

Aspects related to positivity for schistosomiasis: a cross-sectional study in a low prevalence area in Alagoas, Brazil, 2020

10.1590/S1679-49742021000200005

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Abstract

Objective: To analyze aspects related to schistosomiasis positivity in an area of low prevalence in Brazil. **Methods:** This was a cross-sectional study, carried out in the first half of 2020, where we analyzed the proportion of positivity, according to the number of Kato-Katz slides, the diagnostic performance of the test and positivity estimates based on data from the Schistosomiasis Surveillance and Control Program Information System (SISPCE). **Results:** 2,088 slides from 348 individuals were analyzed, with proportion of positivity of 11.8%, 26.7% and 31.0% for 1, 4 and 6 slides analyzed, respectively. There was excellent agreement ($Kappa = 0.91$) between the readings of 4 and 6 slides. The SISPCE data was estimated to be underreported by up to 2.1 times. **Conclusion:** Increasing the number of slides increased Kato-Katz positivity, which can contribute to maximizing the control of the disease as a Public Health problem.

Keywords: Neglected Diseases; Cross-Sectional Studies; Sensitivity and Specificity; Public Health; Schistosomiasis Mansoni; Parasitic Diseases.

*Article derived from the Doctoral thesis entitled 'Epidemiology of schistosomiasis mansoni in a low prevalence area in the state of Alagoas', to be submitted by Israel Gomes de Amorim Santos to the Biosciences and Biotechnology in Health Postgraduate Program, Biosciences and Biotechnology in Health Course, Instituto Aggeu Magalhães, Fundação Instituto Oswaldo Cruz, Pernambuco. The study received financial support from the Alagoas State Research Support Foundation (FAPEAL) and the Ministry of Health: Process No. 60030.000857/2016.

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Introduction

Schistosomiasis is a disease caused by trematode worms of the genus *Schistosoma*. This morbidity is found in more than 78 countries, of which 52 show the need for large-scale treatment campaigns.¹ *Schistosoma mansoni* is the parasite species found in Brazil, which arrived in the country along with the trafficking of enslaved people in the colonization period, initially in states in the Northeast region. When slave labor ended, the parasite and the disease were taken to other regions during the northeastern exodus, and their presence has been of great relevance and magnitude for Brazilian Public Health to date.^{2,3}

In 2019, the Health Surveillance Secretariat of the Brazilian Ministry of Health estimated 1.5 million people infected with *S. mansoni* in Brazil. The disease is found, in an endemic manner, in nine states, while persisting as hotspots in a further nine Federative Units where the maintenance of its epidemiological characteristics can be seen.^{3,5} Due to the relevance of the endemic, health authorities have used several measures to control the mollusks that transmit *S. mansoni* and eradicate the disease in the country.

The Kato-Kratz technique has been effective in diagnosing S. mansoni infection only in areas of high prevalence, a perspective that does not correspond to that of most countries where parasitosis is endemic.

Parasitological stool examination has been the gold standard for the diagnosis of infection and control of morbidity, since its optimization in the 1970s with the creation of the quantitative Kato-Katz technique.⁶ This is the method currently recommended by the World Health Organization for coproparasitological surveys in endemic countries.⁷ Notwithstanding, nowadays, the Kato-Kratz technique has been effective in diagnosing *S. mansoni* infection only in areas of high prevalence, a perspective that does not correspond to that of most countries where parasitosis is endemic. Other methods, such as serologic and immunochromatographic methods based on the detection of worm antigens released in the individual's urine, are promising, although, in terms of sensitivity and specificity, inefficient for replacing the

method recommended by national and international health authorities.⁸⁻¹⁰ However, these methods have been indicated to be used together.¹¹

Given that (i) epidemiological indicators, derived from the activities of the Schistosomiasis Control Program, support the formulation of guidelines for the control and eradication of schistosomiasis mansoni in Brazil, (ii) the Kato-Katz technique has low sensitivity for detecting *S. mansoni* in low prevalence areas and (iii) alternative diagnostic methods cannot yet be used in the routine of municipal health teams, it is undeniable that the diagnostic and logistics guidelines currently used in the Schistosomiasis Control Program must be optimized. As such, it will be possible to know the real epidemiological situation of the disease, contributing to the building of Public Health policies that are more effective in combating the endemic disease.

