Dynamics of *Helicobacter pylori* Detection in Stools During the First 5 Years of Life in Chile, a Rapidly Developing Country

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Background: *Helicobacter pylori* colonization/infection can be transitory or persistent, conditions that have not been thoroughly evaluated in young children. We aimed to characterize the dynamics of *H. pylori* stool detection and to determine host and environmental factors and symptoms associated with persistence.

Method: In a 5-year cohort study, we followed-up infants from birth with clinic visits every 3 months. Symptoms and environmental risk factor survey and a stool sample for *H. pylori* antigen detection were requested in every visit. Secretor/ABH histo-blood group phenotype was determined in saliva.

Results: Overall, 218 of 1456 (15%) stool samples were positive for *H. pylori* and 39 of 96 (41%) children had at least 1 positive sample. Stool detection was transitory in 16 of 39 (41%), persistent in 19 (49%) and undetermined in 4 (10%) children. Persistence was acquired largely during the first 24 months (17/19 cases) and was associated with nonsecretor phenotype (32% versus 0% for transitory infection; $P = 0.02$) and daycare attendance (67% versus 26% for never infected; $P = 0.019$). Symptoms possibly associated with persistence were referred in only 1 child.

Conclusions: Nearly 20% of this Chilean cohort had persistent *H. pylori* stool sample detections during the first 5 years of life, acquired mostly during the first 24 months. Persistence was significantly associated with nonsecretor phenotype and daycare attendance, and possibly associated gastrointestinal symptoms were rare. This relatively common group of young children with persistent *H. pylori* colonization/infection will require further study.

Key Words: *Helicobacter pylori*, persistence, cohort, children, colonization/infection

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Helicobacter pylori is associated with chronic gastritis and duodenal and gastric ulcer, and is considered a class 1 carcinogen for gastric adenocarcinoma and mucosa-associated lymphoid tissue lymphoma.[1] In Chile, a rapidly developing country with a purchasing power parity gross domestic product per capita of approximately US$16,000,[2] gastric cancer mortality rates are among the highest in the world, reaching 20 per 100,000 inhabitants.[3,4] *H. pylori* seroprevalence rates also are high, reaching 73% among adults, with a peak between 45 and 64 years of age.[5]

H. pylori colonization/infection can begin during the first year of life, especially in populations living in lower socioeconomic environments, where up to 30% of children during the first year and 50% during the second year will have at least 1 stool sample positive for *H. pylori*.[6,7] Polyclonal and, more recently, monoclonal antibody-based enzyme-linked immunosorbent assay (ELISA) for *H. pylori* detection in stools have increased the possibility of evaluating colonization/infection in children because of their noninvasive nature.[6–11] The latter has shown high sensitivity and specificity, surpassing 85% and 90%, respectively, in children compared with C-urea breath test (UBT), histology, or culture.[9,11]

H. pylori colonization/infections can be transient or persistent, as identified 15 years ago in a primate model.[12] Strain and host factors determined the transitory or persistent nature of colonization/infection in this model. In a birth cohort study using monoclonal antibody-based stool antigen detection every 6 months, 49% of Bangladeshi children had at least 1 ELISA-positive sample by 2 years of age, but only 3% (8/238 children) had persistent positive samples.[7] The *Pasitos* longitudinal cohort study in United States and Mexican children tested by UBT at 6-month intervals concluded that *H. pylori* prevalence was 16% at 2 years of age and that most infections (77%) were transitory.[13] An extension of follow-up to a mean of 3.8 years showed an overall prevalence of 45%, with only 7% of children presenting with a persistent infection. Interestingly, persistent *H. pylori* infection in older siblings always preceded persistent infection in younger siblings.[14] Persistent infections using UBT were somewhat higher (18%) in a Peruvian cohort followed-up during the first 30 months of age.[15] The importance of understanding the potential role of persistent infections in young children is highlighted by a study from the late 1990s in children of approximately 7 years of age undergoing endoscopy for reasons other than *H. pylori* suspicion.[16] Seven of 8 asymptomatic children with persistent *H. pylori* infections followed-up for 2 years had deterioration in histologic features of the gastric mucosa.

