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HEALTH SCIENCES

Anthelmintic treatment follow up in a rural community in Camamu, Bahia, Brazil

MARINA M.B. FARIAS, NILO MANOEL P.V. BARRETO, WÉSLEI A.C. ARAÚJO, CÍNTIA DE LIMA OLIVEIRA, NOILSON LÁZARO S. GONÇALVES, KAMILA S.S. CAMPAGNOLLO, BERNARDO GALVÃO-CASTRO, MÁRCIA CRISTINA A. TEIXEIRA, NECI M. SOARES & JOELMA N. DE SOUZA

Abstract: Enteroparasites are an important public health problem and the treatment seeks to cure and reduce transmission. The aim of this study was to evaluate the therapeutic efficacy of anthelmintic treatment in individuals living in a rural community area in Camamu, Bahia, Brazil. The parasitological diagnosis was performed by spontaneous sedimentation, Baermann-Moraes and Agar Plate Culture methods. A total of 212 individuals were evaluated. The most frequent helminth was *Trichuris trichiura*, 24.5% (52/212), followed by *Ascaris lumbricoides*, 21.2% (45/212), hookworms, 16.5% (35/212), and *S. stercoralis,* 4.7% (10/212). In the anthelmintic treatment follow up, *T. trichiura* infection presented the lowest parasitological cure rate, only 60.6% (20/33). Hookworm, *Ascaris lumbricoides* and *Strongyloides stercoralis* infections demonstrated cure rates of 70.5 (12/17), 78.1 (25/32) and 100% (5/5), respectively. Individuals who remained infected underwent a new drug therapy. The second parasitological cure rate for *T. trichiura* was 38.5% (5/13), and 66.7% (2/3) and 75% (3/4) for hookworms and *Ascaris lumbricoides*, respectively. *Trichuris trichiura* infection presented the lowest parasitological cure rate at this second evaluation. This reinforces the need to perform a follow-up of all treated individuals. The possibility of drug resistance denotes the necessity for studies to clarify the mechanisms and to evaluate new therapeutic approaches.

Key words: Diagnosis, Enteroparasites, Follow-up, Treatment.

INTRODUCTION

Intestinal parasites are an important public health problem and affect mainly underdeveloped and developing countries, where there are poor sanitary and socioeconomic conditions, associated with difficulties in accessing health services and the deficiency of educational programs (Camello et al. 2016, Oliveira et al. 2018). The Organización Panamericana de la Salud (2018) (The Pan American Health Organization) estimated that 820 million individuals were infected with *Ascaris lumbricoides*, 460 million with *Trichuris trichiura* and 440 million with hookworms. In addition, about 600 million people can be infected with *Strongyloides stercoralis* worldwide (Buonfrate et al. 2020). In Brazil, parasites have a wide geographical distribution and can be found in both rural and urban areas (Schnack et al. 2003). The parasites can cause several injuries to the host's health, such as: electrolyte imbalance, intestinal obstruction, abdominal pain, nausea, weight loss, malnutrition, among others. The characteristics of these organic changes depend on factors related to the parasite - such as the specie and/or parasitic load - as well as the nutritional status and the immune response of

the host (Colli et al. 2014, Dos Santos & Merlini 2010).

The laboratory diagnosis of enteroparasitic infection is performed mainly by parasitological examination of fecal samples through morphological analyzes. Once the diagnosis is made, the treatment aims to cure and reduce transmission. Benzimidazoles are the drugs recommended by the World Health Organization (WHO 2013) for the treatment of some helminths species, as they are low-cost, have a broad-spectrum and are easy to administer (Brasil 2018, Clarke et al. 2019, Urbani & Albonico 2003, Centers for Disease Control and Prevention 2019a). A meta-analysis study demonstrated that both, Albendazole 400 mg and Mebendazole 500 mg, in a single dose, were effective in treating *A. lumbricoides* infection, with cure rates of 95.7 and 96.2%, respectively. For infections caused by *T. trichiura*, benzimidazoles did not render an efficacious outcome with a cure-rate ranging from 30.7 to 42.1% (Moser et al. 2017). Thus, the therapeutic options for *T. trichiura* infection remain a challenge. For *S. stercoralis* infection, ivermectin has the best therapeutic response (Centers for Disease Control and Prevention 2019b).