The objective of this study was to analyze aspects related to schistosomiasis positivity in an area of low prevalence of the disease in Brazil.

Methods

A cross-sectional study was carried out in the municipality of Lagoa da Canoa, Alagoas State, in the months of March and April 2020, the results of which were compared with data reported on Schistosomiasis Control Program Information System (SISPCE) for the period from 2007 to 2016.

The municipality belongs to the state's 7th Health Region (7/10), has a human development index of 0.552 and a social vulnerability index of 0.490, both referring to 2010.¹² Lagoa da Canoa has primary health care centers, a specialized outpatient department and a mixed health and psychosocial care center. Regarding infectious and parasitic diseases, the municipality is endemic for Chagas disease, schistosomiasis and American cutaneous leishmaniasis; and in addition, it is a surveillance area for visceral leishmaniasis and plague.¹³

The study sample was defined considering acceptable error of 3.0% and prevalence of 8.1%, obtained by the ten-year average (2007-2016) of records held on the SISPCE. The local population is estimated at 18,250 inhabitants.¹² Based on these references, a sample of 311 individuals was calculated, and then corrected to 373 as compensation for possible losses.

In the sampling process, individuals were divided proportionally, according to the area of residence: nearly

half of the municipality's population lives in the urban area. Moreover, the population served by each Family Health Strategy health center was taken into account. The residences were randomly selected with the help of community health agents, respecting the distance of 50 to 100 meters between them, from the first selected residence and so on.

Individuals aged 5 years and over with no history of using praziquantel in the six months prior to the research were eligible for the study. Information on the use or not of this anthelmintic or antiparasitic medication was self-reported by the participants or their guardians.

The study variables were used according to the nature of the data:

a) Primary data

- Proportion of positivity for *S. mansoni* infection based on the number of Kato-Katz slides analyzed.

- Proportion of *S. mansoni* infection positivity per location in the municipality.

- Intensity of infection based on the reading of one to six slides analyzed (light [1 to 99 EPG]; moderate [100 to 399 EPG]; heavy [more than 400 EPG]).

b) Secondary data

- Average proportion of *S. mansoni* infection positivity for each location in the municipality, from 2010 to 2016.

Each research participant was verbally informed of the objective of the study and the correct procedure for fecal sample collection. Three fecal samples were collected from each of them, on different days. These samples were collected at each participant's home and stored in a polystyrene box with ice, in order to provide safe transportation to the Human Parasitology and Malacology Laboratory of the State University of Alagoas (UNEAL).

At least four visits were made to collect the material, and individuals who did not deliver all stool samples were excluded from the study.

The material was analyzed using the Kato-Katz method, with two slides per sample. Two trained analysts read the slides. When there was no agreement in 30% of the readings, regarding the presence / absence of eggs or the number of eggs counted, a third analyst, equally trained in the parasitological diagnosis of schistosomiasis mansoni, performed the reading of the slides.¹⁴

Individuals who had at least one *S. mansoni* egg found in the analyzed fecal sample were considered positive for infection. The number of eggs per gram of

feces (EPG) resulted from multiplying the number of eggs counted by a multiplication factor of 24, according to the Kato-Katz method protocol. The number of slides read and the arithmetic mean of the eggs counted on all slides analyzed for each individual were taken into account, for the final presentation of the EPG.

Secondary data used in the study were retrieved from the SISPCE and refer to the period between 2010 and 2016.

The proportion of positivity was checked on the number of positive slides, considering, separately, the reading of one and two slides of each stool sample collected. The proportion of positive cases by analyzing a slide of a sample was compared with the proportion of positive cases by analyzing two slides from the same sample, using the chi-square test of independence, with p-values <0.05 being considered significant. This analysis was performed for each of the three stool samples collected separately.

Co-positivity and co-negativity were established for reading one, two and four Kato-Katz slides, against the reading of six slides, the latter being considered the procedure under analysis in relation to the first reading. Co-positivity was determined by the ratio of the number of true positives to the number of slides diagnosed as positive; and co-negativity, as the ratio between the true negatives and the number of slides determined to be negative.