Our aim was to determine the frequency of persistent and transitory *H. pylori* colonization/infection acquired during the first 5 years of life in a Chilean birth cohort from a semi-rural area, and to characterize the dynamics of *H. pylori* positivity in stool samples obtained every 3 months. We also intended to identify host and environmental factors and potential symptoms associated with transitory and persistent *H. pylori* status.

METHODS

Setting and Population

Mother–infant pairs living in the city of Colina, a semi-urban, middle- to low-income area within the metropolitan region of Santiago, Chile, were enrolled during 2006 to 2007 in a 2-year

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cohort study aimed to understand the dynamics of rotavirus and norovirus infection.[17] New consents were obtained in 2008 to further process stored frozen stools for *H. pylori* antigen detection, to extend follow-up to 5 years of age and to obtain additional information potentially associated with *H. pylori* colonization/infection. Only healthy 1-month-old infants were enrolled and details of subject contact and clinic visit procedures were previously described.[17] Approvals were obtained from the Ethical Committees of the Faculty of Medicine, University of Chile and the Servicio de Salud Metropolitano Norte for the initial and additional protocol and consent forms.

Subject Monitoring and Sample Collection

Monitoring during the first 2 years of life has been reported.[17] Briefly, mothers were scheduled for monthly well baby visits at the Colina outpatient clinic by study personnel. A stool sample was provided at each visit and a saliva sample was obtained once throughout the follow-up period. Conditions for collection, storage and transport of samples have been described.[17] After completion of 18 months, mother–child pairs were invited to participate in the *H. pylori* study and to visit the clinic every 3 months, following the same procedures as indicated up to 5 years of age.

An ad hoc patient record form was designed for the enteric virus study collecting information from the first visit onward on living conditions (household construction fully or partially solid, complete or partial urbanization), exclusive or partial breastfeeding and gastrointestinal symptoms occurring between visits (vomiting, abdominal pain, or distension). A revised patient record form was introduced in October 2008 (subject age range,16–28 months of age), including factors potentially associated with increased *H. pylori* transmissibility (number of individuals living in the household, subject sleeps alone or with an adult, daycare attendance, use of pacifier). This information was collected retrospectively back to the 15-month visit and prospectively until end of follow-up. A pediatric gastroenterologist (AP) clinically evaluated every child with persistent *H. pylori* stool detections at least once per year after status confirmation. For a subset of children, the weight-for-height Z scores were calculated retrospectively extracting values from medical charts at 2, 3 and 4 years of age.

Sample Processing

Stool and saliva samples were stored at −20°C in the Enteric Virus Laboratory, Microbiology and Mycology Program of the University of Chile. Every third month, a stool sample obtained retrospectively and prospectively was tested for *H. pylori* by HpSA ELISA (Premier Platinum HpSA; Meridian Diagnostics, Cincinnati, OH) according to manufacturer's instructions. An OD_{450} ≥0, 14 optical density (OD) was considered positive for *H. pylori*. All stool samples obtained during the first 18 months were tested for norovirus and rotavirus as previously described.[17] Saliva samples were centrifuged and supernatants were processed by ELISA using monoclonal antibodies against H1/H2, Lewis a, Lewis b and A and B histo-blood group antigens as previously described.[18]

Definitions

H. pylori colonization/infection was defined as positive when a child had ≥1 ELISA-positive stool. This detection method does not differentiate between colonization and infection, which is why the term "colonization/infection" is used throughout. Colonization/infection was defined as transitory if 1 or 2 stool samples were positive, as persistent if 3 or more consecutive stool samples were positive, and as undeterminable if a positive sample occurred only in the last visit. Children were classified as secretors if saliva samples were positive for H1/H2 or Lewis b antigens, and as nonsecretors if samples were negative for all or positive only for Lewis a. Secretor individuals were further classified as A, B, AB, or O according to antigen detection in saliva. A symptom was defined

