Significant decrease in the prevalence of *Wuchereria bancrofti* infection in anopheline mosquitoes following the addition of albendazole to annual, ivermectin-based, mass treatments in Nigeria


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Received 1 September 2004, Revised 7 October 2004, Accepted 11 October 2004

A prospective entomological survey was conducted in four sentinel villages in central Nigeria from 1999–2002, to assess the impact of annual, single-dose, mass drug administrations (MDA), with a combination of ivermectin and albendazole, on the transmission of *Wuchereria bancrofti*. As they were also endemic for human onchocerciasis, the four villages had received annual MDA based on ivermectin alone for 7 years prior to the addition of albendazole. Resting *Anophelines gambiae* s.l., *An. funestus* and *Culex* species were collected from 92 sequentially sampled households and dissected. Mosquitoes harbouring any larval stage of *W. bancrofti* were classified as ‘infected’, and those containing the third-stage larvae of the parasite were classified as ‘infective’. Over the 41-month observation period, 4407 mosquitoes were captured and dissected, of which 64% were *An. gambiae* s.l., 34% *An. funestus*, and 1% *Culex* species. The baseline data, from dissections performed before the addition of albendazole to the MDA, showed high prevalences of mosquito infection (8.9%) and infectivity (2.9%), despite apparently good treatment coverages during the years of annual ivermectin monotherapy. Only the anopheline mosquitoes were found to harbour *W. bancrofti* larvae. After the third round of MDA with the ivermectin–albendazole combination, statistically significant decreases in the prevalences of mosquito infection (down to 0.6%) and infectivity (down to 0.4%) were observed (*P*<0.0001 for each). The combination of albendazole and ivermectin appears to be superior to ivermectin alone for reducing the frequency of *W. bancrofti* infection in mosquitoes.

In Africa, human lymphatic filariasis (LF) is caused by *Wuchereria bancrofti*, a filarial nematode that is transmitted in rural areas by anopheline mosquitoes (Ottesen et al., 1997; Molyneux et al., 2000). In many areas south of the Sahara, LF and the human onchocerciasis (river blindness) caused by *Onchocerca volvulus* co-occur (Richards et al., 2000; Hopkins et al., 2002; Molyneux et al., 2003). The drug ivermectin (Mectizan®), which is effective against the microfilariae...
(mff) of both *Wuchereria* and *Onchocerca*, is currently being donated by Merck & Co. for the control of both LF and onchocerciasis in Africa, via mass drug administrations (MDA). For annual MDA based on ivermectin alone, the recommended dose for the control of onchocerciasis (150 µg/kg; Burnham, 1998) is considerably lower than that for the control of LF (400 µg/kg; Brown *et al.*, 2000). Where onchocerciasis and LF are co-endemic, however, the World Health Organization (WHO) recommends that ivermectin be administered, at the lower, ‘onchocerciasis’ dose (150 µg/kg), simultaneously with 400 mg albendazole/subject. Albendazole is being donated by GlaxoSmithKline, and the ivermectin–albendazole combination appears safe (Horton *et al.*, 2000; Makunde *et al.*, 2003) and more effective than ivermectin (at 150 µg/kg) alone in lowering *W. bancrofti* microfilaraemias (Addiss *et al.*, 1997; Ottesen *et al.*, 1997, 1999; Ismail *et al.*, 1998). It is hoped that *W. bancrofti* microfilaraemias can be lowered sufficiently to interrupt all transmission and so eliminate bancroftian filariasis as a public-health problem (Molyneux *et al.*, 2000; Plaisier *et al.*, 2000; Molyneux and Zagaria, 2002).

Against onchocerciasis, the ivermectin–albendazole combination is not considered more effective than ivermectin alone (Awadzi *et al.*, 2003; Makunde *et al.*, 2003).