Agreement between reading one, two and four slides and reading six slides was determined by the Kappa index.¹⁵ This index was stratified into the following categories: no agreement (less than 0.01); poor agreement (between 0.01 and 0.20); weak (between 0.21 and 0.40); moderate (between 0.41 and 0.60); good (between 0.61 and 0.80); and excellent agreement (greater than 0.81). In order to compare the proportions of positive slides according to the number of slides read, the McNemar test was used, with the p-values being determined by the chi-square test.

The positivity of the reading of one, two and four slides was also compared with the reading of six Kato-Katz technique slides, determining the ratio between the positivity resulting from the reading of six slides and the positivity found in reading each of the other slide sets.

The average proportions of positivity reported on the SISPCE for the years 2010 to 2016 were compared with the proportions of positivity from reading one to six slides of the material collected in this study.

A multiplication factor was created based on the difference between the positivity observed in the field study and the data available on the SISPCCE, for the analysis of the positivity estimation per location in the municipality. The calculation of the difference between positivity rates was carried out using the following formula:

$$Dp = pC - pP \quad (1)$$

where Dp corresponds to the difference in positivity, pC to the positivity of the field study and pP to the positivity of the SISPCCE data. Considering that in the field study, the proportion of positivity observed behaved according to the number of slides read - in this case six slides -, the multiplication factor resulted from the sum of the ratios for the differences obtained with the application of formula (1), as follows:

$$Factor = \sum \left(\frac{(Dp) \times \dots \times (Dp)n}{6} \right) / n(Dp) \quad (2)$$

The multiplication factor obtained with formula (2) was used for an empirical estimation of schistosomiasis mansoni positivity in the different locations worked on

by the Schistosomiasis Control Program local team, using the average positivity for the period from 2010 to 2016, calculated according to the SISPCCE data.

The study project was submitted to the Human Research Ethics Committee of the Federal University of Alagoas - Certificate of Submission for Ethical Appreciation N°. 58695716.1.0000.5013 - and was approved: Opinion N°. 3,827,540, issued on February 7, 2020. All participants or their guardians signed (i) a Free and Informed Consent Form or (ii) a Free and Informed Assent Form for children and adolescents.

Results

348 individuals took part in the study. Each of them provided three samples of coprological material for analysis by the Kato-Katz technique. In all, 2,088 slides were examined. The proportion of positivity for schistosomiasis mansoni varied according to the number of slides analyzed. The reading of two slides from each sample resulted in positivity that ranged from 18.4% (64 individuals) to 19.8% (69 individuals). This proportion was greater than that found by analyzing one slide, the variation of which was from 11.8% (41 individuals) to 16.4% (57 individuals), with the differences between the

Table 1 – Schistosomiasis mansoni positivity when reading of one or two Kato-Katz slides analyzed from individuals from a low prevalence area, Alagoas, 2020

Samples	Slide 1 + Slide 2		Slide 1		Slide 2		p-value ^a
	N	Positivity ^b	N	Positivity ^b	N	Positivity ^b	
Sample 1							
Positive							
Yes	64		51		45		
No	284	18.4	297	14.6	303	12.9	p<0.001
Total	348		348		348		
Sample 2							
Positive							
Yes	69		57		41		
No	279	19.8	291	16.4	307	11.8	p<0.001
Total	348		348		348		
Sample 3							
Positive							
Yes	68		50		43		
No	280	19.5	298	14.4	305	12.3	p<0.001
Total	348		348		348		

a) Chi-square test of independence: comparison of the proportions between the sum of the two slides and the result of the reading of each slide; b) Proportion of positivity.

proportions being statistically significant (Table 1). There was an increase in proportion of positivity to 26.7% and 31.0% when four and six slides were analyzed, respectively.

Regarding the intensity of infection, 97 individuals were classified as having light infection and one as having heavy infection when six Kato-Katz slides from three samples of these individuals were analyzed. When two slides of a sample were examined, 58 individuals had light infection and two had heavy infection. Regarding the average number of eggs per gram of feces, in the light infection class, the highest average was 37.8 eggs, in the moderate infection class, it was 229.8 eggs, and in the heavy infection class, 684.0 eggs. (Table 2).

When analyzing the diagnostic indicators of reading from one to six Kato-Katz slides, there was 88.7% co-positivity when comparing the reading of four slides with that of six slides, and 48.0% co-positivity when comparing the reading of six slides with one slide.

