MICROBIOLOGY ARCHIVES
Original Research Article | ISSN: 3041-590X
Journal homepage: https://microjournal.researchfloor.org/

Parasitosis and its genotyping dependent on socio-environmental factors as determinants of health in children of Tlaxcoapan, Hidalgo, Mexico

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ARTICLE INFO

Citation: Sánchez Martínez Diana Verónica, Ponce Macotela Martha, Coronel-Olivares Claudia, Reynoso Vázquez Josefina, Izquierdo Vega Julieta Aleli and Ruvalcaba Ledezma Jesús Carlos (2025). Parasitosis and its genotyping dependent on socio-environmental factors as determinants of health in children of Tlaxcoapan, Hidalgo, Mexico.
Microbiology Archives, an International Journal.
DOI: https://doi.org/10.51470/MA.2025.7.2.22

Received 25 May 2025
Revised 20 June 2025
Accepted 22 July 2025
Available Online August 17 2025

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ABSTRACT

Objective: *Describe the incidence of parasitosis and its genotyping, dependent on socio-environmental factors as determinants of health in children from Tlaxcoapan, Hidalgo.*
Material and methods: *An observational, descriptive, cross-sectional epidemiological study was conducted on 186 children under 5 years of age from the municipality of Tlaxcoapan, with parental consent. Stool samples were collected for a three-series coproparasitological examination through fresh slides, Faust, and Ziehl-Neelsen staining techniques. Socio-environmental factors and symptomatology were assessed through interviews. The cysts from samples that tested positive for Giardia intestinalis (G. intestinalis) were subjected to semi-nested PCR (polymerase chain reaction) of the glutamate dehydrogenase gene. Statistical analysis was performed using SPSS-24.*
Results: *Out of 186 children, 46 (25%) tested positive for parasitic and/or commensal microorganisms in their coproparasitological examination, while 140 (75%) were uninfected. The method with the greatest specificity was the direct (66% positive diagnoses), followed by the Faust method (34%). Ziehl-Neelsen staining showed negative results for Microsporidium, Cryptosporidium and Cyclospora. Genotyping of G. intestinalis denotes two genotypes: AI, characteristic of animals and humans, and AII, primarily observed in humans.*
Conclusions: *Parasitic infection affected 25% of the children studied. The socio-environmental factors with the highest risk were the lack of water purification and high coexistence with domestic animals. Genotyping of G. intestinalis revealed two genotypes, AI and AII. The AI genotype has zoonotic potential, highlighting its importance in public health.*

Keywords: *parasitosis, socio-environmental factors, genotyping, children.*

Introduction

Intestinal parasites jeopardize child growth and development. These organisms cause malnutrition, anemia, and other diseases that lead to poor performance and school absenteeism [1].

The health problem of intestinal parasitosis has been addressed for a long time. Most research has focused on studying its prevalence and risk factors associated with contracting this type of disease. It is estimated that 24% of the global burden of disease and 23% of mortality are attributable to environmental factors [2].

In Mexico, during the 27th epidemiological week of 2016, a total of 2,439,145 intestinal infections caused by other organisms and poorly defined ones were reported, representing 94% of the total reported in 2016 (2,580,694) [3].

At the regional level, numerous studies have been conducted regarding chemical contaminants found in water, soil, and air, due to industrial activity. However, the repercussions of biological contaminants, particularly in water, and their effects on population health, have been neglected.

On the other hand, the number of intestinal diseases caused by other organisms and those poorly defined has exceeded seven thousand cases in recent years, since 2011 [3] in the municipality of Tlaxcoapan. Through this study, it will be possible to determine the current status of parasitosis in the child population, as well as identify the causative organisms. From this, appropriate prophylactic measures can be disseminated to diagnose and prevent gastrointestinal diseases in the population. Currently, the municipality lacks studies addressing this issue.

Objective: Describe the incidence of parasitosis and its genotyping in children from Tlaxcoapan, Hidalgo, based on socioenvironmental factors as health determinants.

Materials and Methods

Type of investigation: The research conducted corresponds to an epidemiological, observational, descriptive, cross-sectional study. The sampling and interviews were carried out in the municipality of Tlaxcoapan (Figure 1), Hidalgo, Mexico, and its three localities: Teocalco, Teltipan, and Doxey. The study included children under five years and 11 months of age residing in the municipality.

Figure 1. Location of Tlaxcoapan in Hidalgo México [4].

Necessary permits were requested from the preschool supervision office and the jurisdiction of Tepeji to approach children in kindergartens. Both symptomatic and asymptomatic children whose parents consented were invited to participate.

Stool samples were collected in preschools within the municipality of Tlaxcoapan, Hidalgo. The experimental work was carried out in the Experimental Parasitology laboratory at the National Institute of Pediatrics (INP).

Meetings were held with parents who agreed to participate, during which instructions were provided on how to collect children's fecal samples. The coproparasitological examination was performed over three alternate days for a serial analysis, and informed consent forms were signed by the parents.

The samples were stored in coolers with refrigerants that maintain an adequate temperature of 4 °C until they were transported to the laboratory for analysis.

In addition, a structured and previously validated survey, designed by the Experimental Parasitology Laboratory of the INP, was applied to parents and/or guardians. The survey collected complementary information regarding household hygiene habits and practices. The data were analyzed using multivariate analysis in SPSS 24, and a Chi-square test was performed.

Three diagnostic techniques were performed: fresh slides, Faust and Ziehl-Neelsen staining. Cysts were identified and quantified using the Faust method, fresh method, and Ziehl-Neelsen staining. In cases of positive parasites detection, DNA (deoxyribonucleic acid) was extracted for genotyping.

G. intestinalis DNA was extracted using a commercial kit (QIAamp DNA Stool Mini Kit, 50; QIAGEN, Cat. No. 51504, Lot No. 142323282). The extracted DNA was stored at -20 ºC (degree Celsius) until use.

The extraction was performed directly from feces at room temperature (15–25 °C), and all centrifugation steps were carried out at room temperature at 20,000 xg (14,000 rpm [revolutions per minute]). The Sheather technique was also used during DNA extraction.

