

# Efficacy of 5-nitroimidazole compounds for giardiasis in Cuban children: systematic review and meta-analysis

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## SUMMARY

Five-nitroimidazole (5-NI) compounds are among the most commonly used medications in the treatment of giardiasis. However, after more than five decades of their initial indication for such treatment, there are some concerns about the efficacy of 5-NIs in giardiasis. This study sought to compare the efficacy of any 5-NI with any other anti-giardial drug for the treatment of Cuban children with giardiasis. We conducted a systematic review and meta-analysis of randomized controlled trials (RCTs). We searched CUMED, EBSCOhost and PubMed databases. Two reviewers independently assessed trial eligibility, trial quality and extracted appropriate data. The primary outcome was the parasitological cure. The effect estimate was the pooled relative risk (RR) with 95% confidence intervals (CI). We included seven RCTs in the systematic review,

involving a total of 1046 children. When the effect of 5-NIs was compared with that of benzimidazole compounds, the pooled effect was significant and favored 5-NIs [the relative risk (RR) is 1.35, 95% CI 1.05 to 1.75], with high heterogeneity (4 studies,  $I^2=79%$ ). Compared with chloroquine, the pooled effects of the 5-NIs were not significant [RR is 0.96, 95% CI=0.79 to 1.18, (2 studies,  $I^2=68%$ )]. Our results support the use of 5-NIs (mainly tinidazole) as first-line therapy for Cuban pediatric patients infected with *Giardia* and may continue being used as reference drugs in future RCTs of giardiasis. These data could help inform policy decisions in Cuba. Caution is needed in extrapolating such data in other settings.

*Keywords:* nitroimidazole, giardiasis

## INTRODUCTION

*Giardia lamblia*, the first protozoan agent associated with human disease, has a world-wide distribution. It is transmitted through the faecal-oral route following direct or indirect contact with environmentally resistant cysts, principally, via contaminated water and food. Giardiasis, the

disease it causes, is an important public health problem, mainly for low- and middle-income countries, where it is considered to be an important cause of morbidity [1]. It is estimated that giardiasis causes annually 184 million clinical cases and an associated 171,100 (115,777-257,315) disability-adjusted life years [2, 3].

In Cuba, a national prevalence survey, conducted in 2009, found an overall prevalence of *Giardia* infection of 6.02%, similar to the former prevalence of 7.2% found in the 1984 national survey [unpublished report]. However, a higher prevalence is reported in other settings including day-

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care centres and primary schools [4-8]. Although *Giardia* affects people of all conditions and ages, children are at greatest risk to present giardiasis, presumably because of poor hygiene as a facilitator of the faecal-oral transmission of *Giardia* cysts [9]. Clinical giardiasis seems to be also a common reason for hospitalisation in paediatric hospitals in Havana, the capital of Cuba [10-12].

Effective anti-giardial chemotherapy began in 1940s with quinacrine (QC), an acridine derivative first introduced for malaria therapy in the 1930s, [13]. QC was replaced by other drugs, as metronidazole (MTZ) and other 5-nitroimidazole (5-NI) compounds [tinidazole (TNZ), ornidazole, secnidazole (SNZ)], and also with nitrofuranes (furazolidone) and aminoglycosides (paromomycin) [14, 15].

Currently, 5-NI compounds are the recommended first line therapy for children and adults infected with *Giardia* [1]. However, some individuals experience treatment failures, despite having received successive courses of treatment that have been documented to result in a cure for most patients [16]. Additionally, non-clinical factors such as patient expectations and demand, and misperceptions about efficacy of anti-giardial drugs, principally MTZ, are important issues in the Cuban population which could impact on the prescription of 5-NIs as first-line drugs to treat giardiasis [17, 18]. Several systematic reviews and/or meta-analyses on the efficacy of treatments for giardiasis have been published, and although the efficacy of anti-giardial drugs is known, there is a lack of knowledge in Cuba, about treatment efficacy within homogeneous groups of children, and its determinants [19-24]. Therefore, the objective of this systematic review and meta-analysis is to properly document and evaluate the current evidence on the efficacy of 5-NIs in the treatment of Cuban children with *Giardia* infection. With this review we hope to produce and provide a reliable, convenient and evidence-based summary of the primary literature in support of policy decisions both in Cuba and other similar countries.

## ■ MATERIALS AND METHODS

This review and meta-analysis was conducted according to the Preferred Reporting Items for Systematic Reviews and Meta-analysis (PRISMA) statement [25].