For any therapeutic treatment of parasite infections there is the risk of drug resistance and reinfection (Orr et al. 2019, Olliaro et al. 2011), which requires a treatment follow-up using parasitological methods with a high sensitivity and the examination of more than one sample. Once the treatment may not lead to a parasitological cure, but only to a reduction in the parasite load, it is possible to misdiagnose the infection due to the low excretion of parasites in the feces. In this study, the therapeutic efficacy of anthelmintic drugs was evaluated in individuals living in a rural community in Camamu County, Bahia, Brazil.

MATERIALS AND METHODS

Study population

All residents of a rural community called Zumbi dos Palmares Settlement (ZPS), Camamu, Bahia, Brazil, were invited to participate in the study, which was carried out from October 2018 to June 2019. The settlement is located 12 km from Camamu County and 335 km from Salvador, the Bahia state capital. It has a total area of 400 hectares, where there are approximately 50 families, adding up to 250 inhabitants. Camamu is located on the Costa do Dendê, on Bahia's southern coast, near the BA-001 highway.

The Committee of Ethics in Research of the Pharmacy School, Federal University of Bahia, Brazil, approved this study, under the registry number 2.616.338/2018. A written informed consent for participation was obtained from each individual who agreed in participate in this study. For individuals under 18 years-old, consent was obtained from their guardians. In addition, children over seven years old also signed an assent form.

Parasitological diagnosis

The parasitological diagnosis was performed by spontaneous sedimentation (Hoffman et al. 1934), Baermann-Moraes (Rugai et al. 1954), and Agar Plate Culture (CPA) (Koga et al. 1992) methods at the Immunoparasitology Research Laboratory, Pharmacy College, Federal University of Bahia, Brazil. The fecal samples were collected without preservative solution and were processed immediately by CPA and Baermann-Moraes methods and after about 6 hours for spontaneous sedimentation.

Treatment and cure control

Patients infected with *A. lumbricoides*, *T. trichiura*, *E. vermicularis* and hookworms were treated with Albendazole 400mg, and those who

were infected with *S. stercoralis* were treated with Ivermectin 200µg/kg. In both cases, the drugs were administered in a double dose regimen, given 15 days apart. Individuals who did not present a parasitological cure with the first treatment, underwent a second drug therapy. For *A. lumbricoides* and hookworms, the first therapy was repeated. For *T. trichiura* infections, the patients were treated with both, Albendazole 400 mg and Ivermectin 200µg/kg, also in a double dose with a 15 days interval.

The treatment follow-up was performed, and three stool samples were analyzed 30, 60 and 90 days after the end of the treatment regimen (both drug doses), using three parasitological methods. Parasitological cure was obtained when there were no parasites in the feces. Individuals infected with protozoa and *E. vermicularis* were not included in the study to assess response to treatment due the small sample size (n <4).

Actions to prevent enteroparasites transmission

After the study, educational activities were carried out through health educational workshops with dialogues and games focused on hygiene and prophylactic activities to prevent the transmission of the enteroparasites found in the community.

Statistical analysis

Statistical analyses were performed using the Statistical Package for Social Science (SPSS) software, version 19.0 for Windows (SPSS Inc., Illinous, Chicago, USA) and Microsoft Excel (Microsoft, Redmond, WA). Qualitative variables were presented in terms of frequency.

RESULTS

A total of 212 individuals were evaluated, 49.5% (105/212) male and 50.5% (107/212) female. The majority were aged between 20 and 59 years-old, 45.3% (96/212), with a mean age of 29.6 ± 21.2 years. According to socioeconomic data, 88.7% (188/212) had a monthly income less than or equal to one Brazilian minimum wage (about USD 250). More than 50% reported not having completed elementary school and only 7.5% (16/212) completed high school. None of the residents had access to piped water, sewage or paved streets. About 80% (168/212) had a bathroom at home, however only 34.4% (73/212) had a sink in it. A total of 78.8% (167/212) reported the habit of walking barefoot and 92.5% (196/212) had direct contact with earth.

An enteroparasite frequency of 72.2% (153/212) was observed. The most frequent helminth was *T. trichiura*, 24.5% (52/212), followed by *A. lumbricoides*, 21.2% (45/212), hookworms, 16.5% (35/212), and *S. stercoralis,* 4.7% (10/212). Both *Giardia duodenalis*, 5.2% (11/212), and the complex *Entamoeba histolytica / dispar / moshkovskii*, 4.7% (10/212), were found among the protozoa (Table I).

All infected patients were treated with were treated with anthelmintics. In the follow-up, after analyses of all three fecal samples (at days 30, 60 and 90 post-treatment), it was observed that *T. trichiura* infection presented the lowest parasitological cure rate, 60.6% (20/33). The treatment of hookworms and *A. lumbricoides* infections demonstrated cure rates of 70.5 (12/17) and 78.1% (25/32), respectively (Table II). Parasitological cure was observed in all individuals treated for *S. stercoralis* infection.