Amplification was carried out using a Gene Amp PCR System 9700 thermal cycler (Applied Biosystems, CA) version 3.12. The reaction mixture had a final volume of 25 µL (microliter) and was prepared in 0.2 mL (milliliter) PCR microtubes.

For amplification, *G. intestinalis* DNA was processed by PCR, carried out in a gel submerged in 100 µL of TBE buffer for 1.5 hours at 60 volts, 10 watts, and 30 milliamperes (mA). Samples 1, 5, and 17 were selected for genetic material amplification. A master mix was prepared to facilitate the amplification process (Table 1).

Table 1. Composition of the master mix

H20 µL	Buffer µL	RE µL	BSA µL	DNA µL
12	2	1	0.4	8
12	2	1	0.4	8
12	2	1	0.4	8
12	2	1	0.4	8 H_20*
48	8	4	1.6	32

Source: *Own elaboration-INP, 2017.*

The agarose gel used was high-resolution and prepared at 0.5% TBE, heated at intervals of 20, 15, and 10 minutes, since it had a thicker consistency. The gel was left until it stopped releasing vapors before adding 8 µL of Ethidium Bromide (BrE) after 120 minutes. Electrophoresis was conducted at 70 volts, 30 mA and 10 watts. Subsequently, 5 µL of the solution was placed in each tube, followed by the addition of 15 µL of DNA. The mixture was centrifuged for 15 seconds and incubated at 37 °C overnight. The agarose gel was prepared at 2% in 30 µL of AL with 5% TBE, dissolving 0.6 g of agar. The gel was hydrated for 15 minutes and then heated in four 15-minute intervals. When it no longer eliminated vapors, BrE was added. Additionally, 5 µL of stop mix was added to each restriction tube (20 µL restriction volume). A 50 bp marker, 8 µL of TE and 2 µL of stop mix were used. Only 5 µL of this mixture was loaded into well 1 of the agarose gel. 100 mL of 0.5 x TE was used to dip the gel. Electrophoresis was run at 70 volts, 30 mA and 10 watts for 90 min. The restriction tubes were stored at -20 °C. Later, 5 µL of the solution was placed in each tube, 15 µL of DNA was added, and centrifuged for a few seconds.

DNA extraction technique for *Blastocystis hominis*

B. hominis positive samples were washed three times with PBS and centrifuged at 2500 rpm for 5 minutes. The samples were transferred in Eppendorf tubes with lysis solution and stored under refrigeration for 48 hours. They were subjected to four cycles of heat-cold shock and incubated at 53 °C for 12 hours.

For the first DNA extraction, 400 µL of phenol and 400 µL of chloroform (stored at -20 °C) were added. The mixture was shaken by inversion for 10 minutes and centrifuged at 12,000 rpm for 15 minutes in an Eppendorf microcentrifuge. The resulting aqueous phase was recovered in another tube, 400 µL of chloroform and 400 µL of phenol were added to those containing the aqueous phase. They were shaken by inversion for 10 minutes, centrifuged at 12000 rpm for 15 minutes in an Eppendorf microcentrifuge. Finally, an extraction was performed using chloroform. The mixture was shaken for 10 minutes and centrifuged at 12,000 rpm for 15 minutes. To the aqueous phase 800 µL of cold absolute ethanol (-20 °C) and 30 µL of 3 M sodium acetate were added.

The tube was mixed and placed at -20°C for 12 hours. Subsequently, the tubes were centrifuged for 15 minutes at 12,000 rpm and the supernatant was decanted. The tubes were placed on absorbent paper to allow ethanol evaporation. The precipitate was resuspended in 30 µL of TRIS-EDTA (TE). The results of the extraction (2 µL of DNA) were mixed with 2 µL stop mix and 4 µL TRIS. The mixture was centrifuged for 10 seconds in an Eppendorf microcentrifuge. A 1% agarose gel, immersed in TAE, was run, for one hour and 30 minutes at 60 volts, 30 milliamperes and 10 watts. To the suspension obtained, 10 µL of RNAasa was added and incubated for 30 minutes at 37 °C. Subsequently, the tubes were tempered, and 400 µL of phenol and 400 µL of chloroform (-20°C) were added. The tubes were manually shaken for 10 minutes and centrifuged briefly. The aqueous phase was recovered in new tubes, and 400 µL of chloroform was added. The solution was manually shaken and centrifuged for 10 minutes. The aqueous phase was transferred to a new tube, and 800 µL of ethanol (-20 °C) and 30 µL of sodium acetate were added. This solution was stored at -20°C for 12 hours.

The Eppendorf tubes containing sodium acetate and ethanol were refrigerated for 48 hours. They were then centrifuged for 10 minutes at 12,000 rpm. The supernatant was decanted, the residual ethanol was evaporated, and the precipitate was resuspended in 30 µL of TE. After resuspension in 30 µL of TE, 2 µL of the solution was transferred to a new tube and mixed with 2 µL of stop mix and 4 µL of TRIS.

The tubes were labeled and briefly centrifuged. The samples were then loaded into wells, using a D500 ladder as a DNA marker. Electrophoresis was performed at 60 volts, 10 watts and 30 milliamperes. The wells showing DNA bands were selected for amplification.

The enzyme used for the amplification of *B. hominis* DNA was Tag DNA Polymerase 5 U/µL, a temperature-resistant enzyme involved in the synthesis reaction. Primers RD3 (5′-GGG ATC CTG ATC CTT CCG CAG GTT CAC CTA C-3′) and RD5 (5′-GGA AGC TTA TCT GGT TGA TCC TGC CAG TA-3′) were used to obtain *B. hominis* DNA bands.

Forty-seven µL of the master mix were added to each tube, along with 3 µL of the DNA sample. The magnesium chloride concentration was adjusted tot 2 mM.

Electrophoresis

The gel mold was prepared by removing the comb, and polymerization was initiated by adding 3 mL of TEMED and 15 mL of 3% ammonium persulfate to the mixture. The solution was mixed and poured into the glass plates to avoid trapping bubbles. The curvature (meniscus) on the surface was removed with a thin layer (1-2 mm) of distilled water or isobutanol, which was very gently deposited on the mixture. The gel was allowed to polymerize (15-20 min). Once the interface between the polymer and water became visible, an additional 10-15 minutes were allowed for the reaction to complete. Excess liquid was then decanted from the surface, and the sample comb was placed in a slightly tilted position (so as not to trap bubbles). The components of the top gel (compactor) were prepared and mixed according to Table 2.