### *Search strategy*

Electronic literature searches are current to March 15, 2018. We searched the following computerized biomedical databases: CUMED, PubMed, and EBSCOhost; all of them from 2000 to 2017. CUMED is a specific Cuban bibliographic database that records the Cuban scientific production in medical and related sciences. It includes bibliographic references and summaries of journal articles, books or book chapters, academic thesis, and congresses communications published in Cuba or outside Cuba by Cuban authors. We used combinations of free and controlled language for the following terms: *Giardia* OR giardiasis for CUMED; *Giardia* OR giardiasis AND Cuba OR Cuban AND "randomized clinical trial" for PubMed; and *Giardia* OR giardiasis AND Cuba OR Cuban, for EBSCOhost. We did not apply language restrictions. If needed the corresponding authors were contacted for further information to assess methodological details and supplementary data. Abstracts and full text papers from eligible studies were reviewed, and those meeting our predefined selection criteria were considered for inclusion.

### *Inclusion and exclusion criteria*

The inclusion criteria were: (i) RCTs evaluating the efficacy of 5-NI with a control condition (placebo, another active treatment); (ii) study population of patients with parasitologically-demonstrated giardiasis and measured parasitological cure (defined as a negative *Giardia* in faecal specimens at the follow-up); (iii) previously untreated patient population, (iv) full text study available or supplementary data provided by corresponding authors on request, and (v) included participants aged <18 years.

We excluded studies if: (i) they included a mixed population of adults and children and responses of the two groups were not differentiated; and (ii) efficacy data (parasitological cure rates) were not available or could not be obtained for the study groups.

### *Study selection and data extraction*

The list of retrieved articles was independently reviewed by two investigators (AAE and PA), and disagreements were discussed and resolved by consensus. The same investigators independently extracted data characteristics and effect sizes for the included studies.

We extracted the following information: (1) study author, and year of publication; (2) study characteristics (study setting, duration of follow-up, and sample size); (3) participant characteristics (participants by treatment arm sex, and age); (4) diagnostic test used for giardiasis and for assessing parasitological cure; (5) presence of signs and/or symptoms at diagnosis; characteristics of interventions (including the type of 5-NI and dose/duration, comparator drug and dose/duration). Another author (JB) reviewed the data extraction forms for inconsistencies and discrepancies were resolved by discussion prior to include data in the meta-analysis.

#### Outcome

The primary outcome measure was the parasitological efficacy defined as the absence of detectable *Giardia* cysts or trophozoites at the end of the treatment in at least two consecutive faecal microscopic examinations.

#### Assessment of methodological quality

Studies that met inclusion criteria were graded for methodological quality using a scale reported by Jadad et al. [26]. Jadad quality scores are based on the description and appropriateness of randomization, adequacy and appropriateness of blinding, and descriptions of withdrawals and dropouts, and can range from 0 to 5, with higher scores indicating better methodological quality (*i.e.*, a score of 0-2 was considered as low quality trial and a score of 3-5 was considered high quality trial). The quality of parasitological diagnostic methods was separately assessed by the scoring system utilised by Zaat et al. and Solaymani-Mohammadi et al. This method evaluates whether techniques are sufficiently described and adequate [19, 20].

#### Statistical analysis

We used as effect size the pooled relative risk (RR) with 95% confidence intervals (CI). A 95% CI for the RR that does not include the value for the null hypothesis of no difference (RR=1) favors 5-NI treatment for all comparisons. Heterogeneity (degree of difference between the results of different trials) was assessed with the  $I^2$  index that estimate the proportion of total variability in pooled estimates that could be attributed to between-trials heterogeneity, rather than by a chance [27].

We pooled effect sizes with the Mantel-Haenszel method using a random effects model and if data

permitted we also estimated a predictive value for the RR that could be expected for new studies [28]. We performed a supplementary analysis pooling efficacy rates within treatments to enhance the interpretation of the comparative analyses. Statistical analyses were performed with the R package using the library *meta* [29, 30].

## RESULTS

#### Search results

The initial search yielded 125 records (65 from CUMED, 52 from EBSCOhost and 8 from PubMed), of which 111 remained after removal of duplicates (see PRISMA flow diagram, Figure 1). Through a review of titles and abstracts, 103 studies were rejected as irrelevant. The remaining potentially relevant seven articles were reviewed and assessed for satisfaction of the inclusion or exclusion criteria. The seven articles met all criteria and were included in the analysis. References cited in the retrieved articles were manually reviewed to identify additional published work not indexed in the databases but we did not find any fitting our inclusion criteria.