In addition, the McNemar test showed good performance in the analysis of six slides, compared to the analysis of one and two slides: McNemar test results of 53.02 and 40.02 respectively; $p < 0.001$ for both results. The Kappa index showed excellent agreement when comparing the reading of four slides with that of six (Kappa: 0.91). With the regard to the difference in the

positivity proportions, the analysis of six slides showed a proportion of 31.0%, that is, 1.2, 1.7 and 2.1 times greater than positivity from reading four (26.7%), two (18.4%) and one slide (14.6%), respectively (Table 3).

Ten out of 12 locations studied that had Schistosomiasis Control Program activities in previous years had higher average proportions of positivity in this study, compared to those reported on the SISPC (Table 4). Furthermore, even considering only the reading of one slide, positivity found in the study was higher than positivity reported for most locations.

The empirical factor for estimating the real positive proportion of schistosomiasis mansoni in the studied locations was equal to 2.1. Before correcting the positivity of several locations in the municipality using this factor, five locations had an average proportion of positivity ranging from 0.9 to 5.0%, for 18 locations this indicator was between 6.2 and 14.8%, while for ten locations this variation ranged from 15.4 to 39.9%. After applying the correction factor, the municipality had two locations with an average positivity proportion of 1.9% and 4.4%, in six locations the indicator ranged from 6.4 to 14.0%, and 25 locations had variation ranging from 16.8 to 84.0% (Table 5).

Table 2 – Intensity of schistosomal infection and average number of eggs per gram (EPG) of feces according to the number of slides read in a low prevalence area, Alagoas, 2020

Sample	Schistosomal Infection		
	Light ^a (average eggs) ^b	Moderate ^a (average eggs) ^b	Heavy (average eggs)
1 st sample / 2 slides	58 (31.6)	4 (196.5)	2 (684.0)
2 nd sample / 2 slides	62 (29.3)	6 (206.0)	1 (528.0)
3 rd sample / 2 slides	58 (37.8)	10 (229.8)	1 (420.0)
2 samples / 4 slides	86 (30.2)	6 (209.0)	1 (552.0)
3 samples / 6 slides	97 (32.3)	9 (193.8)	1 (406.0)

a) Number of individuals infected according to the intensity of *S. mansoni* infection. Intensity of infection: result of the number of eggs per gram of feces, being classified as light (up to 99 EPG), moderate (100 to 399 EPG) and heavy (from 400 EPG); b) Average number of eggs per gram of feces.

Table 3 – Diagnostic performance when increasing the number of slides read regarding schistosomiasis mansoni positivity in a low prevalence area, Alagoas, 2020

KK ^a	6 slides analyzed				Positivity		Relationship between positivity rates
	Co-positivity	Co-negativity	McNemar p-value	Kappa ^b	Reference ^c	6 slides	
1	48.0%	100.0%	53.02 p<0.001	0.56	14.6%	31.0%	2.1
2	60.0%	100.0%	40.02 p<0.001	0.67	18.4%	31.0%	1.7
4	88.7%	100.0%	1.00 p>0.05	0.91	26.7%	31.0%	1.2

a) KK: number of Kato-Katz slides analyzed; b) Kappa index calculated; c) Represents positivity resulting from reading 1, 2 and 4 slides.

Table 4 – Comparison of the average positivity rate obtained in the study and the average positivity rate reported on the Schistosomiasis Surveillance and Control Program Information System (SISPCE), for locations in Lagoa da Canoa, Alagoas, 2020

Locations	Average positivity rate (%) SISPCE Data 2010-2016	Average positivity rate (%) Study Data ^a						
		1	2	3	4	5	6	7 ^a
		Alto do Cruzeiro	23.3	50 ^b	50 ^b	0	0	0
Antunica de Baixo	17.1	0	40 ^b	20 ^b	20 ^b	20 ^b	0	40 ^b
Antunica de Cima	3.2	8 ^b	8 ^b	8 ^b	8 ^b	38 ^b	15 ^b	38 ^b
Barro Preto	11.6	20 ^b	30 ^b	10	0	20 ^b	0	50 ^b
Cavaco	11.4	0	0	10	0	30 ^b	10	40 ^b
Funil	20.6	0	0	8	8	8	0	8
Lagoa da Pedra	12.1	13 ^b	13 ^b	13 ^b	0	0	0	25 ^b
Lagoa do Mato	13.4	9	9	27 ^b	18 ^b	18 ^b	18 ^b	36 ^b
Mata Limpa	10.0	4	0	4	8	0	4	16 ^b
Olho d'Água da Canoa	15.4	28 ^b	33 ^b	22 ^b	22 ^b	17 ^b	11	44 ^b
Pau d'Arco	40.0	6	6	0	6	0	0	6
Zona Urbana	9.4	22 ^b	17 ^b	27 ^b	17 ^b	20 ^b	20 ^b	42 ^b