Table 2. Components of the top gel

Component	Quantity
Deionized water	1.5 mL
Top buffer	630 µL
SDS	33 µL
Acrylamide/bisacrylamide	330µL
TEMED	2 µL
Ammonium persulfate	10 µL

Source: Own elaboration-INP, 2017.

Once the catalysts were added, and the mixture was immediately poured into the mold and the comb was leveled to a horizontal position, ensuring no air bubbles were trapped. The gel was left to polymerize, and the comb was removed by gently sliding it out. Amplified samples were prepared and loaded into the wells. Subsequently, the electrophoresis chamber was filled with a buffer (180 mL in the lower tank and 120 mL in the upper tank). In addition, the gel was placed in the chamber and samples were run at a constant voltage of 200 V. The gel was removed and immersed in fixative for 15 min, in a covered container (to avoid toxic methanol vapors), with gentle agitation. In addition to the fixative, this solution washed out a good portion of SDS from the gel, which interferes with staining.

The gel was subsequently stained with silver nitrate, dried on thick filter paper and between pieces of special cellophane, with methanol and glycerol, with another sheet of cellophane on top, and placed in a vacuum dryer for 1-3 hr. at 60 °C. A D500 DNA ladder was used as a control, and *Giardia duodenalis* samples coded as 4.1, 3.1, 4.2 and 4.3 were loaded into the wells. The second electrophoresis was modified as follows: 1 µL of BrEt, 30 µL of TBE buffer, and 0.3 g of agarose. The wells were seeded with 14 µL of solution (12 µL DNA and 2 µL stop mix) and 10 µL of the control. The gel was run at 100 V, 50 mA. and 10 W for 60 minutes.**Results**

Of the 186 surveys answered by the parents of children who participated in the research, the following results were found: regarding overcrowding, the average number of people living together in a house hold was 5.1. The reported average salary per month was 2,780.00 Mexican pesos (136 USD). Ninety-eight percent of household had access to drinking water at home, while 2% lacked this basic service. Ninety-nine percent sourced their water from a municipal well managed and disinfected by the municipal government. While 1% obtained their water from a dam. Although all children reported drinking purified water, only 22 households reported boiling the water as an additional disinfection method prior to consumption at home.

Ninety-eight percent of the population had an indoor bathroom connected to a drainage system, while only 2% reported using a latrine. Cement floors were reported in 179 households. Five houses had a combination of dirt and cement floors and only one had a dirt floor.

Eighty-eight percent of the study population reported living with domestic animals, including cats, dogs, birds and horses (Figure 2), while 12% of the children had no relationship with any of these animals. 118 children lived with canines, which represents 63% of the population that maintains a relationship with at least one domestic animal. This was followed by felids (12%), birds (8%), sheep (8%), pigs (0.5%) and horses (0.5%).

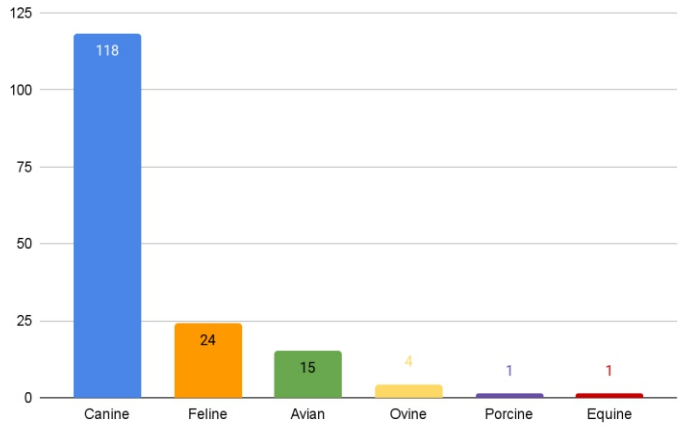

Figure 2. Most frequent domestic animals that coexist with children.
Source: Own elaboration, 2017

Parasitic diagnostics

Coproparasitoscopic methods

Of the three coproparasitoscopic methods (CPM) used, the direct or fresh method had the highest specificity, detecting 66% of the positive diagnoses (40 cases). This was, followed by the Faust method, wich identified 34% of the positive cases (21 cases). The ZiehlNeelsen stain, however, yielded no positive results for *Microsporidium*, *Cryptosporidium* or *Cyclospora* (Figure 3).

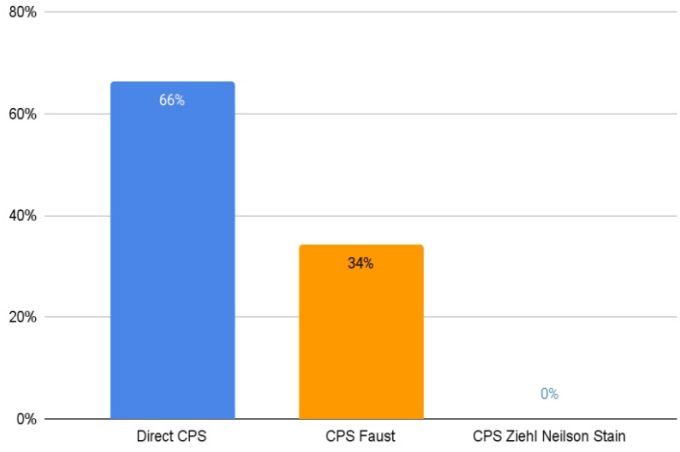

Figure 3. CPM technique according to its diagnostic positivity
Source: INP structured survey, 2017

Incidence of parasitosis

The incidence of parasitosis was 12.9% in the study group and 10.5% in the overall population. The incidence of commensalism was 8% in the study and 6.5% in the total population.

186 children who participated in the study, 46 of them presented some parasitic and/or commensal microorganism in their coproparasitological examination (Figure 4). This represents 25% of children with parasitosis/commensalism and 75% of uninfected children.