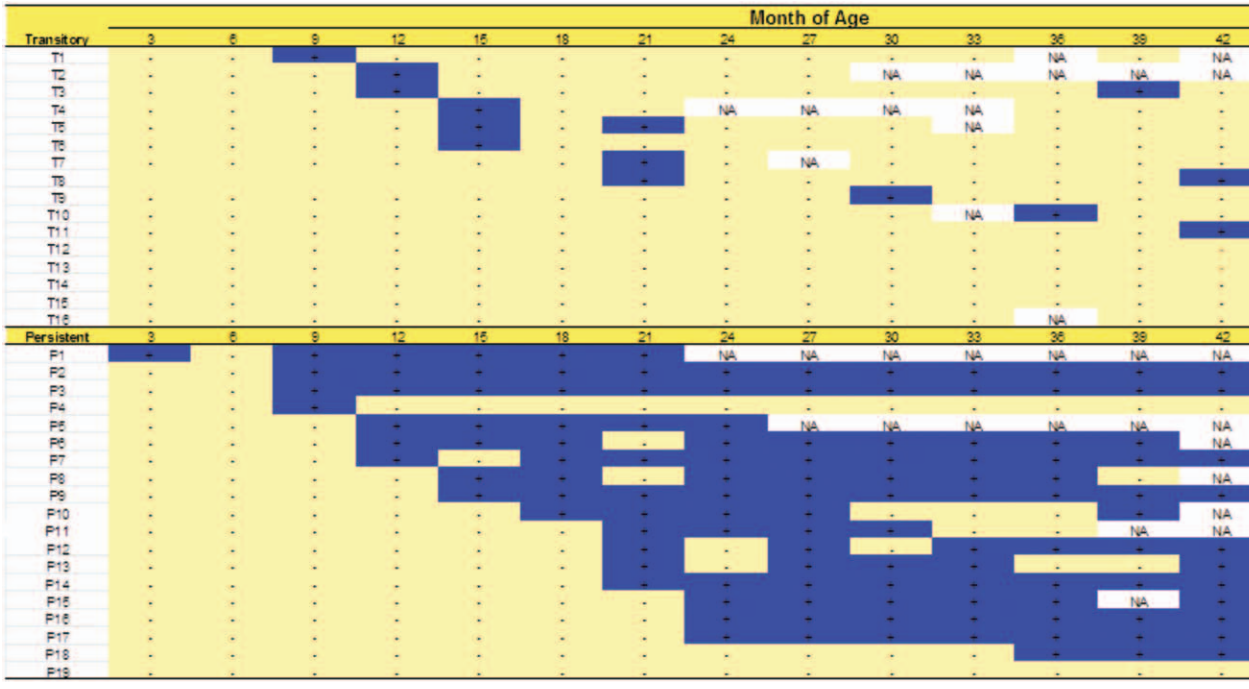

FIGURE 1. Age of children with *Helicobacter pylori* stool positivity for 16 children with a transitory (T) and 19 children with a persistent (P) colonization/infection. NA indicates sample not available.

as "concomitant" with *H. pylori* stool positivity if referral occurred within 3 months before or after a positive stool sample.

Statistical Analysis

Children completing at least 21 months of follow-up and providing ≥6 stool samples were included for analysis. Analysis of subjects with <6 stools was considered insufficient for an adequate characterization. Continuous variables were compared by analysis of variance or Kruskal-Wallis test for 2 groups if variances were nonhomogenous according to Bartlett test for inequality of population variances. Two-tailed χ^2 with Yates correction or Fisher exact test were used to compare categorical variables. The statistical program Epi Info 3.5.3 (January 26, 2011) provided by the Centers for Disease Control and Prevention (Atlanta, GA) was used for statistical analysis.

RESULTS

Cohort Description and Overall *H. pylori* Positivity

A total of 102 mother–infant pairs accepted participation, of these 96 completed the minimum 21-month follow-up period. The median (interquartile range [IQR]) months of follow-up for the 96 subjects was 60 (IQR, 21–60) for a total of 4491 months. We obtained 1456 (97%) stool samples for *H. pylori* testing from a possible total of 1497 according to duration and periodicity of individual follow-ups. A total of 218 of 1456 (15%) samples were *H. pylori*–positive and 39 of 96 (41%) children had at least 1 positive sample during follow-up. For the latter, the overall median number of positive samples per child was 3.5 (IQR, 1–11).

Overall, *H. pylori* positivity increased progressively up to 21 months (Table 1). At 24 months, 26 (27%) subjects declined to continue the prospective follow-up; most of them (22/26) were always negative for *H. pylori.* One child had a persistent colonization starting at 15 months and the other 3 had 1 positive sample at 21 months (undeterminable status). First-time positivity continued throughout the follow-up period, although generally at lower frequency. Fifty-four percent of the 102 children enrolled completed 60 months of follow-up and the cumulative *H. pylori* positivity rate in this population reached 44%.

