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ENTEROPARASITES AND THERMOTOLERANT COLIFORMS IN WATER AND HUMAN FECES OF SECTORS JUAN DE DIOS GONZÁLEZ AND EL MORALITO, COLÓN MUNICIPALITY, ZULIA STATE

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ABSTRACT

In order to determine the presence of intestinal protozoa and thermotolerant coliforms in water samples of sectors Juan de Dios González and El Moralito, Colón Municipality, Zulia State and protozoa and helminths in stool samples in 45 people aged between 0 - 73 years old, was performed a descriptive, prospective, cross - sectional study. By sedimentation by centrifugation was verified the presence of *Blastocystis* spp. in 23 / 25 (92%) of processed samples, a fact which coincided with the presence of thermotolerant coliforms. Similarly highlighted the presence of *Endolimax* sp. and *Giardia* spp. in superficial waters and wastewater. In humans the overall prevalence of intestinal parasites was 71.11% with a clear predominance of *Blastocystis* spp. (62.22%), reflecting a correlation between the species described in water and faeces. Arises the need for to apply standards basic health and environmental measures in the communities evaluated.

KEYWORDS: Enteroparasites, thermotolerant coliforms, superficial waters, Colón Municipality, *Blastocystis* spp., *Giardia* spp., Zulia State.

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ENTEROPARÁSITOS Y COLIFORMES TERMOTOLERANTES EN AGUAS Y HECES HUMANAS DE LOS SECTORES JUAN DE DIOS GONZÁLEZ Y EL MORALITO, MUNICIPIO COLÓN, ESTADO ZULIA

RESUMEN

Con el propósito de determinar la presencia de protozoos intestinales y coliformes termotolerantes en muestras de aguas de los sectores Juan de Dios González y El Moralito, municipio Colón, estado Zulia y de protozoos y helmintos en muestras de heces en 45 personas con edades comprendidas entre 0 - 73 años, se realizó un estudio descriptivo, prospectivo y transversal. Mediante sedimentación por centrifugación verificamos la presencia de *Blastocystis* spp. en 23 / 25 (92%) de las muestras procesadas, hecho que coincidió con la presencia de coliformes termotolerantes. De igual forma destacó la presencia de *Endolimax* sp. y *Giardia* spp. en aguas superficiales y residuales. En seres humanos la prevalencia general de enteroparásitos fue del 71,11% con un claro predominio de *Blastocystis* spp. (62,22%), lo cual reflejó una concordancia entre las especies descritas en aguas y en heces. Es necesario aplicar medidas sanitarias y ambientales básicas en las comunidades evaluadas.

PALABRAS CLAVE: Enteroparásitos, coliformes termotolerantes, aguas superficiales, municipio Colón, *Blastocystis* spp., *Giardia* spp., estado Zulia.

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INTRODUCTION

In water samples, the most important pathogenic protozoa are *Cryptosporidium* spp. and *Giardia* spp. organisms considered markers microbiological quality of water for recreational purposes and for human consumption (1-6). A number of studies suggest the transmission of *Blastocystis* spp. by water and food contaminated with feces, besides this protozoan is frequently identified from humans (7-8). *Cryptosporidium parvum* and *Giardia intestinalis* are clearly associated with disease in humans; its pathogenic role is not questioned. However *Blastocystis* spp. has been linked to blastocystosis or Zierdt-Garavellis disease; its pathogenic role is under discussion and there are epidemiological, clinical and molecular evidence of linkage to irritable bowel syndrome (9-11). In Venezuela there have been several studies on the presence of protozoa in water: Ávila *et al*., (12) emphasize the presence of free-living amoebae (*Naegleria* spp.) and point prevalence of *Blastocystis* spp. in the "Cocuizas" park waters of Maracay City, Aragua State; meanwhile, Arcay and Bruzual (13) emphasize the presence of *Cryptosporidium* spp. in waters of Anare River in humans and domestic and wild animals. More recently Mora *et al*., (14) evaluated the presence of protozoa in surface water and stool samples from individuals in three rural villages in the Montes Municipality, Sucre State, using centrifugal sedimentation, flocculation and Kinyoun and trichrome stains, finding: Amoeba, *Blastocystis* spp, *Endolimax* sp., *Chilomastix* sp. and *Giardia* spp. There are no previous reports on the presence of protozoa and thermotolerant coliforms in superficial waters, wastewater and drinking water in the Colon Municipality, Zulia State. By the above, it became necessary describe the presence of protozoa and thermotolerant coliforms in waters of sectors of Juan de Dios González and El Moralito in the Colon

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Municipality, Zulia State. Likewise some epidemiological factors associated with the presence of intestinal parasites in human feces and water samples in the communities under study were studied.