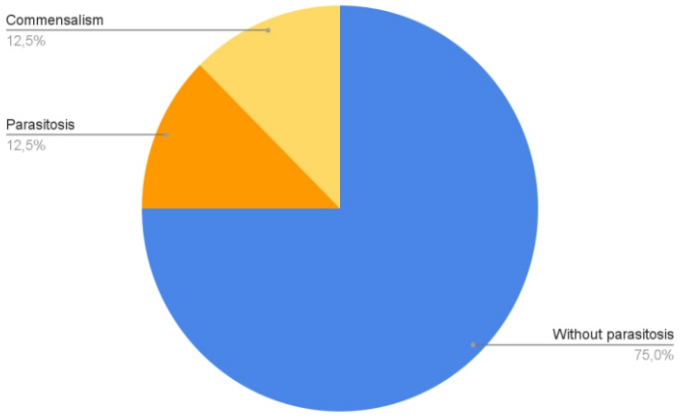

Figure 4. Diagnosis of CPM
Source: Own elaboration, INP, 2017

The most frequent parasite was *B. hominis* (26 cases), followed by *G. intestinalis* (4 cases) and *Entamoeba histolytica-dispar* (1 case). Commensals were also found, with frequency for *Entamoeba coli* (13 cases), *Endolimax nana* (11 cases), and *Chilomastix mesnili* (2 cases) (Table 3).

Table 3. Frequency of microorganisms detected in coproparasitological examinations.

Type of human interaction	Microorganisms	Percentage %
Parasite	*B. hominis*	46
Guest	*E. coli*	23
Guest	*E. nana*	19
Parasite	*G. intestinalis*	7
Guest	*C. mesnili*	4
Parasite	*E. histolytica-dispar*	2

Source: Own elaboration, INP, 2017

According to the literature, 86% of the microorganisms reported were parasites and 14% were commensals. However, it was not ruled out that the latter should have special attention for their treatment.

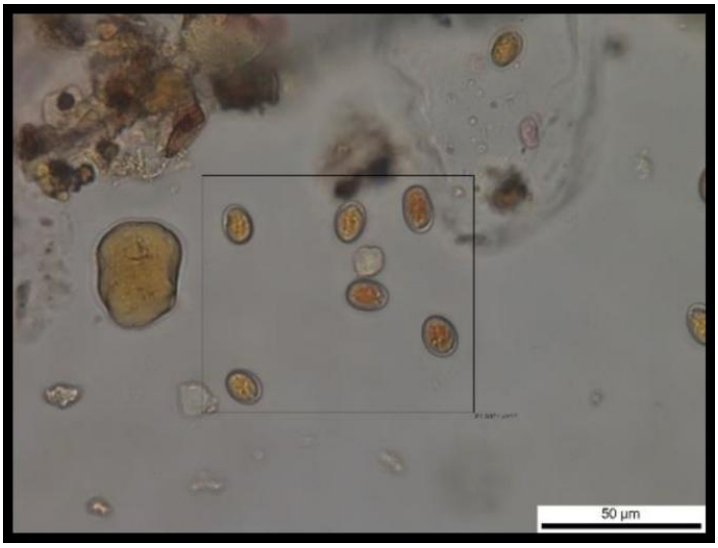

Figure 5. G. intestinalis cysts, stained with lugol (40x) obtained with the CPS Faust technique.
Source: Own elaboration, INP, 2017.

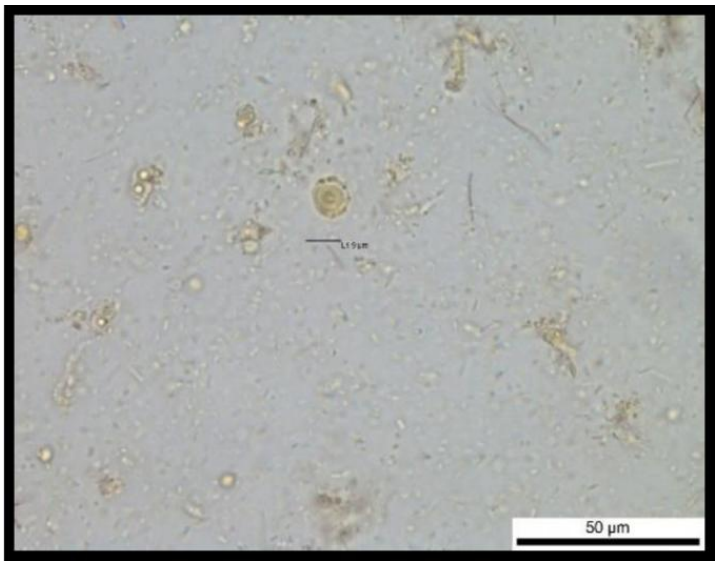

Figure 6. B.hominys cysts, stained with lugol (40x) obtained with the direct CPS technique
Source: Own elaboration, INP, 2017

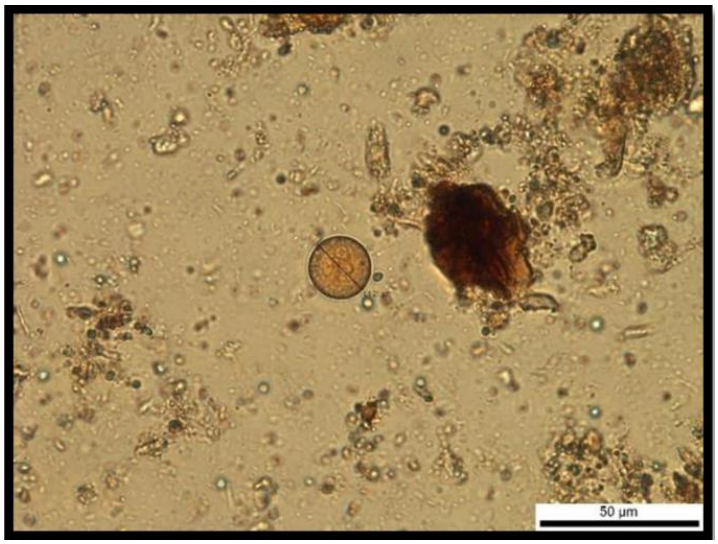

Figure 7. E. coli cysts, stained with lugol (40x) obtained with the CPS Faust technique
Source: Own elaboration, INP, 2017