#### Description of included studies

In the present analysis 5-NIs were compared with benzimidazoles (3 comparisons), chloroquine (2 comparisons), aminosidine (1 comparison) and nitazoxanide (1 comparison) [31-37]. The key characteristics of the included studies are presented in Table 1.

#### Study characteristics and methodological quality

The seven studies (Table 1) included a total of 1046 children. All studies were conducted in outpatient population. Samples sizes were small (ranging from 92 to 256, duration of 5-NI regimen varied from 1 day, 5 days, to 7 days. All trials were open. The post-treatment follow-up time varied across the studies from 7 days to 21 days [31-37]. By the quality of studies evaluated by Jadad scale; all studies were considered of high quality (Table 2). All studies were described as randomized, and all of them described appropriately the randomization procedure. Although none of the studies were appropriately blinded for participants, in all of them the laboratory personnel checking the post treatment fecal samples were unaware of the treatment allocation. Loss of follow-up did not

occur in six studies whereas one study reported patient withdrawals [37].

*Parasitological examination*

In all studies, the absence of detectable *Giardia* trophozoites and/or cysts in the fecal microscopy during the follow-up period was required to declare the children cured. All studies implemented two different parasitological methods for diagnosis at the same time (direct fecal microscopy in combination with ether concentration technique). All trials scored 8 points out of a maximum of 15, indicating a modest quality. It was mainly due to the relatively great weight of the item "repeated examinations at follow-up". Inter-observer variation was not described in any trial (Table 3). The quality of parasitological examinations was assessed according to the following:

- Description of technique: Two points for a description of the laboratory technique. Rate 0 if there is only a statement about "parasitological examination" without further description.
- Adequate techniques: in each study, we first distinguished whether a fresh stool specimen, fixed or a duodenal aspirate (where a duodenoscopy and brush) was used. Then the examination technique was scrutinized, scoring as tabulated.
- Credit points:
  - one credit point if more than one technique is used at the same time;
  - one credit point if fresh and fixed stools are examined at the same time;
  - one credit point if stool specimen and duodenal aspirate are used at the same time.
- Repeated examinations: Rate 3 when more than one stool specimen was examined at dif-

	<i>Fresh</i>	<i>Fixed</i>	<i>Duodenal aspirate</i>
Direct (saline, eosine, Lugol)	0	0	1
Concentration (Faust, Ritchie, etc.)	2	3	0
Immunological (ELISA)	3	3	0
Permanent stained smear	0	4	4

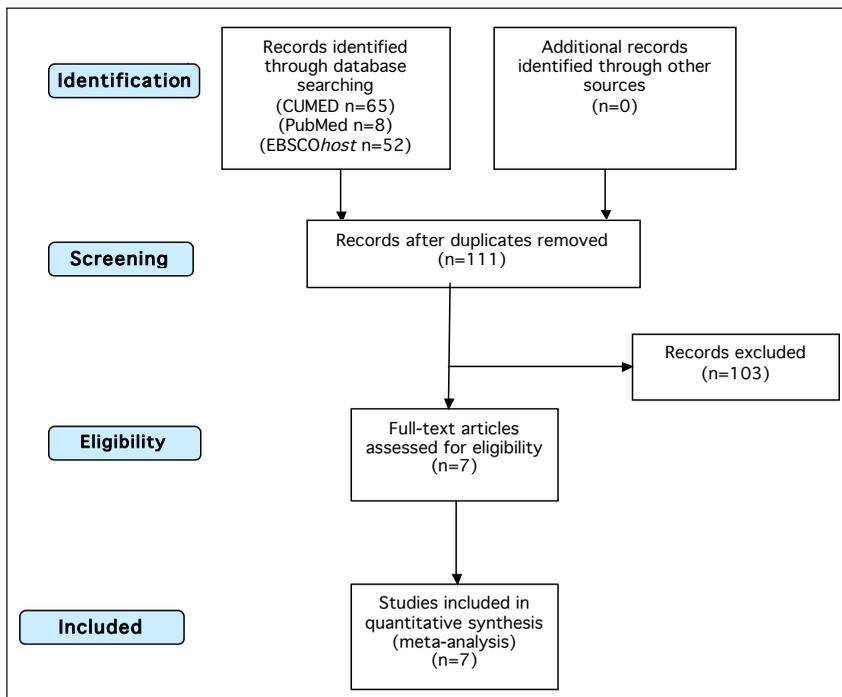


Figure 1- Search strategy for identification of articles (Flow chart).