Within Africa, Nigeria is thought to have the greatest numbers of people at risk of LF (Lindsay and Thomas, 2000; Hopkins *et al.*, 2002) and the second largest population at risk of onchocerciasis, after the Democratic Republic of Congo (WHO, 1995; Jiya, 1998). A pilot initiative to integrate LF-elimination efforts in Nigeria into a mature, MDA-based programme for the control of onchocerciasis began in 2000 (Hopkins *et al.*, 2002). The baseline evaluations for this initiative revealed that there were villagers with LF antigenaemia in most (90%) of the 149 villages that had been receiving ivermectin monotherapy for onchocerciasis since 1993, the prevalence of such antigenaemia varying from 0%–67% (mean=22.4%). Hydrocele (Eigege *et al.*, 2003) and entomological surveys (unpubl. obs.) in 1999 showed that the prevalences of hydrocele and of human and mosquito infection with *W. bancrofti* inside the onchocerciasis-endemic zones (where there had often been several rounds of MDA based on ivermectin alone) were similar to those in the areas where onchocerciasis was not endemic (where there had been no MDA). Together these observations indicated that ivermectin monotherapy would not interrupt *W. bancrofti* transmission. The main aim of the present study was to determine if the addition of albendazole to ivermectin-based MDA would produce a measurable decrease in the prevalence of mosquito infection with *W. bancrofti*.

**MATERIALS AND METHODS**

**Study Area**

Plateau and Nasarawa states are located in central Nigeria and together are formed of 30 administrative districts called local government areas (LGA). Twelve of these LGA are designated as mass-treatment zones for the control of onchocerciasis and have received annual MDA, based on ivermectin-alone, since 1993 (Richards *et al.*, 1996; Hopkins *et al.*, 2002). LF mapping, performed in 1999 and based on a commercial, rapid immunochromatographic (ICT) card test that detects AD12.1 filarial antigenaemia (ICT Diagnostics, Balgowlah, Australia) — now produced as the NOW® ICT filariasis kit (Binax, Portland, ME) — indicated that all 30 LGA were endemic for LF and therefore needed mass treatments with the ivermectin–albendazole combination (Weil *et al.*, 1997; Hopkins *et al.*, 2002). Rapid entomological surveys, in which resting mosquitoes caught in randomly selected household compounds were dissected, have since been conducted in villages both inside and outside the onchocerciasis-endemic
zones (unpubl. obs.). The principal local vector species identified in these surveys were *Anopheles gambiae* s.l. and *An. funestus*; *Culex* species constituted <3% of the mosquitoes caught (unpubl. obs.). For the present study, four ‘entomology sentinel villages’ were selected from the villages where these entomological surveys had been conducted, so that the impact on *W. bancrofti* transmission of adding albendazole to the ongoing ivermectin-based MDA (for the control of onchocerciasis) could be serially monitored.

**Entomology Sentinel Villages**

The criteria for selecting an ‘entomology sentinel village’ included: (1) a longstanding ivermectin-treatment programme for onchocerciasis, with good reported coverage (i.e. >65% of the total population); (2) a prevalence of filarial antigenaemia (as detected by ICT cards) of >40%; (3) a prevalence of infection (with any larval stage of *W. bancrofti*) in the anopheline mosquitoes of 3%; (4) a location >10 km from the border of any ivermectin-untreated area and >10 km from any other villages; (5) easy vehicular access during the rainy season; and (6) a village leadership and household-compound residents willing to allow the research team to enter houses to collect mosquitoes routinely, over the course of several years.

The four communities chosen as entomology sentinel villages were Seri (population 1406), Lankan (1478), Gbuwhen (361) and Maiganga (512). In previous, ICT-card-based surveys (Hopkins *et al.*, 2002; Eigege *et al.*, 2003) of ‘convenience’ samples of the adult residents, Seri was found to have the highest prevalence of *W. bancrofti* filarial antigenaemia (62%; *N*=50), followed by Maiganga (54%; *N*=50) and then Lankan (47%; *N*=70) and Gbuwhen (47%; *N*=30). Mass monotherapy with ivermectin for onchocerciasis began in Maiganga in 1992, in Lankan and Gbuwhen in 1993, and in Seri in 1995. MDA based on the ivermectin–albendazole combination were launched in July 2000 in three of the four sentinel villages (Seri, Lankan, and Gbuwhen), and in October 2000 in Maiganga.