Transitory and Persistent *H. pylori* Stool Detection

Stool detection was transitory in 16 of 39 children with positive samples (41%), persistent in 19 (49%) and undetermined in 4 (10%). The latter group included the 3 children dropping out of the study at 21 months and 1 child with a positive sample at 60 months. Colonization/infection dynamics according to age is displayed in Figure 1. Transitory *H. pylori* stool detection occurred throughout the first 5 years of life. Persistence in contrast began mostly (17/19) during the first 24 months of life and as early as 3 months in 1 child. Children with persistence tended to be *H. pylori*–positive in a majority of the stool samples; only 2 of 19 children (patient 4 and patient 10 in Fig. 1) had a prolonged period of negative stool samples between positive samples. In only 1 patient persistence may have spontaneously ceased (patient 11), but this cannot be assured.

Host and Environmental Factors and *H. pylori* Stool Detection Status

Children with persistence had a first positive ELISA sample occurring at younger age, higher OD_{450} readings suggesting higher bacterial load, and a lower probability of having a secretor phenotype compared with children with transitory *H. pylori* detection (Table 2). For secretor-positive children, a trend toward higher type B prevalence in both persistent (2/13; 15.4%) and transitory (2/15; 13.3%) *H. pylori* detection (4/27; 14.8% combined) compared with always negative (1/43; 2.3%) cases was observed (P = 0.07). Exclusive breastfeeding at 6 months of age was significantly more common in the persistent group compared with noninfected group, as was attendance at daycare at 36 months of age (a similar nonsignificant trend was observed for other months).

For a majority of host and environmental factors, no difference was observed among children with stools never, transitorily, or persistently positive for *H. pylori.* For these factors, only overall results are provided (95% confidence limits provided unless otherwise specified) as follows: female, 44.8% (34.6–55.3%); living in a solid household, 56.4 % (33.4–54.2%); with complete urbanization, 87.2% (78.8–93.2%); median number of individuals living in the household at the time of any clinic visit, specifically shown for 15 months was 4 (IQR, 3–5), at 30 months was 4 (IQR, 3–5) and at 48 months was 3 (IQR, 3–5); children sleeping alone at the time of any clinic visit, specifically shown at 15 months was 42% (31.1–53.5%), at 30 months was 49.4% (38.1–60.7%) and at 48 months was 50% (35.5–64.5%); children using pacifier at the time of any clinic visit specifically shown at 15 months was 18.2% (9.1–30.9%), at 30 months was 12% (5.0–23.3%), and at 48

TABLE 1. Overall *Helicobacter pylori* Positivity by Trimester in a Cohort of Chilean Children Followed-up During the First 5 Years

	Age (mo)									
H. pylori Results	3	6	9	12	15	18	21†	24	27	30
Children at follow-up	102	100	98	97	96	96	96	70	69	68
% *H. pylori*–positive*	1	2	5	8	10	9	19	19	20	19
First-time positive/negative results‡	1/102	1/99	4/96	5/91	5/85	1/80	9/79	1/39	3/44	0/40
% First time positive	1	1	4	5	6	1	11	7	0	3
Cumulative positivity§	0	2%	4%	7%	16%	18%	25%	30%	30%	32%
	Age (mo)									
	33	36	39	42	45	48	51	54	57	60
Children in follow-up	68	68	65	62	62	59	59	56	56	55
% *H. pylori*–positive*	18	19	18	21	21	29	22	18	21	22
First time positive/negative results‡	0/38	2/38	0/33	1/30	1/30	4/25	0/21	1/18	0/17	0/16
% First-time positive	0	5	0	3	3	16	0	6	0	0
Cumulative positivity§	32%	35%	35%	35%	35%	42%	42%	44%	44%	44%

*At least 90% of samples were available for each individual month period.
†First follow-up period ended and 26 (27%) subjects declined to continue follow up, including 23 children always negative for *H. pylori* and 3 children with 1 or more positive samples.
‡First-time positive/negative results in subjects remaining after discounting children with 1 or more previously positive samples and always negative drop-outs between visits.
§For the 55 children completing 60 months of follow-up, any child with 1 or more *H. pylori*–positive sample.