**Table 1 - Characteristics of the randomized controlled trials included in the meta-analysis**

Author, Year [Reference]	Study design	No. of randomized participants	Age (y)	Sex M/F	Disease characteristics	Diagnostic test	Antigiardial drug regimens (No. of participants)	Efficacy	Follow-up
Mendoza et al., 2003 [31]	Open-label, RCT 2 parallel arms	92	2-5	47/45	Symptomatic and asymptomatic	Direct wet mount and formol ether sedimentation	ABZ, 400 mg/d for 5 days (49)	17/49 (34.6%)	Day 7, 14 & 21 after treatment
							TNZ, 50 mg/kg (max. 2g) s.d. (43)	31/43 (72.0%)	
Escobedo et al., 2003 [32]	Open-label, RCT 2 parallel arms	146	5-15	80/66	Symptomatic	Direct wet mount and formol ether sedimentation technique methods	MBZ, 200 mg tid for 3 days (73)	57/73 (78.1%)	Days 3, 5 & 7 after treatment
							SNZ, 30 mg/kg s.d. (73)	58/73 (79.4%)	
Escobedo et al., 2003 [33]	Open-label, RCT 3 parallel arms	165	2-15	58/57	Symptomatic	Direct wet mount and formol ether sedimentation technique methods	ABZ, 400 mg/day for 5 days (60)	37/60 (62.0%)	Day 7 & 10 after treatment
							CQ, 10 mg/kg twice daily for 5 days (50)	43/50 (86%)	
							TNZ, 50 mg/kg (max. 2g) s.d. (55)	50/55 (91.0%)	
Núñez et al., 2004 [35]	Open-label, RCT 2 parallel arms	256	1-5	122/134	Asymptomatic	Direct wet mount and formol ether sedimentation technique methods	Aminosidine, 35 mg divided into 3 doses for 7 days (59)	54/59 (91.5%)	Day 7, 14 & 21 after treatment
							MTZ, 25 mg/kg divided into three doses for 7 days (89)	71/89 (79.8%)	
Cañete et al., 2006 [34]	Open-label, RCT 2 parallel arms	122	5-15	63/59	Symptomatic	Direct wet mount and formol ether sedimentation	MBZ, 200 mg tid, 1 day (61)	39/61 (63.9%)	Day 3, 5 & 7 after treatment
							TNZ, 50 mg/kg, (max. 2g) s.d. (61)	50/61 (81.9%)	
Escobedo et al., 2008 [37]	Open-label, RCT 2 parallel arms	166	5-14	87/79	Symptomatic and asymptomatic	Direct wet mount and formol ether sedimentation	NTZ, 7.5 mg/kg bid for 3 days (85)	58/74 pp (78.4%) 58/85 iit (68.2%)	Day 5 & 10
							TNZ, 50 mg/kg, (max. 2g) s.d. (81)	57/63 pp (90.5%) 57/81 itt (70.3%)	
Cañete et al, 2010 [36]	Open-label, RCT 2 parallel arms	122	5-15	70/52	Symptomatic	Direct wet mount and formol ether sedimentation	CQ, 10 mg/kg bid for 5 days (61)	52/61 (85.2%)	Day 3, 5 & 10 after treatment
							MTZ, 15 mg/kg divided into three doses for 7 days (61)	45/61 (73.7%)	

**Table 2 - Jadad scoring of included studies**

Author [Reference]	Study described as randomized	Method used to generate the sequence of randomization described and appropriate	Study described as double blind	Method of double blinding described and appropriate	Study described withdrawals and dropouts	Total Jadad score
Mendoza et al., [31]	1	1	1	1	1	5
Escobedo et al., [32]	1	1	1	1	1	5
Escobedo et al., [33]	1	1	1	1	1	5
Núñez et al. [35]	1	1	1	1	1	5
Cañete et al., [34]	1	1	1	1	1	5
Escobedo et., [37]	1	1	1	1	1	5
Cañete et al., [36]	1	1	1	1	1	5

**Table 3 - Parasitological quality of included studies**

Author [Reference]	Parasitological examination				
	Description	Adequate	Repeated	Interobserver variation	Total*
Mendoza et al., [31]	2	3	3	0	8
Escobedo et al., [32]	2	3	3	0	8
Escobedo et al., [33]	2	3	3	0	8
Núñez et al. [35]	2	3	3	0	8
Cañete et al., [34]	2	3	3	0	8
Escobedo et., [37]	2	3	3	0	8
Cañete et al., [36]	2	3	3	0	8

\*Range 0-15; 15 indicates optimal laboratory technique

ferent follow up time, otherwise rate 0.