**Longitudinal Sampling**

Mosquitoes were collected, in the same household compounds, from July 1999–November 2002, with each sentinel village being visited for 3–4 days every 2–3 months. The 41-month observation period included baseline measurements, which lasted approximately 12 months per community, during the ‘pre-albendazole period’ of ivermectin monotherapy, followed by a period that encompassed three rounds of MDA with the ivermectin–albendazole combination. All the household compounds (each a cluster of huts and houses enclosed by a fence or mud wall) that were easily accessible in each village were visited and the study was described to their residents. The compounds where families agreed to participate were numbered and sequentially visited. During each morning of a working visit, the indoor-resting (primarily blood-fed) female mosquitoes in odd-numbered compounds were caught, following the recommendations of the World Health Organization (WHO, 2002), for dissection (see below). During each afternoon of a working visit, pyrethrum ‘knockdown’ collections of the mosquitoes in even-numbered compounds were performed, using standard techniques; the mosquitoes so collected were sorted by species, counted, and placed in tubes with desiccant for future laboratory testing (to be described elsewhere).

**RESTING CAPTURES AND DISSECTIONS**

Two collectors entered the odd-numbered compounds between 06.00 and 11.30 hours and, using flashlights and aspirators, collected all the mosquitoes that could be identified in the huts and houses where humans resided. Seven to eight compounds
could be sampled during each morning's work. The collected mosquitoes were immediately transferred to screened paper cups (which were labelled with the compound numbers) and kept alive in a cool box containing moist towels. In the afternoon, each female mosquito was killed, identified (as *Anopheles gambiae* s.l., *An. funestus*, other *Anopheles* species, *Culex*, or another genus), and separated into head, thorax and abdomen on a glass slide. Each of these body parts was teased apart in a drop of normal saline under a binocular dissecting microscope. The slide preparation was then passed to a trained microscopist (D.P. or A.K.), who looked for the first-, second- or third-stage larvae of *W. bancrofti*, using a regular light microscope at ×100–×500.

**Treatment Coverage**

The total number of people treated in each village in each year during the period of monotherapy (from 1993–1999) and combination therapy (from 2000–2002) was determined by reviewing the village treatment registries and annual summary treatment statistics kept by the local and state Ministry of Health offices. Treatment coverage in each village in each year was then calculated, as a percentage of the ‘population denominator’ — the total population of the village in that year (as estimated by a census prior to or during the MDA in that year). If no census had been conducted in a particular year, the village was assumed to have the same population as observed in the previous year's census. ‘Aggregate annual treatment coverages (%)’ — 100 × (the sum of all treatments given in each village over a certain number of years)/(the sum of all the corresponding annual ‘population denominators’ for that village) — and ‘interval treatment coverages (%)’ — 100 × (the sum of all treatments provided in a village over the entire 1993–2002 period)/(the sum of all the corresponding annual ‘population denominators’ for that village) — were also calculated for each village.

**Analysis**

The data on each dissected mosquito were individually coded and keyed into a computer database. ‘Infected’ mosquitoes were defined as those harbouring the first-, second- or third-stage larvae of *W. bancrofti*. Each mosquito found to contain at least one of the parasite’s human-infective third-stage larvae (L₃) was considered ‘infective’ (and thus the infective formed a subset of the infected). Mosquitoes containing only mff were not considered in the analysis.

The collected data were analysed using version 6.04d of the Epi Info software package (Centers for Disease Control and Prevention, Atlanta, GA) and version 8.0 of the SAS software (SAS Institute, Cary, NC). Categorical time intervals were constructed for the dissections performed over six periods: the interval prior to the addition of albendazole, and then 1–6, 7–12, 13–18, 19–24 and 25–30 months after starting albendazole. The percentages of the dissected mosquitoes found infected and infective were calculated for each interval, along with 95% confidence intervals. Differences between communities and time intervals were compared, as categorical data, in $\chi^2$ tests.

**Ethical Review**

The study protocol was approved by the Ministries of Health of Plateau and Nasarawa states in Nigeria and by the Institutional Review Board of Emory University in Atlanta (Georgia, U.S.A.).