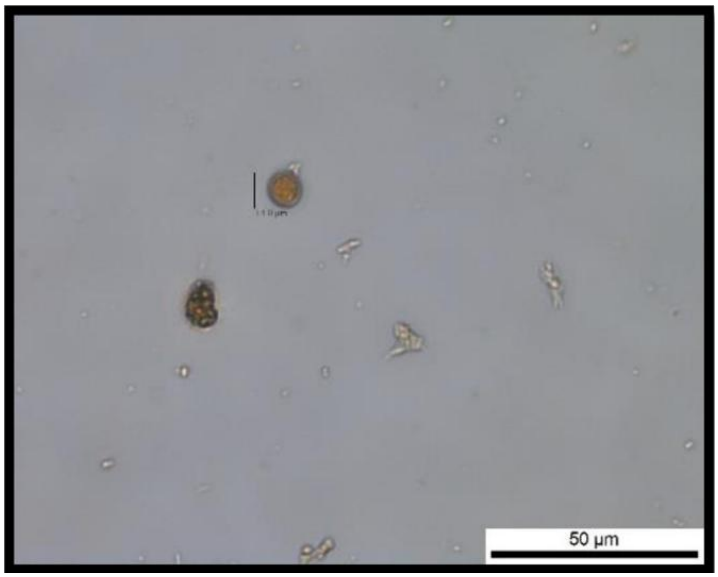

Figure 8. C. mesnili cysts, stained with lugol (40x) obtained with the CPS Faust technique
Source: Own elaboration, INP, 2017

Symptomatology

Regarding the symptoms reported by parents at the time stool samples were collected, 98% of children with parasitosis presented at least one symptom, such as: diarrhea, meteorism, bruxism, and pruritus. Only three cases were asymptomatic, corresponding to those with *B. hominis* and commensals such as *E. coli*, *C. mesnili,* and *E. nana*. The most common symptoms observed were meteorism, dark stools, constipation, irritability and hyporexia, while blood in stools and vomiting were reported in only two cases.

For *B. hominis,* the most frequent symptoms in the study population were meteorism, followed by cough, urticaria, dark stools, and constipation. Only one case of blood in stool, insomnia and asthenia was reported, while two cases were asymptomatic.

The predominant symptoms for *G. intestinalis* in children are headed by meteorism, dark stools, abdominal pain and anal pruritus. Since, from weight loss, nasal pruritus, myalgia, bruxism, general malaise, irritability, tenesmus, cough, insomnia and constipation were each reported only once. The symptomatology of the parasite with the lowest incidence, *E. histolytica* (only two cases) included nasal pruritus, meteorism, abdominal pain, general malaise, headache and cough.

Regarding commensal organisms, the symptomatology associated with *E. coli* primarily dark stools, meteorism, irritability, hyporexia and anal pruritus and in only one occasion, there was myalgia, vomiting, asthenia, nausea, nasal pruritus, and an asymptomatic case were reported.

The most frequent symptoms corresponding to *E. nana* were: meteorism, constipation, cough, irritability. And those that occurred only once were insomnia, asthenia, nausea, and also an asymptomatic case.

Finally, the commensal *C. mesnili* was present in only two patients. One of them exhibited hyporexia, anal pruritus, bruxism, myalgias, tenesmus, dark stools, blood in stools, and diarrhea, while the second one was asymptomatic.

There were two cases in which only *G. intestinalis was found,* and 20 cases with *B. hominis* alone. Likewise, two cases had both *E. histolytica* and *B. hominis*, as well as *G. duodenalis* and *B. hominis*.

In relation to commensal organisms, one case was reported for *C. mesnili*, and seven cases for *Entamoeba coli* and *E. nana,* respectively. Two more cases were reported where two commensals coexisted in infants *Entamoeba coli* with *E. nana,* and *C. mesnili* with *E. nana*, respectively.

Finally, some children had both parasites and commensals. One child had *G. intestinalis* and *E. coli* and two children had *B. hominis* with *E. nana* and *B. hominis* with *E. coli*, respectively.

The prevalence of parasitism in the study is 12.9%, while commensalism was 8%, covering the period from December 2016 to June 2017 in the municipality of Tlaxcoapan.

The average age of the participating children was 4.3 years, with an average height of 105.7 centimeters and an average weight of 18 kg (Table 4).

Table 4. Average of quantitative variables

Average of quantitative variables		
Age (years)	**Height (cm)**	**Weight (Kg)**
4.3	105.7	18

Source: Own elaboration, INP, 2017

Bivariate Analysis

2x2 cross tables

In the contingency tables, the relationship between the children's symptoms and the diagnosis obtained from the coproparasitoscopic examination was analyzed using the SPSS Statistics 24 program.

Chi-Square Test

The Chi^2 test, compares the observed and expected frequencies in each category to test whether all categories contain the same proportion of values or whether each category contains a user-specified proportion of values. All 186 records were valid for the test and 0 had invalid data. Therefore, 100% of the data were used.

The confidence interval for the difference between the theoretical mean and the observed mean at the confidence level is 95%. In these data, 0 means that there is no possibility that this hypothesis is acceptable. Therefore, if the p-value is less than or equal to the desired significance level, the null hypothesis will be rejected.

The symptoms that presented statistical significance for their value equal to or less than 0.05 were blood in stool, dark stool, constipation, meteorism, hyporexia, nausea, vomiting, pallor, parasite elimination, cough, insomnia, bruxism and convulsions (Table 5).