MATERIALS AND METHODS

Study type. The research was descriptive, prospective and transversal (14-15).

Study area. In the Bolivarian Republic of Venezuela, the Colón Municipality is located at latitude 10° 39 ' N and longitude: 71° 37' W, the capital is San Carlos de Zulia. It has an area of 3,368 km^2, five parishes, with an average annual temperature ranging from 25 to 30°C, according to the census of population and housing in 2001, the population was estimated in 107,821 inhabitants (density = 32.0 inhabitants / km^2). The main river of the Municipality is the Escalante, with numerous tributaries along its route (16).

Population and samples. During the months of January to June 2013 were processed in the bacteriology laboratory of the Universidad Nacional Experimental Sur del Lago "Jesús María Semprum" UNESUR a total of 25 water samples from areas of Juan de Dios González: wastewater "AR" (n = 5), drinking water "AP" (n = 5), Caño (n = 5) and El Moralito: Caño El Padre (n = 5), Sector Las Brisas Kilometre 5 "Km 5" (n = 5). The sampling was non probabilistic, intentional or convenience. For humans were processed 45 stool samples, distributed as follows: 28 were for Juan de Dios González and 17 El Moralito. The age of participants ranged from 7 months to 73 years old. The approach followed in this case was included people residing in the nearby river, sewer, or water used for direct consumption (intake or recreational purposes).

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Legal, Ethical and written consent of parents and guardians. In the case of children, their parents and guardians were asked written permission and informed consent to collect data and samples, in accordance with the Declaration of Helsinki of the World Medical Association (17). The results were given to all participants at no cost.

Coproparasitoscopic analysis. Stool samples were observed for a period of no more than two hours from collection through direct examination of fresh (0.85% physiological saline and lugol) between film slide and slide cover slip and Kato - Katz modified. For samples of liquid consistency was used methylene blue or Quensel (18-19).

Water samples analysis. In each of the selected sectors 1L processed, taken about 30 - 50 cm below the water surface (AR, Caño, Caño El Padre, Km 5) and 500 mL drinking water (AP). The samples were collected in plastic containers, clean and screw caps. Mora *et al*. (14) describe a detailed processing of surface water, which was applied with some modifications in this study protocol. Briefly, the water collected was allowed to stand for a period of 48 hours, then 900 mL was discarded and the pellet (50 - 100 mL) was stirred and aliquots were transferred to plastic tubes with a capacity of 15 mL. In this part the samples were processed in duplicate: first, aliquots were centrifuged at 1,500 *g* for 10 minutes, the pellets were placed a drop of Lugol's solution for the preparation observed under the microscope at 10X and 40X light for search protozoa. Second, mixed in equal parts water and an aliquot of a physiological saline solution 0.85%, using gauze, the mixture was filtered, stirred vigorously and allowed to stand for 30 minutes for subsequent observation. To determine the most probable number (MPN) of coliform thermotolerant was realized a presumptive

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test and a confirmatory test in accordance with the second revision of the 1104 standard COVENIN (1996). Quickly, volumes of 1 mL or 100 uL was performed for each of the samples and / or dilutions respectively were added to 1% peptone water (10^{-1}, 10^{-2}, 10^{-3}) in each of 3 or 5 tubes with sterile of Lauryl Sulfate Tryptose broth, double concentrate. Tubes were then incubated at 35° C $\pm$ 1°C for 24 h $\pm$ 2 hours. At the end of this period, each tube was shaken to observe the production of gas and / or effervescence. In the tubes considered a positive test result presumption (by the presence of gas in Durham tubes after 48 hours of incubation) were inoculated using a calibrated loop sterile 3 mm, until broth enrichment for coliforms (*Escherichia coli* broth) previously placed in 45°C $\pm$ 0.2°C, followed by incubation in a water bath at the same temperature for 24 $\pm$ 2 hours, the tubes are checked and gas formation taken as positive. Then read the corresponding most probable number (MPN) in the results table respectively.

Statistical analyses. The results were presented as absolute values, percentages and in order to assess the possible independence between age groups (categorical variables), Fisher's exact test or Chi-square ($\chi 2$) were selected; and probability values to a confidence level of 95% for epidemiological variables considered. The use of Odds Ratio helped evaluate the risk factors using the Win Episcope 2.0 program (20).