TABLE 2. Differing Characteristics According to *Helicobacter pylori* Stool Detection Status Among the 96 Subjects Completing at Least 21 Months of Follow-up

Characteristic	*H. pylori* Detection Status			
	Never (N = 57)	Transitory (N = 16)	Persistent (N = 19)	*P**
First positive month, median (IQR)	NA	30 (15–48)	18 (12–24)	0.02
Optic density of samples, median (IQR)	NA	0.4 (0.3–1.0)	1.8 (1.5–2.5)†	0.001
Child secretor phenotype (%)	83%	100%‡	68%‡	0.02§
Exclusive breastfeeding at 6 mo (%)	21%‡	20%	44%‡	0.04§
Attending daycare at 36 mo (%)¶	9/34 (26%)‡	6/12 (50%)	10/15 (67%)‡	0.019§

*Significant by analysis of variance for age analysis and Kruskal-Wallis test for 2 groups for OD analysis because of nonhomogenous variances, by Fisher exact test for secretor phenotype, and for exclusive breastfeeding analysis, and by χ^2 with Yates correction for daycare attendance analysis.

†Represents the median (IQR) of the mean OD values for all persistent cases.

‡Indicates which proportions are being compared for the *p* value of the line.

§Indicates significance comparing the two results marked by ‡.

¶Data not available for all participants as indicated. Similar albeit nonsignificant trend among never and persistent groups were observed at 27 months (5/44 [11%] vs. 4/15 [27%]), 30 months (6/46 [13%] vs. 4/16 [25%]), 33 months (6/43 [14%] vs. 6/17 [35%]), 39 months (9/32 [28%] vs. 8/16 [50%]), and 42 months (8/30 [27%] vs. 8/15 [53%]).

NA indicates not applicable.

months was 8% (1.7–21.4%). Overall percent of children with at least 1 diarrhea episode during the first 18 months was 70.8% (95% confidence limits: 60.7–79.7%) and median number of diarrhea episodes was 2 (IQR, 1–2), none of which differed according to *H. pylori* infection status. A trend toward a higher proportion of children with 3 or more norovirus or rotavirus asymptomatic or symptomatic infections during the first 18 months of life was observed for the transitory group (29%) compared with the always negative group (14%; $P = 0.18$), but not for the persistent group (22%).

Symptoms and *H. pylori* Stool Detection Status

One or more symptoms (vomiting, abdominal pain, or distension) were referred by 30% of parents during any given visit. Abdominal pain was reported as the sole symptom by 15.5% of parents and distension or vomiting was reported by 2% each. Presence of 2 different symptoms at any given visit was reported by 9% of parents, with only 1 parent reporting all 3 symptoms, albeit at different visits. In an attempt to determine a possible relationship with *H. pylori*–positive stool detection, we analyzed presence of symptoms according to stool detection status and to the timing of stool positivity. Persistence was not associated with an increase in overall symptom reporting compared with never or transitorily stool-positive children. Only in 5 cases, 1 transitory and 4 persistent, was the presence of 1 or more symptoms and stool *H. pylori* detection "concomitant" as defined. One child (patient 3 in Fig. 1) with repeated referral of all 3 symptoms, although at different time points, had persistent stool positivity. None of the 16 of 19 persistent cases in patients followed-up by the pediatric gastroenterologist at 55 months of age had an abnormal physical finding. The median weight-for-height Z scores for 22, 12 and 11 never, persistent and transitorily colonized/infected children at 2, 3 and 4 years of age did not differ between or within groups (data not shown).