Table 5. Test statistics Chi^2

Symptoms	Registry (n)	Chi^2	Significance
Diarrhea and parasitosis	186	2.161	0.142
Blood in stool and parasitosis*	186	26.449	0
Dark stools and parasitosis*	186	9.013	0.003
Tenesmus and parasitosis	186	0.213	0.644
Constipation and parasitosis*	186	10.345	0.001
Meteorism and parasitosis*	186	29.557	0
Abdominal pain and parasitosis	186	0.122	0.727
Hyporexia and parasitosis*	186	8.615	0.003
Irritability and parasitosis	186	1.37	0.242
Nausea and parasitosis*	186	7.934	0.005
Vomiting and parasitosis*	186	9.143	0.002
Fever and parasitosis	186	1.493	0.222
Weight loss and parasitosis	186	3.439	0.064
General malaise and parasitosis	186	3.213	0.073
Asthenia and parasitosis	186	1.635	0.201
Pallor and parasitosis*	186	7.875	0.005
Elimination of parasites and parasitosis*	186	30.42	0
Cough and parasitosis*	186	23.273	0
Insomnia and parasitosis*	186	13.754	0
Cephalea and parasitosis	186	3.111	0.078
Bruxism and parasitosis*	186	4.298	0.038
Anal itching and parasitosis	186	0.831	0.362
Nasal itching and parasitosis	186	1.038	0.308
Seizures and parasitosis*	186	31.373	0
Urticaria and parasitosis	186	1.5	0.221
Myalgias and parasitosis	186	2.817	0.093

Source: *Own elaboration, INP, 2017. (*) With statistical significance*

Table 6 shows the Chi^2 test statistics for the socio-environmental conditions of the structured survey of the INP, which was applied to parents at the time of delivery of coproparasitoscopics. Significance was 0 for all conditions and 0.001 for those who boil water (Table 6).

Table 6. Test statistics Chi2 socio-environmental conditions

Relationship with parasitism	Registries (n)	Chi^2	Asymptotic significance
Piped water	186	135.007	0
Water supply		136.007	0
Water consumption		138.007	0
Boiling water		11.021	0.001
Intradomiciliary drainage		134.007	0
Apartment at home		127.182	0
Living with animals		70.723	0

Source: *Own elaboration, INP, 2017*

For the calculation of the Chi^2 with respect to symptomatology and environmental conditions, marginal homogeneity tests were performed. These tests compare changes in response, using the Chi^2 distribution, and are useful for detecting response changes caused by experimental intervention in before-and-after designs.

In addition, two other non-parametric tests were performed: the Wilcoxon test and the Mc Nemar test. All three tests yielded similar levels of significance for the different variables. However, we report only the Chi^2, since it is the most commonly used for this type of study, according to the consulted literature.

Molecular results

Genotyping

As mentioned above, DNA was extracted, amplified and restricted from the four *Giardia intestinalis* positive samples.

The amplified products were analyzed using a polyacrylamide gel (Figure 6), where the AI genotype (located in column four) and AII genotype (corresponding to column one, two and three) were identified. The molecular marker used was 50 base pairs, probably with a mixture of genotype D, since bands of 120 and 250 base pairs were observed.

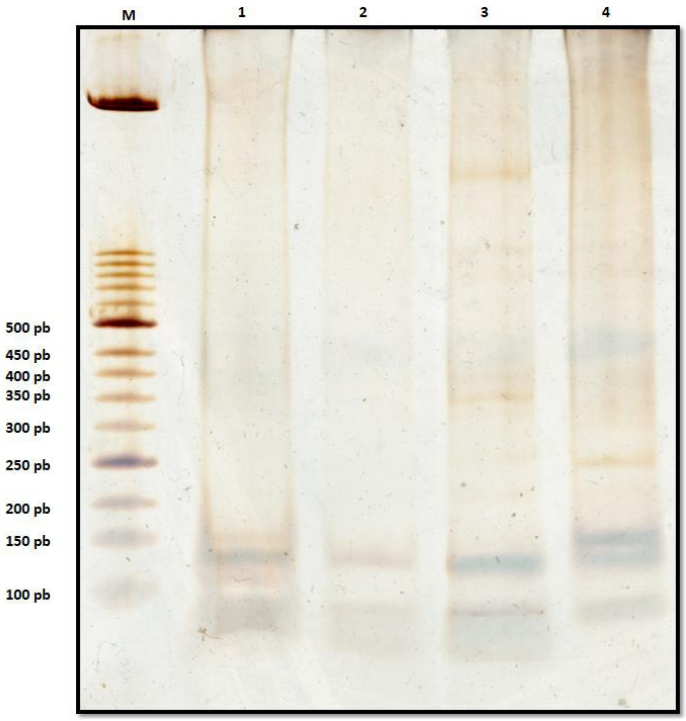

Figure 6: *5% polyacrylamide gel, stained with Silver, showing the restriction fragments of the 432 bp product of the Glutamate dehydrogenase gene with the Nla IV restriction enzyme.*
M: *50 bp molecular size marker;* ***1-4:*** *Children samples. Column 1-3 corresponds to genotype AII and column 4 to genotype AI*
Source: *Own elaboration, INP, 2017.*

Regarding *B. hominis* and *E. histolytica*, genotyping could not be achieved due to the low number of cysts found in the feces, which resulted in an insufficient amount of genetic material to achieve successful amplification with an adequate amount of DNA.

Discussion

The literature reports that in recent years, *Blastocystis* sp. has accumulated evidence supporting the acceptance of its pathogenic. However, these arguments are not yet known by the vast majority of health professionals involved in the diagnosis, treatment, and control of intestinal parasitosis.

Consequently, at the level of health care and in some academic circles, *Blastocystis sp.* is still considered a commensal or, in other cases, a parasite of "unknown pathogenicity" [5, 6]. Its prevalence and incidence at a global level is increasing, it continues to be an underestimated health problem. The average frequency is 10% in developed countries and up to 50% in developing countries [7], with reaching an increasing frequency. For the present research, however, *Blastocystis* sp., was considered a parasitic species due to its clinical manifestations and even resistance to the dewormer granted by the Ministry of Health in mass deworming campaigns.

On the other hand, it is important to consider the growing prevalence of infection by *Blastocystis* sp. as an emerging parasitosis, since most studies carried out on practically the entire planet and the best knowledge about its multiple impacts on human health. Therefore, according to the results obtained, it is possible to support a certain criterion, where there was a higher incidence compared to *G. intestinalis*.