a) The numerical sequence (1 and 2; 3 and 4; 5 and 6) represents the pair of slides from each sample collected. Number 7 represents the average positivity considering the reading of the 6 slides as a single result; b) Average positivity observed in the study greater than the average positivity reported on the SISPCE for each location in the municipality.

Table 5 – Average schistosomiasis mansoni positivity estimated according to a multiplication factor resulting from field survey and data from the Schistosomiasis Surveillance and Control Program Information System (SISPCE), for locations in the city of Lagoa da Canoa, Alagoas, 2010-2016

Locations	Average Recorded Positivity ^a	Average Estimated Positivity
Alexandre	6.3	13.2
Alto do Cruzeiro	23.3	48.9
Antunica de Baixo	17.1	35.9
Antunica de Cima	3.2	6.7
Barro Preto	11.6	24.3
Barro Vermelho	0.9	1.9
Boa Vista I	3.0	6.4
Campestrinho	34.1	71.6
Capim	11.1	23.2
Cavaco	11.4	23.9
Chã do Pau d'Arco	14.8	31.1
Folha Miúda de Baixo	2.1	4.4
Funil	20.6	43.3
Jenipapo	8.5	17.8
Jurema	9.7	20.3
Lagoa da Braúna	6.2	13.1
Lagoa da Canoa	9.4	19.8
Lagoa do Mato	13.4	28.2
Lagoa da Pedra	12.1	25.5
Lagoa das Varas	10.7	22.5
Lagoa Grande	8.0	16.8
Lagoa Queimada	11.3	23.8
Mata Limpa	10.1	21.1
Olho d'Água da Canoa	15.4	32.4
Padre Cícero	6.7	14.0
Pau d'Arco	39.9	84.0
Pintada	5.0	10.6
Riacho Grande	20.0	42.0
Riacho Fundo	11.3	23.7
Riacho Fundo de Cima	20.0	42.0
Santa Clara	26.1	54.8
São José III	8.0	16.9
Sizília	16.9	35.5

a) Recorded on the Schistosomiasis Surveillance and Control Program Information System (SISPCE); b) Estimated from factor 2.1: empirical factor, constructed from the difference between the positivity found in the study and the positivity rates reported for various locations in the municipality studied.

Discussion

The results of this study showed that the increase in the number of Kato-Katz slides examined significantly raised positivity for the disease, with greater agreement in measuring the proportion of positivity as the number of slides increased. The application of an empirical estimation factor suggests that positivity in several locations in the municipality is underestimated, a relevant fact from an epidemiological perspective, within a context of eliminating the disease in areas of low schistosomiasis *mansoni* prevalence.

Possible inconsistencies in the data obtained from the SISPCE, relating to the methodology used in the analysis of *S. mansoni* infection positivity and data input to the system, along with the non-adjustment of the study data, may represent a limitation for this research, concerning the comparison of the positivity rates found, although a long period (seven years) was chosen for the collection of data that comprised the average positivity used in the analysis, with a view to better data stability.³ Furthermore, possible errors of underestimation or overestimation of data based on the empirical factor created require caution in their interpretation. This limitation can be minimized with the validation of this factor and its use in different epidemiological scenarios of the disease.

The high positivity found in the study, resulting from the increase in the number of slides analyzed, shows a very worrying scenario, either because of the current guidelines, or in view of the goals established for the control and eradication of parasitosis. In 2012, an integrated plan for strategic actions to eliminate certain neglected diseases, including schistosomiasis, was launched. The objective of the plan was to eliminate schistosomiasis as a Public Health problem in Brazil by 2015.