RESULTS AND DISCUSSION

Fecal contamination of drinking or surface waters is one of the most disturbing problems in developing countries (3-4). It is argued that the presence of microorganisms in normal conditions is beneficial for surface water, but problems can arise when they present in concentrations and compositions altering water quality. In table I are

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presented in percentage terms protozoa and fecal coliforms in water samples, highlighting *Blastocystis* spp. in 92% of them and the complete correspondence with the presence of thermotolerant coliforms, whose number was consistently higher in 1,100 (> 1,100 CFU / mL; three tubes Durham), indicating fecal contamination of water of sectors Juan de Dios González and The Moralito, Colón Municipality. Also, were described the presence of the pathogen *Giardia* spp. and the commensal *Endolimax* sp. (see table I). This coincides with the results of Ávila *et al*., (12) and Mora *et al*., (14) for different regions of Venezuela. However, in the Colon Municipality of Zulia State, the prevalence was significantly higher (92%) compared with 25.33% obtained by Mora *et al*., (14) for *Blastocystis* spp. This could be explained by the smaller number of samples analyzed in this study (n = 25) compared with 75 surface water samples tested in the state of Sucre (14). Another important difference between these two schemes of work is the use of trichrome stain, Kinyoun stain, flocculation technique of surface waters and technical of Ritchie modified for human feces; which provides a clear added value in terms of sensitivity at scheme followed by Mora *et al*., (14). In this work the quantification of MPN of coliform thermotolerant was included to corroborate the parameter fecal contamination of water including the drinking water (see table I).

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Table I. Protozoa and thermotolerant coliforms in water samples of sectors Juan de Dios González and El Moralito, Colón Municipality, Zulia State.

	Water samples*									
Microorganisms	**Juan de Dios González**						**El Moralito**			
	AR		AP		Caño		Caño el Padre		Km 30	
Protozoa	n	%	n	%	n	%	n	%	n	%
Blastocystis spp.	5	100	3	60	5	100	5	100	5	100
Giardia spp.	5	100	0	0	0	0	4	80	0	0
Chilomastix sp.	5	100	0	0	5	100	5	100	5	100
Iodamoeba sp.	0	0	0	0	0	0	4	80	1	20
Endolimax sp.	5	100	0	0	5	100	3	60	4	80
Otras amebas	5	100	0	0	5	100	5	100	5	100
Thermotolerant coliforms*	5	100	3	60	5	100	5	100	5	100

*** From each of the samples were taken five replicates. AR = wastewater, AP = drinking water, * the most probable number (MPN) of thermotolerant coliforms was > 1,100 CFU / mL, n = number of samples where protozoa or thermotolerant coliforms were observed.**

The number of thermotolerant coliform obtained for drinking water samples (AP) (see table I) exceeded 100 times the level permitted by the COVENIN Venezuela standard for drinking water (less than 10 CFU / mL). The containers of these samples were contaminated with fecal matter. In 2006 Di Giovanny *et al*., (21) performed a field study in the Juarez Valley of Mexico to investigate the potential transmission of *Cryptosporidium* and *Giardia* to sheep livestock grazing on forage irrigated with reclaimed wastewater, and the potential for disease transmission back to humans. These

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authors concluded there was no evidence of zooanthroponotic transmission of *Cryptosporidium* or *Giardia*. In this report, *Giardia* spp. was described in water samples of sectors Juan de Dios González and Caño El Padre (table I), which represents a potential risk to people living in the vicinity of these tributaries of the Escalante River in the Colon Municipality. There are international regulations and standardized techniques for estimating intestinal pathogens in water samples (21-25). In the work of Di Giovanny *et al*. (21) the United States Environmental Protection Agency Method 1623 immunofluorescent assay (IFA) revealed high levels of pathogens in reclaimed wastewater, with 183 to >7000 *Giardia* cysts and 9 - 762 *Cryptosporidium* oocysts detected per litre. Infectious Cryptosporidium oocysts were detected in the reclaimed wastewater using the cell culture focus detection method (FDM). Polymerase chain reaction (PCR) analyses revealed reclaimed wastewater contained the *C. parvum* bovine (zoonotic) genotype, human-specific *C. hominis* subgenotypes, and *G. lamblia* assemblage A genotypes (A2 and A3). Despite high levels of *Cryptosporidium* and *Giardia* in the reclaimed wastewater, these pathogens were rarely found on the forage plants, possibly due to environmental attenuation. Moreover, the highest concentrations of indigenous oocysts were detected by method 1623 with the HV filter, which provided a sufficient number of oocysts for further confirmation of infectious potential. Confirmation of species and potential infectivity for all positive protozoan samples was made by using a nested PCR restriction fragment polymorphism assay and the focus detection method most-probable-number assay, respectively. The methodology and results described provide useful information for the establishment of pathogen numeric standards for reclaimed effluents used for unrestricted irrigation (22). Figure 1 shows the total number of parasitized and non-

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parasitized humans, with an overall prevalence of 71.11% for the communities of Juan de Dios González and El Moralito, similar to that obtained by the population of Orinoco La Peña, Sucre State (77.60%) by Mora *et al*., (14) and lower than the 27.3% obtained in Ciudad Bolivar, Bolivar State by Cermeño *et al*., (26). In sectors Juan de Dios González and El Moralito *Blastocystis* spp. was the most prevalent intestinal parasite in 62.22% of human fecal samples tested (Table II), was not difference by age and sex ($p > 0.05$) (data not shown).