In general, *Blastocystis* spp. is pleomorphic and presents six different morphotypes. Its pathogenicity continues to be a matter of controversy, even 100 years after its initial description. *Blastocystis* spp. are associated with intestinal disorders such as diarrhea, inflammatory bowel disease, irritable bowel syndrome, ulcerative colitis) as well as extraintestinal disorders including urticaria and iron deficiency anemia [8].

The incidence of infection has been determined in several investigations; however, the results are primarly based on samples mostly associated with symptomatic patients. Although it has already been mentioned that blastocystosis is a cosmopolitan infection, it occurs mostly in tropical areas where climatic and hygienic-sanitary conditions (including close contact with animals) facilitate the fecal-oral transmission of the infective form of the microorganism. In the present study, its prevalence was found to be less than 46%, despite the high coexistence with animals.

Currently, *B. hominis* represents a transitional parasite, being one of the most prevalent in humans [6]. Just two decades ago, reports of its prevalence began to emerge, but in recent years, it has increased rapidly.

In Mexico, the presence of *B. hominis* has not been systematically studied, and there are insufficient studies of its frequency, so that epidemiology and prevalence are underestimated. This is due to factors such as the selection of CPM techniques, the difficult in identifying the parasite, and the deep-rooted concept that it is a commensal organism.

According to the publications of the Unified Information System of the National Epidemiological Surveillance System of the Ministry of Health in Mexico, which is governed by the International Code of Diseases, cases of intestinal parasitosis are reported weekly. In this publication, *B. hominis* is placed among the "other intestinal infections due to protozoa". The cumulative report for 2016 in the country reported 59,491 cases of IIDP for both sexes [6].

On the other hand, a previous study found *G. duodenalis* to be the most frequent parasite among children; however, it was difficult to establish a precise relationship with some socioeconomic indicators such as biweekly family income, number of inhabitants in the household, and the absence of basic items for the family [9].

The development of infants during the preschool years, as well as morbidity from intestinal parasitic infections, can have irreversible consequences on children's physical and cognitive development.

The population knows how often they should deworm their children; however, there are those who let the event pass by not participating in health campaigns when they are summoned.

In Mexico, a frequency of *G. lamblia* has been reported to range from 3% to 60% [10], with rates as high as 68.5% among infants, preschoolers, and schoolchildren [11]. Several studies have evaluated the association between sociodemographic factors and *G. duodenalis* infection; however, the consistent risk factors include age, water suppy, and living with animals [12]. A previous study in the metropolitan area, of Toluca, Mexico, indicates that 37.2% of dogs tested positive for parasites, including *Giardia* spp., which implies a zoonotic risk for those living with them [13].

In contrast, the presence of protozoan organisms such as *E. nana*, which has a similar life cycle to *Giardia* [14], was found in this study, so it could be an important factor that conditions the existence of *G. intestinalis* in this research.

Regarding the epidemiology of *E. histolytica*, the prevalence of infection differs from one area to another, as does the severity of disease among patients. Differences in prevalence are often depend by the screening methods used and the number of tests performed [15].

Regarding the symptoms, the literature reports that *B. hominis* is associated with dark stools, diarrhea, pruritus, urticaria, myalgia, insomnia, constipation, bruxism, headache, tenesmus, weight loss, meteorism, and even asymptomatic cases. Urticaria, in particular, is related to other parasitological findings, and *B. hominis* has also been significantly associated with patients exhibiting symptoms such as abdominal pain, and abdominal inflammation. Additionally, the consumption of unboiled water has been identified as the only statistically significant factor associated with infection [8].

The symptoms of *G. intestinalis* include pruritus, bruxism, weight loss, headache, pallor, weight loss, bloating, and diarrhea. These symptoms align with those reported in the literature, which also indicates watery or pasty diarrhea, anorexia, abdominal distension, flatulence, and, occasionally, headache, low-grade fever, allergic manifestations (arthralgias, myalgia, urticaria). Currently, worldwide, it is also asymptomatic or in other studies the presence of irritable bowel syndrome, food allergies and urticaria is reported [16, 17] [18]. In children aged 0-4 years, it has also been linked to growth and cognitive deficiencies [19].

For *E. histolytica*, about 10% of infected patients present symptoms [20]. However, in the present study, only one case of 189 (2%) exhibited symptoms, including myalgia, pruritus, and. As mentioned above, the symptoms of parasitosis are varied and even asymptomatic. This lack of symptoms could limit the necessary attention to this public health issue in infants. However, the literature mentions painful hepatomegaly, with possible irradiation to the right hypochondrium, epigastrium, right shoulder and even back, fever, cough, dyspnea, pain during deep inspiration, and diaphragmatic hypomobility. Infrequent jaundice and the absence of bowel sounds, pleural or pericardial rubbing are considered warning signs. Cases with pericardial, cutaneous, cerebral, and other metastases are rare but important to mention, as they can go unnoticed.

As for the commensal organisms, they showed intermediate frequencies, including *E. coli*, *E. nana,* and *C. mesnili*. At least *E. nana* and *E. coli* have been frequently observed in other studies, however, it is important to identify the risk factors that favor their presence in the environment and their role in the infection of children.

The prevalence of cases of intestinal infections is minimal due to the lack of accessible methods with high sensitivity methods for identifying the causative microorganisms. The techniques used in the local laboratories of the municipality correspond to microscopy, which lacks specificity for a correct and timely diagnosis. Additionally, the nature of the parasites makes their prevalence intermittent, so laboratory tests often yield negative results when conducted. Although the population consumes purified water, they do not boil or chlorinate it, thus not reducing the risk of contamination at home. It is necessary to allocate more economic resources to monitoring and sanitary surveillance of water and food consumed at home. Moreover, protozoa such as *G. intestinalis* should be included in the surveillance systems, since it is one of many organisms that are resistant to chlorination, a method implemented in the study area. Giardiasis is currently a re-emerging disease that should be considered due to its high global frequency, which is also asymptomatic.