**RESULTS**

There were a total of 226 household compounds in the four villages, of which 92 provided the 4407 live resting mosquitoes collected for immediate dissection. Most (64.6%) of the mosquitoes dissected were *An. gambiae* s.l. but 33.9% were *An. funestus* and a few were of *Culex* or other genera (Table 1). The percentages of the...
collections represented by *An. gambiae* s.l. and *An. funestus* varied markedly from one village to another, and in Gbu when more *An. funestus* than *An. gambiae* s.l. were caught (Table 1). Overall, 3.55% of the *An. gambiae* s.l. but only 1.00% of the *An. funestus* dissected were found infected (*P*<0.001; Table 1). Mosquitoes belonging to *Culex* species represented only 1.25% of the collections and none was found infected.

In Figure 1, the prevalences of mosquito infection recorded when the MDA were based on ivermectin alone are compared with those recorded at least 24 months after the addition of albendazole (i.e. after the third round of MDA based on ivermectin–albendazole). The overall prevalences of both infection (8.9%) and infectivity (2.9%) during the pre-albendazole period were significantly higher that those recorded for infection (0.6%) and infectivity (0.4%) after the third round of treatment with the combination (*P*<0.0001 for each). When the corresponding data for each village were compared, it appeared that, over the same period, the prevalences of mosquito infection had fallen significantly in all of the sentinel villages except Seri (Table 2). For all four villages combined, the fall in the prevalence of mosquito infection, compared with that recorded pre-albendazole, was

FIG. 1. Data from the dissections of the indoor-resting anopheline mosquitoes collected in sequentially examined households during the period 1999–2002. Each household was visited every 2–3 months. The ‘infected’ mosquitoes harboured any larval stage of *Wuchereria bancrofti* whereas the ‘infective’ held third-stage larvae. The graphs show the results recorded prior to the use of albendazole in the annual mass drug administrations (MDA) but after 7 years of MDA based on ivermectin alone (■), and those recorded 25–30 months after the addition of albendazole to the ivermectin-based MDA, 1–6 months after the third round of treatment with the ivermectin–albendazole combination (□).

### TABLE 1. Aggregate results of the mosquito dissections, split by village

<table>
<thead>
<tr>
<th>Village</th>
<th>Gbuwhen</th>
<th>Lankan</th>
<th>Maiganga</th>
<th>Seri</th>
<th>All four</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of mosquitoes dissected</td>
<td>1271</td>
<td>1311</td>
<td>957</td>
<td>868</td>
<td>4407</td>
</tr>
<tr>
<td><em>Anopheles funestus</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>% of mosquitoes from village</td>
<td>62.55</td>
<td>10.22</td>
<td>48.07</td>
<td>2.44</td>
<td>33.97</td>
</tr>
<tr>
<td>Prevalence of infection (%)</td>
<td>0.25</td>
<td>2.99</td>
<td>1.74</td>
<td>0.00</td>
<td>1.00</td>
</tr>
<tr>
<td><em>Anopheles gambiae</em> s.l.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>% of mosquitoes from village</td>
<td>35.64</td>
<td>89.55</td>
<td>51.52</td>
<td>83.71</td>
<td>64.62</td>
</tr>
<tr>
<td>Prevalence of infection (%)</td>
<td>0.44</td>
<td>4.94</td>
<td>1.42</td>
<td>4.67</td>
<td>3.55</td>
</tr>
<tr>
<td><em>Culex</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>% of mosquitoes from village</td>
<td>1.81</td>
<td>0.23</td>
<td>0.42</td>
<td>2.88</td>
<td>1.25</td>
</tr>
<tr>
<td>Prevalence of infection (%)</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.81</td>
<td>0.16</td>
</tr>
<tr>
<td>OTHER GENERA</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>% of mosquitoes from village</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.16</td>
</tr>
<tr>
<td>Prevalence of infection (%)</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
</tr>
</tbody>
</table>
already significant after the second round of treatments with the ivermectin–albendazole combination (Fig. 2).