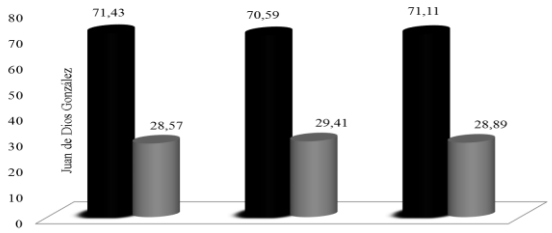

Figure 1. Parasitized and non parasitized population of sectors from Juan de Dios González and El Moralito, Colón Municipality, Zulia State. The black bar represents people parasitized and the gray bar represents people non parasitized.

Table II. Intestinal protozoa and helminths in the people of sectors Juan de Dios González and El Moralito, Colón Municipality, Zulia State.

	Juan de Dios González		El Moralito		Total	
Protozoa	n	%	n	%	n	%
Blastocystis spp.	18	64.29	10	58.82	28	62.22
Endolimax nana	10	35.71	9	52.94	19	42.22
Giardia duodenalis	3	10.71	1	5.88	4	8.89
Entamoeba coli	1	3.57	4	25.53	5	11.11
Iodamoeba butschlii	1	3.57	0	0.00	1	2.22

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Helmints						
Trichuris trichiura	3	10.71	2	11.76	5	11.11
Ascaris lumbricoides	2	7.14	2	11.76	4	8.89
Ancilostomideos	1	3.57	0	0.00	1	2.22

n = number of people parasitized.

Figure 2 shows the prevalence of mono-parasitism described in the sector Juan de Dios González (35.71%) and the bi-parasitism in the El Moralito (41.18%), and the samples non parasitized or polyparasitism in both communities. Chourio - Lozano et al., (27), estimated the prevalence of intestinal parasites in 78 children aged between two months and 12 years old, treated at the University Hospital of Autonomous Maracaibo, Zulia State. The prevalence of mono-parasitism was also found predominantly in groups of malnourished children, oncology, and clinically healthy children of the Maracaibo City. The polyparasitism predominated in immunocompromised children with HIV / AIDS.

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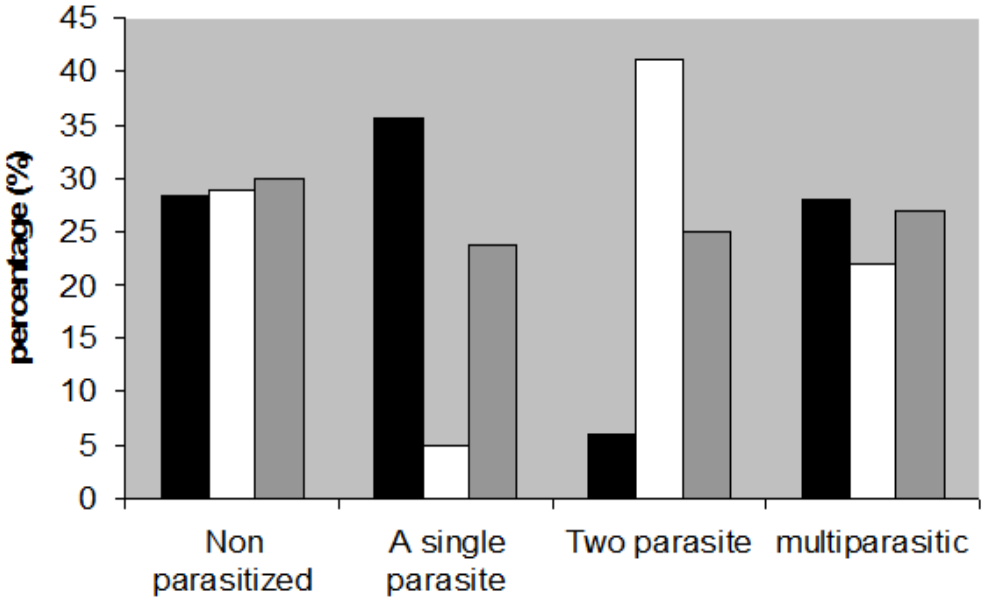

Figure 2. Distribution according to the number of parasites present in feces of people of Colón Municipality. The black bar represents people from Juan de Dios González.