The treatment follow-up was performed by the analysis of three fecal samples 30, 60 and 90 days after the drug administration (Figure 1). For all infections, except *S. stercoralis*, a decrease

Table I. Number of individuals infected with enteroparasites living in the Zumbi dos Palmares Settlement, Camamu, Bahia, Brazil (n = 212).

Parasite	Number of positive samples (%)
Infected individuals	153 (72.2)
Non-infected individuals	59 (27.8)
Individuals with one parasite	60 (28.3)
Individuals with two or more parasites	93 (48.4)
Helminths	
Trichuris trichiura	52 (24.5)
Ascaris lumbricoides	45 (21.2)
Hookworm	35 (16.5)
Strongyloides stercoralis	10 (4.7)
Enterobius vermicularis	8 (3.8)
Protozoa	
Entamoeba histolytica/díspar/moskovskii	10 (4.7)
Giardia duodenalis	11 (5.2)
Entamoeba coli	73 (34.4)
Endolimax nana	67 (31.6)
Iodamoeba bütschlii	18 (8.5)

in the parasitological cure rate was observed throughout the follow-up period.

Individuals who did not present parasitological cure underwent a second drug therapy and, again, *T. trichiura* infection demonstrated the lowest parasitological cure rate, 38.5% (5/13). The second treatment for *A. lumbricoides* and hookworm infection presented cure rates of 75 (3/4) and 66.7% (2/3), respectively (Table III).

DISCUSSION

Intestinal parasitic infections are associated with poor sanitary and socioeconomic conditions, which is experienced by a large part of the Brazilian population, especially in rural areas (Camello et al 2016, Fonseca et al 2010). In this study, an elevated frequency of enteroparasites was observed, 72.2% (153/212). These data are similar to the results presented in other studies in Brazilian rural areas, where prevalence rates range from 69.5% to 76.9% (Souza et al 2016, Neres-Norberg et al. 2014). Among the intestinal helminthiasis, *T. trichiura* infection had the highest frequency, 24.5% (52/212), followed by *A. lumbricoides*, 21.2% (45/212), hookworm, 16.5% (35 / 212), and *S. stercoralis* infections, 4.7% (10/212). This data corroborates with other studies carried out in Brazil in different areas (Cunha et al. 2013, Eustachio et al. 2018, Inês et al. 2011, Neres-Norberg et al. 2014). Environmental conditions, such as a hot and humid weather, associated with a deficiency of basic sanitation, enabled the development and transmission of geohelminths in the Zumbi dos Palmares Settlement. The high prevalence of infections by commensal protozoa, such as *Entamoeba coli*, 34.4% (73/212) and *Endolimax nana*, 31.6% (67/212), is also an important indicator of fecal-oral contamination (Soares et al. 2019), which reflects the absence of health guidelines for preventing the transmission of enteroparasites and confirms data from other studies (Vilar 2017, Neres-Norberg et al. 2014, Cunha et al. 2013).

The treatment of parasitic infection aims to cure and, consequently, reduce transmission. The drug recommended by the Brazilian Ministry of Health (2018) for control and treatment of geohelminths (*A. lumbricoides*, hookworms and *T. trichiura*) is Albendazole 400mg, single dose (Brasil 2018), which provides high cure rates for ascariasis, as demonstrated in a meta-analysis study by Moser et al. (2017), where the cure rate reached 95.7%. This has also been demonstrated in other studies, with cure rates ranging from 98.2 to 99.4% (Moser et al. 2017, Tefera et al. 2015, Vercruysse et al. 2011). For hookworm infections, some authors have demonstrated cure rates

Table II. Treatment follow-up of individuals infected with helminths, residing in the Zumbi dos Palmares Settlement, Camamu, Bahia, Brazil, after the analyses of three fecal samples 30-, 60- and 90-days post-treatment.