A national survey on the prevalence of schistosomiasis and geohelminths, conducted at the same time as the plan, found that Brazil had not reached the eradication goal, and that the state of Alagoas continued to be one of the Federative Units with the highest schistosomiasis morbidity prevalence.¹⁶⁻¹⁸

It is important to emphasize the relevance of the logistics of the results observed within the scope of the municipal actions of the Schistosomiasis Control Program teams, both for the achievement of the goals of the integrated plan and for the results of the national

survey, as both follow the norms of Brazil's highest health authorities. Reading one or two Kato-Katz slides per individual is no longer effective in estimating schistosomiasis prevalence in low prevalence areas and thus contributes to case underreporting. Studies that analyzed the influence of the increase in the number of Kato-Katz slides examined, new methods or the combination of several diagnostic methods, found that the Kato-Katz technique had underreported the prevalence of the disease when compared to the other methods, despite the increase in the number of slides examined to raise its sensitivity.^{9,10,19-22}

It can be seen that the Schistosomiasis Control Program teams perform surveys in the same areas successively, for technical and operational reasons.²³⁻²⁵ In this study, the municipality was sampled according to its urban and rural population composition, having taken into account the number of Primary Health Care Centers and the population served by each one. For most locations, the positivity found in this study was higher than that reported by the local Schistosomiasis Control Program survey. The use of a representative survey of the municipality's population, associated with a reduction in the number of participating individuals and an increase in the number of slides analyzed for each of them, proved to be more effective in revealing schistosomiasis prevalence in the locations studied.

Given the scenario described, it is possible to envisage strategies to optimize the work of the Schistosomiasis Control Program at the municipal level:

1) Integration with the actions of community health agents with the function of guiding or collecting material for coproparasitological examination in their area of coverage, as recommended by the Ministry of Health and academic evidence of its effectiveness.²⁵⁻²⁷ This strategy was conceived due to the work model adopted by the municipality studied, where community health agents work actively to promote individuals' access to care services, early diagnosis and timely treatment.

2) Random but representative selection of the individuals included in the survey sample, year by year, in order to know the real schistosomiasis positivity in the different locations in Lagoa da Canoa and the epidemiological scenario of the entire municipality, rather than just restricted places. This measure is equally efficient in controlling morbidity, reducing prevalence, evaluating and monitoring actions.

3) Reduction in the number of participating individuals and increase in the number of slides examined for each individual. This strategy is shown to be necessary due to its rationality, since the average number of slides read, in the municipality analyzed, is 2,000 per year, which represents 2,000 people. In this study, 2,088 slides from 348 individuals were read, with positivity being at least two times greater among the individuals evaluated, compared to the positivity of the individuals covered by the local Schistosomiasis Control Program surveys.

Without correction of positivity, only five locations would have had positive individuals and those who live with them being treated, and three locations would have had collective treatment. After correcting positivity, positive individuals and those who live with them were found to be being treated in 12 locations, and collective treatment was found in 13 locations. This is very important data, since it shows that even without a representative survey and with an increase in the number of slides per individual, these being measures proposed in this study, the treatment categories recommended by the Ministry of Health are no longer aligned with the country's epidemiological reality, mainly due to the parasitological analysis model

recommended by the Ministry of Health and practiced by Schistosomiasis Control Program technical staff.

Taken as a whole, the study data show that collecting three samples of fecal material and reading six Kato-Katz slides increase schistosomiasis positivity in an area of low endemicity. In conclusion, it is evident that schistosomiasis mansoni continues to be a major problem for municipalities in the endemic area and a priority to be included in health promotion policy agendas in Brazil.

Authors' contributions

Santos IGA designed the study, collected, analyzed and interpreted the data, drafted and revised the manuscript. Santos Júnior EG, Alves LC and Brayner AF analyzed and interpreted the data, and reviewed the manuscript. Bezerra LP, Cirilo TM, Silva LO, Machado JPV, Lima PD, Souza MRB, Gomes SC, Silva GIL, Damasceno IA, Alencar VJB, Carvalho MMV, Ramos RES, Gomes DS and Paz WS collected and analyzed the data, and reviewed the manuscript. All authors have approved the final version of the manuscript, guaranteeing its accuracy and integrity.

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Received on 07/04/2020

Approved on 10/31/2020

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