Aggregate annual treatment coverages in each of the villages ranged from 63%–86% (Fig. 2). There was no significant difference between the coverage achieved in each village during the pre-albendazole period (1993–1999) and that achieved after albendazole had been added to the annual MDA (2000–2002). Seri (which, interestingly, was the only village where no statistically significant decrease in the prevalence of mosquito infection was observed) had the highest interval treatment coverage (86%). The interval treatment coverages for

![Graph](image-url)

**FIG. 2.** Aggregate annual treatment coverages for the period of ivermectin monotherapy (■), and after the addition of albendazole (□). For the period 1999–2002, the prevalences of mosquito infection are also shown (○), with their 95% confidence intervals indicated by vertical lines. The prevalences shown run from that recorded in 1999 (12 months prior to the addition of albendazole to the ivermectin-based mass drug administrations) to those recorded 1–6, 7–12, 13–18, 19–24 and 25–30 months after the addition of the albendazole, and so encompass three annual rounds of treatment with the ivermectin–albendazole combination.

### TABLE 2. The prevalences of infection among the mosquitoes dissected in the pre-albendazole period and those dissected after three annual rounds of mass-treatment with the ivermectin–albendazole combination, split by village

<table>
<thead>
<tr>
<th>Village</th>
<th>Pre-albendazole</th>
<th>After three rounds of ivermectin–albendazole</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. of mosquitoes:</td>
<td>Prevalence of infection and (95% confidence interval) (%)</td>
</tr>
<tr>
<td></td>
<td>Dissected</td>
<td>Found infected</td>
</tr>
<tr>
<td>Gbuwen</td>
<td>60</td>
<td>2</td>
</tr>
<tr>
<td>Lankan</td>
<td>235</td>
<td>23</td>
</tr>
<tr>
<td>Maiganga</td>
<td>72</td>
<td>7</td>
</tr>
<tr>
<td>Seri</td>
<td>61</td>
<td>4</td>
</tr>
<tr>
<td>All four</td>
<td>428</td>
<td>36</td>
</tr>
</tbody>
</table>

*P* values indicate statistical significance.
Maiganga, Lankan and Gbuwhen were 82%, 78% and 63%, respectively.

DISCUSSION

The high prevalences of infection and infectivity recorded in the mosquitoes at the start of the present study indicate that 5–7 years of annual mass treatments with ivermectin alone failed to interrupt transmission of *W. bancrofti* in the study area, where *An. gambiae* s.l. appears to be the main vector. In Burkina Faso, Kyelem et al. (2003) similarly reported that 5 years of semi-annual mass treatments with ivermectin alone (targetted at onchocerciasis) reduced but did not interrupt the transmission of *W. bancrofti*.

During the present study, the addition of albendazole to the ivermectin-based MDA led, after three rounds of treatment, to significant reductions in the prevalences of mosquito infection in three of the four sentinel villages. Annual mass treatment with ivermectin–albendazole therefore appears superior to annual ivermectin monotherapy for lowering or stopping the transmission of *W. bancrofti*, at least in rural areas of sub-Saharan Africa. The present results appear to represent the first entomological confirmation of the importance of albendazole in MDA-based programmes of LF control (Addiss et al., 1997, 2004; Ismail et al., 1998; Ottesen et al., 1999; Dunyo et al., 2000), although the present study has some limitations that need to be considered. Firstly, as there were no human-landing catches, it was impossible to determine vector biting rates or transmission potentials. Therefore, although statistically significant decreases in the prevalences of mosquito infection and infectivity were observed, the abundance and human-biting activity of the vectors may have been sufficient to maintain the transmission of *W. bancrofti* after albendazole had been added to the MDA. In a recent study in Papua New Guinea, Bockarie et al. (2002) showed that MDA-driven reductions in community levels of microfilaraemia and mosquito infection did not always overcome the abundance of human-biting mosquitoes and in some communities, therefore, transmission potentials remained unacceptably high. A second weakness of the present study is the lack of information on the prevalences and levels of microfilaraemia in the residents of the sampled household compounds. No attempt was made to determine the prevalences of mosquito infection with the first-stage larvae of *W. bancrofti* (which would give some indication of the prevalence of microfilaraemia in the humans on which the collected mosquitoes had fed). Unfortunately, the prevalences of mosquito infection with the third-stage larvae of the parasite (which were recorded) are less likely to mirror closely the infection status of the residents of the sampled compounds. Female mosquitoes that have ingested blood containing mf need to oviposit before the parasites develop into L3, and therefore usually leave the compound in which they took the infective bloodmeal, in search of a suitable oviposition site. These females, when seeking subsequent bloodmeals, presumably enter other compounds and become randomly distributed throughout the community. Thus, the present data on mosquito infectivity are probably better indices of community transmission than of human-infection status in any particular households. A third weakness of the present study is that treatment coverage was estimated per village, not per individual household compound. A sampled household may have held an infected resident who had repeatedly refused treatment and continued to infect mosquitoes biting in his or her compound. Repeated sampling in that compound could then have spuriously increased the present estimates of transmission in the community at large. Such bias appears to be the most likely reason why one village (Seri) appeared to have persistently high prevalences of mosquito infection despite having the
highest interval treatment coverage (86%) of the four sentinel villages. There was, however, no evidence of the mosquito infections that were detected clustering by compounds in any of the four villages (data not shown), indicating that the present data were not influenced by a few infected villagers who repeatedly refused treatment.