DISCUSSION

H. pylori was detected in stools from nearly half of children during their first 5 years of life in a middle- to low-socioeconomic area of Santiago, Chile, of whom 20% and 17% had persistent or transitory cases, respectively. Overall, *H. pylori* stool detection rate is comparable with a report of Portuguese children, also reaching 50% at 5 years of age using 6-month sampling.[19] In Bangladeshi children, cumulative positivity at 2 years of age reached 50%.[7] It is not surprising that colonization/infection rates were higher at earlier ages in a country with higher poverty indicators compared with Chile.[2] The rate of persistent infections was significantly higher in Chilean compared with Bangladeshi children. It is probable that the more frequent sample testing used in our study allowed a more accurate reflection of persistent infection status in middle- to low-income settings. The *Pasitos* cohort study also reported lower rates of persistent infection;[13] a possibly lower sensitivity of the UBT in young children[13,20] and nonidentification of persistent cases because of the relatively low number of tests performed per child (1–4 tests) may explain part of this difference. Frequency of persistent infections reached 3.7% at 24 months in a cohort of Japanese children followed-up with stool antigen detection every 4 months.[21] Thus, a different *H. pylori* detection rate and particularly persistence between Chilean children as compared with North American and Japanese children cannot be excluded. In the Portuguese study, persistence was not evaluated. A few of the transitory cases, especially those with readings near the cut-off value, could represent false-positive results because this test has been validated only in symptomatic individuals.[22,23]

Several novel findings of this study associated with persistence can be addressed. The majority of cases began in children before 2 years of age and, somewhat surprisingly, host secretor phenotype was less common in persistent compared with transitorily colonized/infected children. In vitro studies have suggested that Lewis B and H antigens, present in mucosa of secretor individuals, could be receptors for *H. pylori* attachment,[24] contrasting with our results that suggest that the presence of these antigens could protect against persistent colonization/infection in asymptomatic children. If this result is sustained in larger series, then the role of the host factors in dynamics of infection should gain importance. Blood type B had a marginal association with *H. pylori* positivity differing from other studies that more commonly report type O in infected adults[25,26] and possibly A in children,[7] although the scope of this association remains unclear.[27]

A small number of environmental factors associated with *H. pylori* persistent or transitory colonization/infection could be identified. An increased probability of daycare attendance in persistently infected children was an expected finding because it has been repeatedly associated with an increased risk for transmissible pathogens.[28] A higher probability of breastfeeding in the persistent group at a marginal significance is an interesting finding because it also would oppose common thinking. Several possible explanations could be speculated if this finding is sustained in larger series.

H. pylori persistence was not associated with identifiable symptoms or physical abnormalities. Only 4 of 19 children had a possible concomitance between symptom referral and stool positivity, and in 1 child these symptoms were multiple and repeated

overtime. This could be a case to benefit from endoscopy, a procedure restricted mainly for older symptomatic children.[29] The gastroenterologist did not find clinical merit for endoscopy in any child followed-up in this study. Thus, we conclusively demonstrate that only a small fraction of young *H. pylori*–positive children present symptoms confirming other results.[6] We hope to provide further insight in the potential impact of persistence through a currently ongoing cohort study of 300 children followed-up from 1 to 3 years of age using genome-wide analysis to identify host response signals to colonization/infection and real-time polymerase chain reaction applied to stools to identify specific bacterial virulence factor–associated genes.

Several weaknesses of this study should be addressed. This study is an extension of a viral enteric pathogen study in which a part of the tested samples were retrospective and the reconsent process for continuing follow-up led to a 27% drop-out of mostly *H. pylori*–negative children at the 21-month visit. Testing frozen samples could have reduced stool antigen detection yield, but this does not seem to occur.[30] The drop-out of children with negative samples could bias toward an increased proportion of children with positive samples after 18 months. This was balanced by periodical analysis of the rates of first-time positive cases. Nevertheless, a small overrepresentation of positive cases in children at 5 years of age is possible. In addition, colonization could not be differentiated from infection in this study. It is possible that one-time positive samples, especially those with readings below OD_{450} of 0.5 (10/16 transitory cases, data not shown) may not be attributable to *H. pylori*. Finally, the numbers of children in the different groups for comparison of host and environmental factors are relatively small, a situation we hope to overcome in our ongoing, large, second cohort study.

In conclusion, *H. pylori* persistence was common in Chilean children during the first 5 years of life and significantly associated with nonsecretor phenotype and daycare attendance. Further characterization of persistence in the 5- to 10-year age interval, including more children in the different *H. pylori* status groups, and studies of host signals and bacterial factors associated with persistence will further improve our understanding of the relevance and impact of *H. pylori* colonization/infection in children. A comprehensive understanding of the dynamics of *H. pylori* infection/colonization in children should lead to more rationale treatment and prevention strategies in countries with high *H. pylori* prevalence rates and gastric cancer mortality.

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