- Interobserver variation: Rate 3 when inter/intra observer variation is described or mentioned; otherwise rate 0.

#### 5-NIs versus benzimidazoles

There are 4 studies giving evidence for this comparison including 232 participants randomized to 5-NIs (73 to SNZ, 159 to TNZ), and 243 participants randomized to benzimidazoles (134 to mebendazole (MBZ), 109 to albendazole (ABZ)) [31-34]. The pooled effect was significant and favored 5-NIs (RR=1.35, 95% CI=1.05 to 1.75,  $I^2=79%$ ) with a wide prediction interval (0.43 favouring benzimidazoles to 4.22 favouring 5-NIs). By interventions, SNZ efficacy was similar to MBZ (1 study, 146 participants, RR=1.02, 95% CI=0.86 to 1.20). TNZ was significantly better than ABZ (2 studies, 207 participants, RR=1.67, 95% CI=1.19 to 2.36,  $I^2=55%$ ).

TNZ was significantly better than MBZ (1 study, 122 participants, RR=1.28, 95% CI=1.03 to 1.60). See figure 2.

#### 5-NIs versus chloroquine

There are 2 studies giving evidence for this comparison including 116 participants randomized to 5-NI (61 to MTZ, 55 to TNZ), and 111 participants randomized to chloroquine [33,36]. The pooled effect was not significant (RR=0.96, 95% CI=0.79 to 1.18,  $I^2=68%$ ). There was not enough information to estimate a prediction interval. By interventions, MTZ was worse than chloroquine but not statistically significant (1 study, 122 participants, RR=0.87, 95% CI=0.72 to 1.04). TNZ was not significantly different to chloroquine (1 study, 105 participants, RR=1.06, 95% CI=0.92 to 1.22). See figure 3.

#### 5-NIs versus aminosidine

We found one study including 89 participants randomized to MTZ and 59 participants rand-

omized to aminosidine. The study presented adequate methods of randomization and allocation concealment, but inadequate follow-up [35]. The effect was significant and favored aminosidine (RR=0.87, 95% CI=0.77 to 0.99).

*5-NIs versus nitazoxanide*

We found one study including 81 participants randomized to tinidazole and 85 participants randomized to nitazoxanide. The study presented adequate methods of randomization, allocation