PARASITE	Infected individuals (n)	Treated individuals		Parasitological cure rate (n)
		Without follow-up*	With follow-up**	
T. trichiura	52	19	33	60.6 (20/33)
A. lumbricoides	45	13	32	78.1 (25/32)
Hookworm	35	18	17	70.5 (12/17)
S. stercoralis	10	5	5	100 (5/5)

*** Without follow-up = treated individuals who did not collected post-treatment fecal samples.**
**** With follow-up = treated individuals who collected post-treatment fecal samples.**

between 79.5 and 87.8% (Clarke et al. 2019, Moser et al. 2017, Vercruysse et al. 2011). Vercruysse and collaborators (2011) evaluated the therapeutic response of *A. lumbricoides* and hookworms to this treatment, analyzing one fecal sample, thirty days after treatment. They demonstrated cure rates of 98.2 and 87.8%, respectively. This corroborates with the results found in this study, when only one stool sample, after thirty days, was evaluated. However, after analyzing three samples, the cure rate decreased to 78.1 and 70.5%, respectively, reaffirming the need for at least three stool samples to assess cure control.

Benzimidazoles have limited efficacy for *T. trichiura* infections. Some studies have demonstrated low cure rates, between 42.1 to 59.9% (Moser et al. 2017, Tefera et al. 2015, Vercruysse et al. 2011). Adegnika and colleagues presented a cure rate of 67% with two doses of Albendazole (400 mg). In this study, a parasitological cure rate of 60.4% was found, which is in agreement with the results described above. The hypothesis of genetic resistance of these parasites to benzimidazoles, through a polymorphism in the nucleotides, has not yet reached conclusive results (Matamoros et al. 2019, Hansen et al. 2013). Therefore, other studies are essential to elucidate the parasite genetic factors in inducing drug resistance. A meta-analysis study demonstrated an increase in the cure rate after the combination of two drugs, Albendazole and Ivermectin (Clarke et al. 2019). However, there are controversial results for this same combination, with therapeutic efficacy ranging from 27.5 to 38%, but with a significant reduction in egg count (Knopp et al. 2010, Speich et al. 2015). In this study, individuals resistant to the first treatment with Albendazole (400mg) were treated with the combination of Albendazole / Ivermectin and a cure rate of only 38.46% (5/13) was obtained. Factors associated with the parasite, such as single-nucleotide polymorphisms (SNPs) in the beta-tubulin gene, are associated with resistance to benzimidazoles in nematodes. In fact, a recent study detected this *T. trichiura* polymorphism in Brazil (Oliveira et al. 2022). Also, reinfection should be considered, as well as host-specific mechanisms related to the low therapeutic response. Due to the COVID-19 pandemic, it was not possible to continue the treatment follow-up. About two years later there was a return to the community, where new parasitological examinations were carried out and the infected individuals were referred for treatment at the Brazilian Medical Health Service and the project was discontinued.

For *S. stercoralis* infections, the treatment with Ivermectin 200µg / kg in a single dose, can reach 88% efficacy. When a second dose is administered, the cure rate increases to 96% (Zaha et al. 2002, Repetto et al. 2018). In this

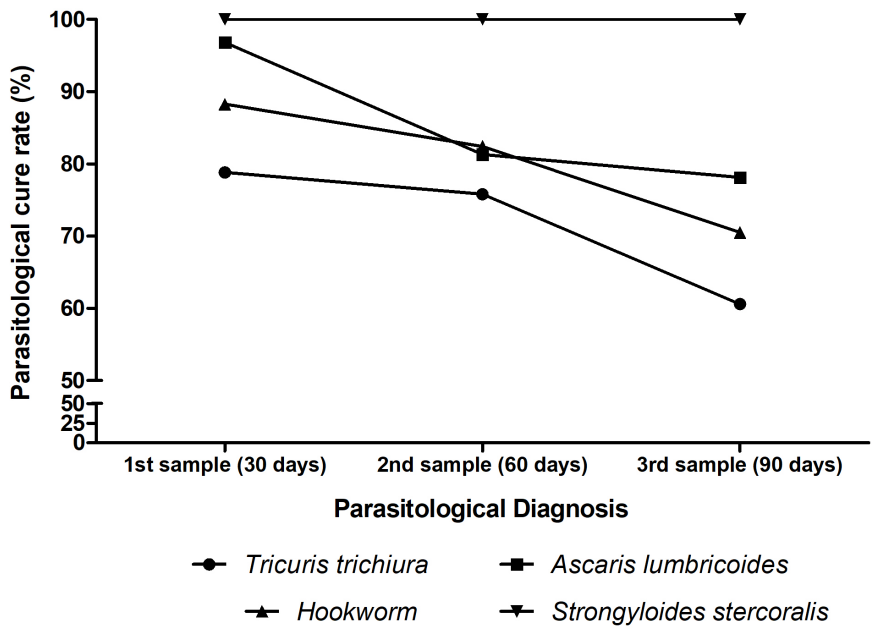

Figure 1. Parasitological cure rate of patients infected with *T. trichiura* (n = 33), *A. lumbricoides* (n = 32), hookworms (n = 17) and *S. stercoralis* (n=5) through the analysis of three fecal samples analyzed 30, 60 and 90 days post-treatment.