Regarding the techniques that reported positive diagnoses, the direct method reported diagnoses in 66% of cases for *B. hominis*, *E. coli*, *E. nana*, *C. mesnili*, and *G. intestinalis.* Faust's method, however, only reported 33% of positive cases for *Giardia intestinalis*, *E. histolytica,* and commensal organisms such as *E. coli*, *E. nana* and *C. mesnili*. Therefore, no difference was found between the two techniques used, despite the fact that the direct method is the oldest that exists, and that, according to the literature, it is a fast method, but not very safe, since it has the possibility of false negatives, so it is necessary to repeat it in some cases of suspicion in the diagnosis.

According to the literature reviewed, a high incidence of *G. intestinalis* was expected. However, the observations made indicate that Faust's method [21] did not have the specificity expected for this research. This could be explained by the massive deworming campaigns in which some of the participants participated, prior to the collection of their samples. The diagnosis of this parasite relies on identifying characteristic cysts in formed feces or cysts and trophozoites in liquid feces. However, it is important to note that examining duodenal contents is also required for establishing a diagnosis, as cysts production of can be sporadic and these may not found in feces during a fecal smear examination is performed for eggs and parasites. Therefore, according to the literature and the findings of this research, a series of three or more tests on alternate days is recommended, since trophozoites (vegetative state) adhere to the villi by suctor discs [22]. In the present research, three of the four cases reported as positive did not provide complete samples. In one case, however, with a single sample submitted for analysis, a diagnosis for *G. intestinalis* was confirmed due to the abundance of cysts found.

It has been stated that water is a factor that affects public health and poses a risk when it is contaminated. Nevertheless, the study population stated that they had access to drinking water services, regardless of the presence of parasitosis.

For over a century, Mexico City wastewater has been utilized as part of the irrigation system in the Tula Valley and the municipality of Tlaxcoapan [23]. This is a direct source of fecal exposure. Studies on the biological and chemical pollutants found in the Tula River and surrounding soils are currently lacking, aside from the potential health hazards to the municipality. Regarding socio-environmental conditions, water is recognized as a determinant of public health and, at the same time, a potential risk when contaminated. However, the study population claimed to have access to drinking water services, wich does not eliminate the risk of parasitosis.

In the municipality of Tlaxcoapan and the Tula Valley, wastewater from Mexico City has been used for over 100 years as part of the irrigation system [23], representing a direct source of exposure to feces. Currently, there is a significant lack of studies regarding biological and chemical contaminants present in the Tula River, as well as nearby soils, overlooking the associated health risks for the local population.

Although only 1% of the study population reported obtaining water supply from a dam and 99% from the municipal well, the quality of the liquid upon reaching homes is unknown. Therefore, a treatment of the vital liquid should always be added at the domestic level (boiling, filtering, chlorinating and solar disinfection). However, those who reported the supply of water from the municipal well harbor the positive cases as well as the one who sourced water from a dam, so this factor was not considered a determining risk. Regarding boiling water, 97% of participants stated that they did not use boiling as a disinfection system, and positive cases of *Giardia* and *Blastocystis* were found among them. This represented a significant risk factor, consistent with studies on the prevalence of giardiasis in Colombia, wich demonstrate that hygienic conditions are closely related with the persistence of the protozoan [24].

In addition, the high coexistence with animals such as cats, pigs, horses, sheep, birds and mainly dogs, in 88% of the population, is closely related to the molecular genotyping results of *Giardia intestinalis.*

The direct characterization of *G. intestinalis* cysts using the PCR technique has proven effective in clarifying the zoonotic role of the different genotypes [25]. In this research, the characterization of positive samples for *G. intestinalis* was successful despite the low number of cysts or the presence of PCR inhibitors, depending on the characteristics of the samples. Therefore, eight genetic groups or assemblages of *Giardia* morphologically similar but genetically distinct have been identified. Assemblages A and B are considered zoonotic. A (AI and AII) and B (BIII and BIV) infect humans, among other animals. In contrast, the other six assemblages (CH) are host-specific. These genetic differences contribute to the complexity of giardiasis epidemiology [26].

Dogs can represent a risk to public health due to cross-infection by zoonotic assemblages. This risk was evidenting the study population, where such coexistence was demonstrated. According to Godinez Galaz 2020 diagnosis, assemblage A was the only one detected, present in in 100% of the samples, with 83% classified as AI and 17% as AII. These findings suggest that dogs in central Mexico may play a significant role in the zoonotic transmission of this parasite, as confirmed by similar results in this research for the state of Hidalgo. This reinforces the association of genotypes A and B with human infections [27]. Therefore, it is necessary to carry out more studies on mechanisms and routes of transmission, considering different study areas, age groups, temporal factors, and socio-environmental conditions.

In addition, it is essential to implement molecular techniques in the health sector to accurately identify risk factors by area and address specific health programs for each region and/or population.

Conclusions

The incidence of parasitosis in children under five years of age in Tlaxcoapan, Hidalgo, was 12.9%, while the incidence of commensalism was 8%.

The socio-environmental risk factors identified in this population were: failure to boil water prior to consumption, living with animals, and having dirt floors, in order of frequency of positive diagnoses.

B. hominis, G. intestinalis and *E. histolytica* were identified through direct coproparasitoscopic examination and Faust method, while all cases were negative for the Ziehl- Neelsen stain. However, the differences between the results of the direct CPM and the Faust technique suggest greater specificity for direct coproparasitoscopic techniques. Therefore, it is necessary to complement any diagnosis with more than one technique to rule out false-negative cases.

Regarding the genotyping of *G. intestinalis*, the presence of the AI and AII genotypes found in the CPM suggests zoonotic potential. However, it is necessary to carry out more studies to clarify the relationship between these subtypes and transmission via specific domestic animals. This factor was found to be predominant in the study population, despite the low incidence of the parasite. In this sense, several research lines are recommended and feasible in the immediate future: investigating the clinical significance of the protozoan subtypes circulating in the area and exploring their potential zoonotic behavior. In the case of *B. hominis,* it is necessary to continue the standardization of methods that allow for the amplification of its DNA, as its genetic material was not successfully amplified in this study

Conflict of interest. The authors declare that there are no conflicts of interest for the publication of this article.

Artificial intelligence. The authors declare that no AI tools were used in the development of this research.

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