence of intestinal parasites and deleterious nutritional conditions in children population with harmful effects on their health. Furthermore, multiparasitism is a common characteristic of Amerindian children, with reported extreme cases with up to 6–7 species at the same time in a single individual (Brandelli et al., 2012; Escobar-Pardo et al., 2010). Our findings agree with those studies and also are comparable with works performed in Mbyá Guaraní villages from the central area of Misiones province (Navone et al., 2006; Zonta et al., 2010). However, the parasitological pattern and the main determinant factors assessed were different and can be related to the relative location of the villages and their socio-environmental context.

In a recent work that we carried out with non-indigenous children from urban and rural areas of Puerto Iguazú city, we detected a parasite and multiparasitism prevalence of 58.8% and 34.1%, respectively. *G. duodenalis* and *E. vermicularis* were the most prevalent parasites, while STH parasites were detected at very low frequencies in children but appeared as the main environmental contaminator when soil and animals samples were surveyed (Rivero et al., 2017a). For Mbyá- Guaraní children in the same area, we here describe a prevalence of 87.8% and 70.0% for intestinal parasites and multiparasitism, respectively, being significantly higher than those found in that work. Also, infections with hookworms and *S. stercoralis* were considerably higher in Mbyá-Guaraní children. Previous studies from a decade ago also described high prevalence of these STH (Zonta et al., 2010) illustrating persistent inequalities at Mbyá-Guaraní people's living conditions, which barely get better even in communities located closer to more commercial and touristic cities. It is important to pinpoint that even though we combined the outcome of several microscopy procedures to obtain a more accurate picture and to improve the parasitological diagnosis, the elevate *S. stercoralis* prevalence may still be underestimated as this study did not use specific culture or molecular techniques for its detection. Hence the threat posed by this pathogen must be considered potentially

Table 5
Multivariate models (GLMM) combining the most important predictor variables for parasite infection in the Mbyá Guaraní villages around Iguazú. The *Estimate* parameters in these models represent the rate of change in the odds of the response variable for a given unit change in the predictor variable. GLMMs incorporated the families as a random effect.*.

Fixed effects	Estimate	Std. Error	p
Parasite infection			
(Intercept)	6.485	1.759	< 0.001
Age	0.735	0.143	< 0.001
Previous deworming treatment	5.038	1.389	< 0.001
Hand washing	−10.148	2.266	< 0.001
Wearing shoes	−5.073	2.020	0.012
Any symptom	3.450	0.926	< 0.001
Multiparasitism			
(Intercept)	0.821	0.090	< 0.001
Age	0.023	0.008	0.006
Stunted	−0.429	0.104	< 0.001
Stunted when age increases	0.055	0.014	< 0.001
Hand washing	−0.391	0.098	< 0.001
Wearing shoes	−0.358	0.105	0.001
Anal itching	0.267	0.070	< 0.001
Fortín Mbororé village	−0.203	0.086	0.017
Infection with STH			
(Intercept)	7.684	1.603	< 0.001
Male children	1.345	0.469	0.004
Age	0.494	0.090	< 0.001
Stunted	−2.704	0.677	< 0.001
Overweight & obese	0.752	0.773	0.330
Overweight & obese when age increases	−0.267	0.113	0.018
Hand washing	−4.091	0.878	< 0.001
Wearing shoes	−6.688	1.208	< 0.001
Mother literacy	−3.481	1.439	0.016
Fortín Mbororé village	−5.417	1.358	< 0.001
Infection with *E. vermicularis*			
(Intercept)	−13.821	2.487	< 0.001
Male children	−2.155	0.457	< 0.001
Age	0.519	0.082	< 0.001
Hand washing	−5.807	2.476	0.019
Wearing shoes	4.857	2.049	0.018
Thumb sucking	4.235	1.185	< 0.001
Anal itching	5.008	0.707	< 0.001
Grinding teeth	6.331	1.478	< 0.001
Infection with *G. duodenalis*			
(Intercept)	−0.640	0.580	0.270
Age	−0.243	0.052	< 0.001
Stunted	−1.819	0.445	< 0.001
Stunted when age increases	0.342	0.067	< 0.001
Overweight & obese	−1.053	0.438	0.016
Overweight & obese when age increases	0.148	0.062	0.017
Hand washing	−1.599	0.462	0.001
Playing with soil	1.095	0.393	0.005
Stomach-ache	2.333	0.326	< 0.001

* Statistical analyses were developed using R software version 3.4.3 through library *lme4*.

greater. We also mention that parasite intensity of infection was not assessed in this study, being a limitation to describe in detail the associations between nutritional status and parasites. However, our models outcomes indicate the likelihood of intestinal parasite infections acting as factors associated with low nutritional status.