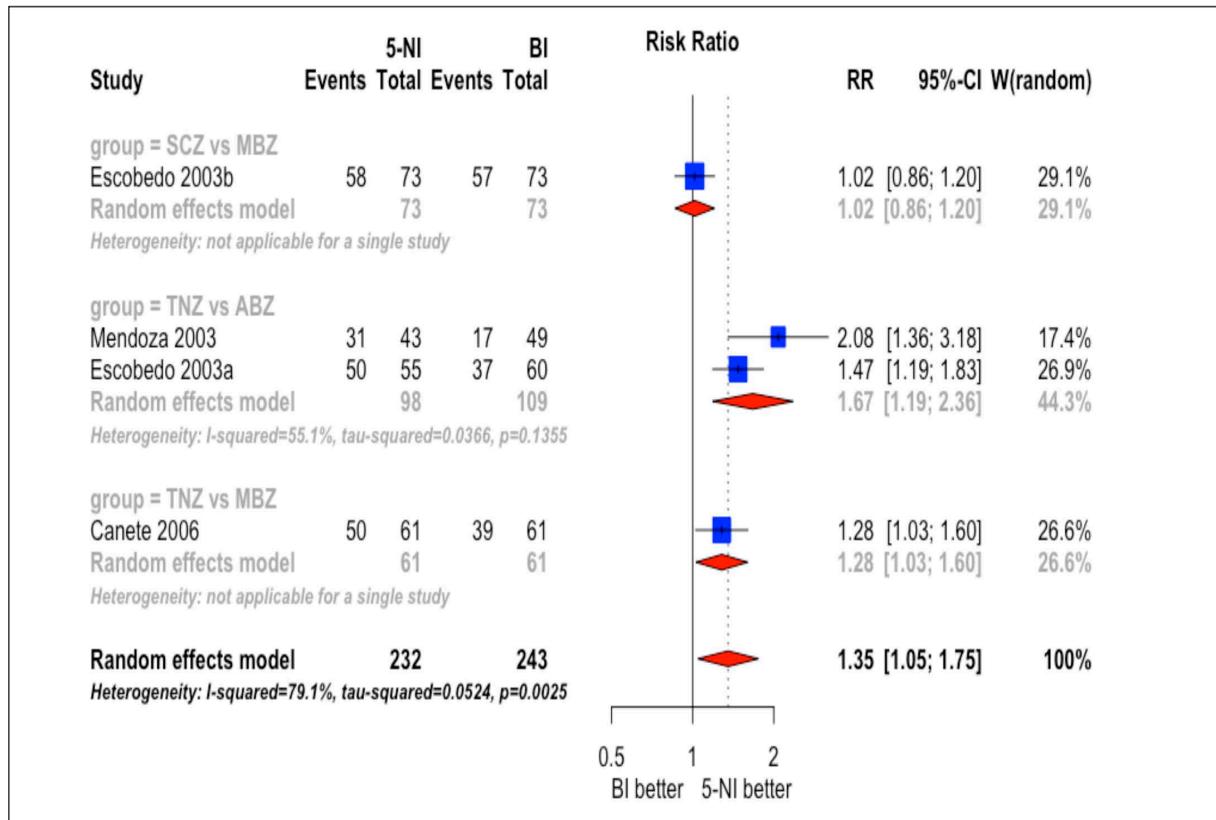


Figure 2 - Forest plot showing the effects of 5-NIs and benzimidazole compounds in giardiasis in Cuban children.

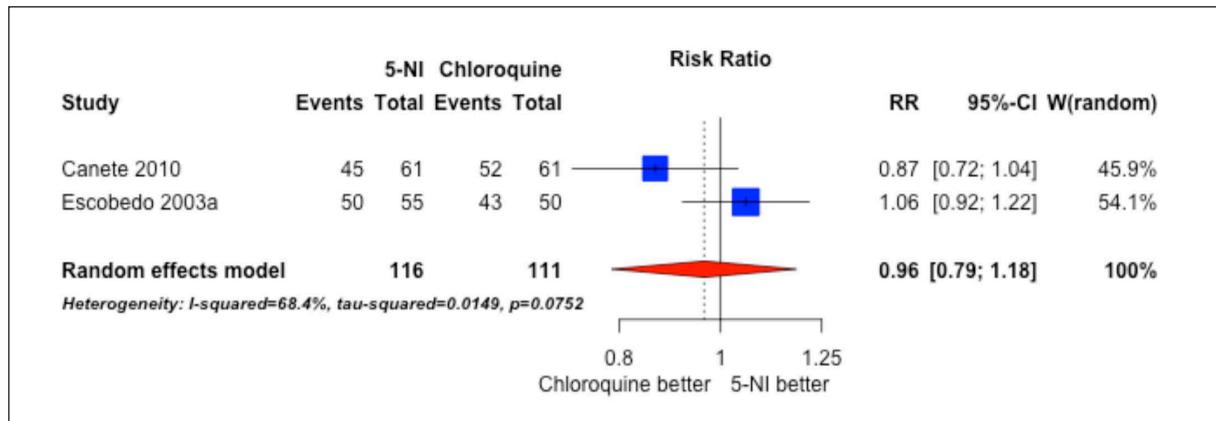


Figure 3 - Forest plot showing the effects of 5-NIs and chloroquine in giardiasis in Cuban children.

concealment and follow-up [37]. The effect was not significant (RR=1.03, 95% CI=0.84 to 1.26).

## ■ DISCUSSION

This systematic review with meta-analysis provides a PRISMA-compliant, internationally accessible, and timely review on the efficacy of 5-NI compounds for *Giardia* infection in Cuban children. We identified, extracted, and evaluated data from a collection of clinical studies of various treatments for *Giardia* infection carried out in Cuba between 2000 and 2017.

Whether 5-NIs therapy in giardiasis should continue as first-line therapy is currently under debate. Our results showed that in a population of <18 years of age Cuban children with *Giardia* infection, assisted in the outpatient setting, there was a statistically significant therapeutic benefit favoring the use of 5-NIs, supporting the recommendation of the Cuban pediatric guidelines, which recommend 5-NIs as first-line treatment for giardiasis in patients [38].