As the prevalence of mosquito infection is gradually reduced by repetitive rounds of MDA, entomological-impact monitoring (at least that based on traditional dissection methods) will become ever more difficult, and ever larger sample sizes will be required to prove that further decreases in mosquito infection are statistically significant. The use of PCR-based assays, or other molecular techniques, to detect *W. bancrofti* in mosquito ‘pools’ may be one solution to these problems (Williams et al., 2002). Such techniques have already been successfully used in the entomological monitoring of onchocerciasis-control schemes (Katholi et al., 1995), although the ability to separate and test *Simulium* heads (which, in infected *Simulium*, may only carry the L₃ of *O. volvulus*, not the younger larvae) is a distinct advantage in the PCR-based monitoring of LF. As the first-, second- and third-stage larvae of *W. bancrofti* occur in both the heads and thoraces of infected mosquitoes, it is currently impossible to separate infective mosquitoes from other infected mosquitoes using molecular methods. Even PCR-based estimates of the prevalence of infection with the (first-, second- and third-stage) larvae of *W. bancrofti* may be made over-estimates, unless the abdomens of the mosquitoes (which may contain mff that cause PCR positivity) are carefully removed from the samples being tested. For these reasons, mosquito sampling and handling techniques, and the treatment status of the people residing in the sampled households, will become even more important issues when molecular assays are deployed for the entomological monitoring of LF.

MDA based solely on ivermectin may not interrupt the transmission of *W. bancrofti* in all areas where LF and onchocerciasis overlap (Plaisier et al., 2000) but the scale of the ivermectin distribution already in place throughout the Nigerian federation is immense — there were, according to unpublished data from the African Programme for Onchocerciasis Control, >20 million ivermectin treatments in 2003. There is therefore an unprecedented logistical opportunity to scale up the current LF-control programme in the country, quickly and relatively simply, by the addition of albendazole to the ivermectin in the areas already being treated for onchocerciasis (Hopkins et al., 2002). To enable such collaboration, areas where LF and onchocerciasis are co-endemic need to be mapped, and both the LF- and onchocerciasis-control programmes need to articulate the benefits of programme integration to local programme managers, international technical committees, and the donor community (Molyneux, 2004).

ACKNOWLEDGEMENTS. The authors thank R. Ajigbeda, B. Bagnall, D. Blaney, D. Colley, I. Dhillon, M. Iwamoto, D. Hopkins, J. Jiya, N. Kruse, T. Lehmann, W. Mathai, E. Mathieu, S. Sullivan, A. Wright and P. Wuchet for their help. The entomology described was carried out with support from GlaxoSmithKline, the Emory University Lymphatic Filariasis Support Center, and the Bill and Melinda Gates Foundation. The Mectizan® and albendazole used in the MDA in the study area were donated by Merck & Co and GlaxoSmithKline, respectively. The Carter Center assisted the Ministry of Health’s programme for onchocerciasis control in Plateau and Nasarawa states, in partnership with Lions Club’s SightFirst Program, Lions District and the African Program for Onchocerciasis Control.
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