Mbyá villages are characterized by highly uniform poor housing conditions and are affected by the worst WASH indicators of the region (INDEC, 2004-2005INDEC, -, 2005INDEC, 2004-2005, 2010). This can explain why these variables were not good predictors of IPIs and, when combined, conditions at the individual level were best suited to understand the risk factors. Our findings suggest that the high prevalence of multiparasitism in the Mbyá-Guarani community has become the rule rather than the exception and in general, both parasitism and multiparasitism infections had common risk factors mostly related with individual conditions and habits of the children.

Mothers' literacy has been pointed out as one key factor underpinning infectious diseases and closely related to children and family health (Buor, 2003). Indeed, this factor appears as an important socioeconomic aspect defining STH infections in our results and also in other areas (Mamatha and Munirathnamma, 2014; Quihui et al., 2006; Rivero et al., 2017a). Mothers' literacy can also be related with other very important factors that we found in relation to children personal hygiene and habits. Several international health organizations argue that nations should make substantial efforts to educate and empower women in order to maximize success in health outcomes for them and their families (CEPAL, 2016; Kar et al., 1999).

Considering protozoan parasites occurrence, the presence of *G. duodenalis* in Mbyá children was the highest reported in the province being a similar rate to the reported prevalence from children of Puerto Iguazú city (Rivero et al., 2017a). Mbyá children from villages of the central area of the province also presented a high prevalence of *Giardia* (Zonta et al., 2010). However, the main protozoan parasite detected in that area was *B. hominis*. The amoeba *E. coli* had high prevalence in our study in coincidence with that study in the central area. Although *E.coli* is not pathogenic, its detection is a valuable descriptor indicating fecal contamination underlying reduced sanitation conditions and poor environments (Sard et al., 2011). Recurrent stomach-ache and diarrhea episodes reported by mothers were associated with *G. duodenalis*. In fact, acute and chronic intestinal infections in humans have been attributed to this parasite. The specific analyses explaining *G. duodenalis* presence comprised a set of individual factors combined with household factors. Giardia was the only parasite which presented a differential pattern when considering specific village prevalence. Fortin Mbororé village had higher prevalence than YJV. Therefore, this finding supports the need to apply a comprehensive monitoring strategy that incorporates and links both water quality data and human disease data in a spatial database.

Considering the percentage of children (20%) that have ever been exposed to a previous deworming treatment, we found an association with current parasitic infections suggesting unsolved intrinsic or extrinsic conditions that favor parasitic infections. Accordingly, we assume that deworming approaches must be accompanied by sanitary improvements. Deworming national programs performed a decade ago (PROAPS-REMEDIAR, 2007) did not include all the Mbyá villages and there is not a current initiative to address this issue. These public-health actions should be promptly considered, including inter-cultural perspectives (Kujawska et al., 2017; Sy, 2009), considering the parasitological pattern as well as the wide range of risk factors associated with intestinal parasitic infections evaluated in urban and rural children of the area (Rivero et al., 2017a).

Age-specific playing habits conjointly with Mbyá lifestyle, e.g. poor shoe wearing and hand washing habits favor more contact with the routes of transmission of parasite infective stages. In this regard, it is worth mentioning that although a small proportion of families reported footwear use and regular hand washing of their children, these children were clearly less affected by parasites. Local participation in the development of intercultural strategies to encourage healthy habits is essential (Rivero et al., 2017b; WHO, 2012) together with public health actions sustained over time.

5. Conclusion

Similar to other countries worldwide, in Argentina indigenous populations constitute a socioeconomically disadvantaged segment (Montenegro and Stephens, 2006). Mbyá-Guaraní villages were characterized by households with significant housing constraints, very limited access to improved WASH capacities and incomes, and deficient structural situation that need to be upgraded in order to improve the population's response capacity. Indeed, drug administration and health education is limited to isolated efforts developed by researchers during parasitological surveys. Public policies are needed to guarantee clean water, sanitation, hygiene education access, and community deworming programs, including an intercultural approach to guide efforts

of all the partners involved. Considering the magnitude of these infectious diseases, especially affecting young children from populations with high levels of nutritional vulnerability, the call to action results imminent.

Conflict of interest statement

The authors declare no conflicts of interest.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.actatropica.2018.08.015.

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