In the present systematic review and meta-analysis, TNZ is regarded as the best available option for the treatment of *Giardia* infection in Cuban children. This drug, with a similar structure to MTZ but with a longer half-life and more favorable side effect profile, has been used for decades in Cuba. Due to its pharmacokinetics, it is possible to be used as a single dose therapy. The good tolerance of TNZ is also considered an advantage in terms of patients' compliance. All of these features result in superior cure rates compared with other anti-giardial drugs for the treatment of giardiasis. However, as 5-NI drugs share common mechanism of activation by *Giardia* trophozoites, it is wise to suspect that a cross-resistance between them could occur. However, variations in pharmacokinetics and bioavailability of drugs have been noted that may influence their efficacy in clinical setting and, in the case of TNZ, it may be used in cases where a previous MTZ treatment has failed [39]. Other extra benefits of TNZ include that symptoms like diarrhoea have been reported to ameliorate earlier when treating with TNZ in comparison with MTZ and parasitological cure rates have also been proven earlier, higher or comparable when both drugs are evaluated [40-44].

### *Strengths and limitations*

As with any meta-analysis, the strength of the findings reflects the quality of the underlying data, potential for publication bias, and heterogeneity. The comprehensive search, attempts to identify data in a Cuban database. All included studies had a Jadad quality score of 5 and the authors of the included studies were contacted to retrieve unpublished data. However, we must also consider its limitations. First, the sample size of this meta-analysis was relatively small, which may reduce the power of statistical analysis. Second, only published studies were included in the present meta-analysis; thus, publication bias may have occurred. Third, we have not been able to relate the outcome of the studies to the dose and dosing of the drugs given. Fourth, there was no data to make conclusions regarding ornidazole due to lack of availability of this drug in the Cuban market. Fifth, all studies referred to a generic diagnosis of "giardiasis", without distinction among acute, recurrent or the chronic forms of this parasitic disease. Finally, although the analysis is based on patients enrolled in RCTs, they may not be representative of the broader patient population seen in clinical practice. Enrolled patients have strict eligibility criteria and those who agree to participate may have higher adherence profile compared to unselected patients and be very different from the general population. Despite these limitations, the value of this meta-analysis lies in its combination of data from several Cuban RCTs, which resulted in demonstration of differences in some aspects of the effectiveness of the treatments. Most of the differences observed were of marginal statistical significance, and RCTs with large sample sizes would be required to manifest these differences.

How can pediatricians and family doctors use the results of this meta-analysis? From the studies identified, it is reassuring to see that treatment effects of 5-NIs have been monitored in Cuba during the last years, and alternative treatment options actively sought. However, limited or no data exist for some drugs; some of them deserve more studies. Our analysis, which included recently trials published since the year 2000 to 2017, supports the use of 5-NIs (specifically, MTZ, TNZ and SNZ) as drugs of choice for first-line therapy of *Giardia* infections in Cuban children. There is no reason to be reluctant to prescribe such thera-

py due to its well-documented efficacy. Benzimidazole compounds and chloroquine may offer an alternative to 5-NIs but do not make them obsolete. MTZ and TNZ have been in clinical use for years with extensive amount of post-marketing data available. More post-marketing information regarding its efficacy for *Giardia* infections in children is needed on ornidazole for comprehensive pharmacovigilance.

## ■ CONCLUSIONS

This study extends the results of previous meta-analyses and offers an objective picture of the current status of the 5-NIs efficacy in *Giardia* infections in Cuban children. Our results also support that 5-NIs may continue being used as reference drugs in future RCTs in children with giardiasis. Of the drugs tested, TNZ appears to be the most efficacious option currently available for treatment of *Giardia* infections in Cuban children. Well-designed, multi-centre *Giardia* eradication trials in children are currently lacking and are critically needed, as some treatment options have not yet been tested in Cuba (*i.e.*, ornidazole), where the prevalence of this infection and associated disease burden in paediatric population seems to be high. Despite availability and use of 5-NIs for more than 5 decades, our data support the Cuban recommendations that these drugs be considered as first-line therapy for giardiasis.

### Authorship contribution

All authors contributed to the design of the study, planning, analysis, and interpretation of the results as well as to the writing of the paper. All authors have approved the final version for publication. All authors had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

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### Disclosure

Authors have no conflict of interests, and the work was not supported or funded by any drug company.

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