Table III. Follow-up of the second drug therapy of individuals infected with helminths, residing in the Zumbi dos Palmares settlement, Camamu, Bahia, Brazil, 30 days after the second drug regimen.

Helminths	Infected individuals (n)	Treated individuals		Parasitological cure rate (n)
		Without follow-up*	With follow-up**	
T. trichiura	13	0	13	38.5 (5/13)
A. lumbricoides	7	3	4	75.0 (3/4)
Hookworm	5	2	3	66.7 (2/3)

*** Without follow-up = treated individuals who did not collected post-treatment fecal samples.**
**** With follow-up = treated individuals who collected post-treatment fecal samples.**

work, in individuals submitted to treatment with two doses, with an interval of 15 days, a parasitological cure of 100% was observed. This was confirmed through the analysis of three stool samples with three parasitological methods, one of which was agar plate culture – the gold standard for strongyloidiasis diagnosis (Inês et al. 2011).

Thus, in this study, it was possible to observe a high frequency of enteroparasites in a rural community, the Zumbi dos Palmares Settlement, Camamu, Bahia, Brazil. The treatment of *A. lumbricoides*, hookworms and *S. stercoralis* infections demonstrated high cure rates with the therapeutic protocols already used. However, a low cure rate was found in the treatment of *T. trichiura* infection, even when a second therapy with a combination of anthelmintics was administered. In this manner, the cure control of anthelmintic therapies with at least three stool samples and different parasitological methods, are essential to evaluate the drug efficacy and control of parasitic transmission. Studies evaluating resistance to anthelmintics are still very limited, as are those evaluating new therapeutic options. Parasitic diseases mainly affect populations living in vulnerable socioeconomic conditions and an increase of public investment is necessary to interrupt the cycle of poverty and disease.

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MARINA M.B. FARIAS[1]
https://orcid.org/0000-0003-3972-7138

NILO MANOEL P.V. BARRETO[1]
https://orcid.org/0000-0002-1397-1362

WESLEI A.C. ARAÚJO[1]
https://orcid.org/0000-0001-6491-6702

CÍNTIA DE LIMA OLIVEIRA[1]
https://orcid.org/0000-0002-5355-8695

NOILSON LÁZARO S. GONÇALVES[2]
https://orcid.org/0000-0001-5076-2976

KAMILA S.S. CAMPAGNOLLO[1]
https://orcid.org/0000-0002-2794-076X

MÁRCIA CRISTINA A. TEIXEIRA[1]
https://orcid.org/0000-0003-0477-5092

BERNARDO GALVÃO-CASTRO[3]
https://orcid.org/0000-0002-0644-6471

NECI M. SOARES[1]
https://orcid.org/0000-0003-1409-9884

JOELMA N. DE SOUZA[1]
https://orcid.org/0000-0002-1456-9009

[1]Universidade Federal da Bahia/UFBA, Faculdade de Farmácia, Departamento de Análises Clínicas e Toxicológicas, Rua Barão de Jeremoabo, 147, Ondina, 40170-115 Salvador, BA, Brazil

[2]Centro de Pesquisas Gonçalo Moniz (Fiocruz), Laboratório de Saúde Pública/LASP, Rua Waldemar Falcão, 121, Candeal, 40296-710 Salvador, BA, Brazil

[3]Centro Integrativo e Multidisciplinar de Atendimento ao Portador de HTLV, Escola Bahiana de Medicina e Saúde Pública (CHTLV/EBMSP), Av. Dom João VI, 275, Brotas, 40290-000 Salvador, BA, Brazil

Correspondence to: **Joelma Nascimento de Souza**
E-mail: joelmandesouza@gmail.com; joelmans@ufba.br

Author contributions

Conceptualization, Resources, Supervision, Funding Acquisition N.M.S.; Methodology, Writing-Review and Editing J.N.d.S.; N.M.S., and M.C.A.T.; Formal Analysis and Data Curation M.M.B.F. and J.N.d.S.; Investigation, M.M.B.F., N.M.P.V.B, W.A.C.A., C.d.L.O., N. L. S. G., K. S. S. C., B. G. C.; Writing—Original Draft Preparation, M.M.B.F.; Visualization, J.N.d.S. and N.M.S.; Project Administration, N.M.S. and M.C.A.T.