With regard to partnerships between parasites, of the 23 possible associations between species of protozoa and helminths, in 16 of them were presence of *Blastocystis* spp. and *Endolimax* nana (data not shown). In the table III, among the factors epidemiological considered, the strata IV (Méndez - Castellanos clasification) and the previous history of intestinal parasitism were significantly associated with the presence of intestinal parasites in water samples and feces of humans of sectors Juan de Dios Gonzalez and El Moralito, Colón municipality. Calchi-La Corte *et al*., (27) reported the high prevalence of *Blastocystis* spp. and other commensals parasites in stool

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samples from neighboring Santa Rosa de Agua, Maracaibo, Zulia State; mainly due to poor environmental conditions in the community, along with the lack of personal hygiene habits of its people. Also they infer that an important risk factor is the type of housing (stilt houses) on Lake Maracaibo, where villagers living with water contaminated with sewage. Table III shows the main epidemiological factor associated with intestinal parasites were a family history of prior infection (probability "p" exact hypergeometric = 0.011, two-tailed p = 0.01645 exact).

Table III. Epidemiological aspects associated with intestinal parasites in inhabitants of the sectors Juan de Dios González and El Moralito, Colón Municipality, Zulia State.

Variable	Intestinal parasite						Comment
	Present		Absent		Total		
	n	%	n	%	n	%	
Social stratum							
Stratum III	5	11.11	5	8.89	9	20.00	**X^2 = 3.01; 2 degrees of freedom; p = 0.222; Cramer's V = 0.2558
Stratum IV	20	44.44	6	13.33	26	57.78	
Stratum V	8	17.78	2	6.67	11	24.44	
Overcrowding							*p exact hypergeometric = 0.1624; p two-tailed exact = 0.4112
Present	27	60.00	9	20	36	80.00	
Absent	5	11.11	4	8.89	9	20.00	
Water quality for human comsuption							
Adequate	2	4.44	1	2.22	3	6.67	*p exact hypergeometric = 0.4544; p two-tailed exact = 1.0
Inadequate	30	66.67	12	26.67	42	93.33	
Handwashing							
Adequate	19	42.22	8	17.78	27	60.00	**X^2 = 0.04; 1 degree of fredom; p≥0.5; OR = 0.97; range (1.31 to 0.72)
Inadequate	13	28.89	5	11.11	18	40.00	
Washing food							
Adequate	17	37.78	13	28.89	30	66.67	*p exact hypergeometric = 0.2159; p two-tailed exact = 0.3634
Inadequate	15	33.33	0	0.00	15	33.33	
Excreta disposal							
Adequate	18	40.00	9	20.00	27	60.00	*p exact hypergeometric = 0.1964; p two-tailed exact = 0.5136
Inadequate	14	31.11	4	8.89	18	40.00	
Garbage collection							

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Adequate	20	44.44	10	22.22	30	66.67	*p exact hypergeometric = 0.1872; p
Inadequate	12	26.67	3	6.67	15	33.33	two-tailed exact = 0.4917
Denture							
Spotted / mottled	30	66.67	0	0.00	30	66.67	This factor shows no association with intestinal parasites, is included because it is an endemic area for dental fluorosis.
Unblemished	2	4.44	13	28.89	15	33.33	
History of intestinal parasites							
Present	15	33.33	1	2.22	16	35.56	*p exact hypergeometric = 0.011; p
Absent	17	37.78	12	26.67	29	64.44	two-tailed exact = 0.01645
Prior antiparasitic treatment							
Present	10	22.22	1	2.22	11	24.44	*p exact hypergeometric = 0.082; p
Absent	22	48.89	12	26.67	34	75.56	two-tailed exact = 0.1361

* The calculation of the value of probability was performed using Fisher's exact test, because the 2X2 contingency tables had observed frequencies below 5; ** the calculation of Chi square test was performed with Yates correction. p = probability, n = number of observations for each variable.

CONCLUSIONS

Blastocystis spp. is present in 92% of water samples tested (drinking water, surface waters, and wastewater) in sectors of Juan de Dios Gonzalez and El Moralito in the municipality Colón, Zulia state. Likewise, the presence of *Endolimax* sp. and *Giardia* spp. coincides with the presence of thermotolerant coliforms, and the finding of these same organisms in feces of humans, was due to consumption of drinking water and foods contaminated with fecal matter. The programming, implementation and monitoring of basic health measures for treatment of surface waters, the solid waste collection, proper hand washing and food are needed in the communities included in this study.

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