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High prevalence of *Wuchereria bancrofti* infection as detected by immunochromatographic card testing in five districts of Orissa, India, previously considered to be non-endemic

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ABSTRACT

India accounts for over one-third of the world's burden of lymphatic filariasis (LF). Although most coastal districts of Orissa state (eastern India) are LF-endemic, the western districts of Orissa are considered non-endemic. During a large-scale insecticide-treated bed net/microfinance trial, we tested one randomly selected adult (age 15-60 years) for LF from a random sample of microfinance-member households in five districts of western Orissa, using immunochromatographic card testing (ICT). Overall, 354 (adjusted prevalence 21%, 95%CI 17-25%) of 1563 persons were ICT positive, with district-wide prevalence rates ranging from 15-32%. This finding was not explained by immigration, as only 3% of subjects had ever lived in previously known LF-endemic districts. These results therefore suggest ongoing autochthonous transmission in districts where LF control programs are not operational. Our results highlight the importance of broad, systematic surveillance for LF in India and call for the implementation of LF control programs in our study districts.

1. Introduction

Lymphatic filariasis (LF) is a major vector-borne public health problem affecting more than 120 million people in over 80 endemic tropical and subtropical countries.[1] Caused by the nematode parasites *Wuchereria bancrofti, Brugia malayi,* and *B. timori,* manifestations of LF include subclinical infection, chronically disabling lymphedema, hydrocoele, and elephantiasis.[2]

Circulating microfilariae are responsible for transmission of LF; many elimination and control efforts therefore attempt to interrupt transmission by decreasing the prevalence of persons with microfilaremia in the population. Annual doses of albendazole plus either diethylcarbamazine (DEC) or ivermectin are effective at reducing the prevalence of microfilaremia in a population.[3] Given the high efficacy and cost-effectiveness of this approach, the current strategy for eliminating LF involves identification of endemic areas followed by regular administration of these drugs to most of the population, a strategy known as mass drug administration (MDA).[3–5] WHO guidelines call for MDA programs in areas with an LF prevalence of 1% or higher.[6]

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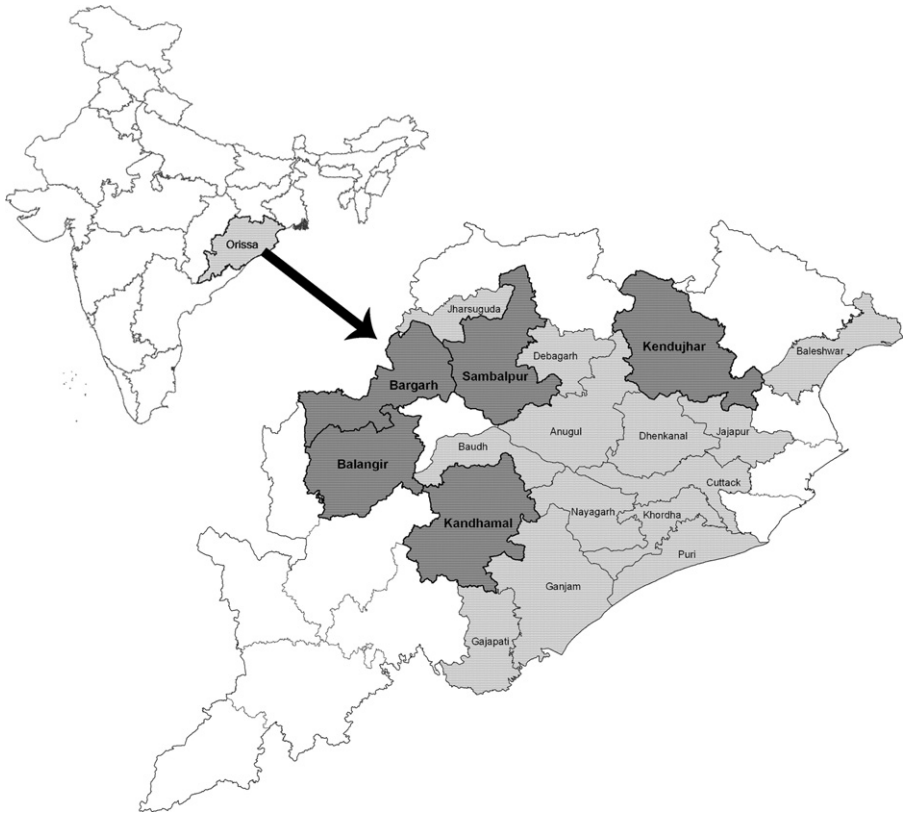

Figure 1. Study districts and known LF-endemic districts in Orissa state, India. Dark gray indicates the five study districts. Light gray indicates non-study districts previously known to be LF-endemic (microfilaremia > 1%).

Successful elimination of LF requires accurate mapping of endemic regions to effectively target MDA programs. This is particularly important in India, which bears over one-third of the global LF burden with approximately 48 million people infected.[7] However, MDA programs in India have expanded without comprehensive or systematic district-level LF prevalence data, leading to a need for more accurate and complete data.[7–9]

Orissa (Figure 1) is the most impoverished Indian state.[10] Although detailed and systematically collected data on LF in Orissa have not been reported, a compilation of national survey data and selected publications in 2000 estimated the prevalence of LF (persons with microfilaremia or clinical filarial disease) at 8%.[7] The same study identified Orissa's eastern (coastal) districts as one of four high priority regions in India for targeted control due to their hyperendemicity (>10% combined microfilaremia and clinical filarial disease).[7] As a result, both small-scale epidemiological studies and MDA programs in Orissa have focused on these coastal districts.[11–17]

Despite this focus on Orissa's coastal districts, evidence from these LF mapping activities[7] and from a 2005 district-level microfilaremia surveillance survey by Babu BV and others (unpublished data) suggests that other districts in western Orissa may also be LF-endemic. Therefore, we undertook a survey that sought to more precisely document LF prevalence in these districts where LF control programs are not currently ongoing.

2. Materials and methods

2.1. Study site

Data were collected during 2007-2009 in 141 villages across the five districts of Bargarh, Balangir, Kandhamal, Kendujhar, and Sambalpur, located in western Orissa state, India (Figure 1). At the time of the 2001 Indian national census, Orissa had a population of 36.8 million people distributed across 30 districts. The populations of the five districts in this study totaled approximately 5.8 million and ranged from about 648 000 in Kandhamal to 1 562 000 in Kendujhar.[18]

2.2. Study design and population

The data were collected as part of a randomized, stratified, community-based trial of sustainable insecticide-treated bed net (ITN) distribution strategies using microfinance loans written by micro-lender Bharat Integrated Social Welfare Association (BISWA). Villages, the primary sampling unit, were selected from a list of the 878 villages with ongoing BISWA operations in the five study districts. The number of villages sampled from a given district was proportionate to the district population, with the additional condition that the number of villages sampled in each block (administrative unit) was a multiple of three, given that there were three study arms in the

randomized trial. A total of 141 villages were selected in this manner. Within each village, fifteen households that were members of the microfinance group BISWA were randomly selected for participation in the study. In villages with fewer than fifteen microfinance-member households, all microfinance-member households were selected.

2.3. Blood testing

In the spring of 2007, a baseline questionnaire and blood testing were performed in the selected households. During these visits, one randomly selected person aged 15–60 years was tested for the presence of *W. bancrofti* antigenaemia using the Binax NOW Filariasis immunochromatographic card test (ICT) (Inverness Medical, Princeton, NJ, USA). This methodology has been well-validated for the detection of *W. bancrofti* antigenaemia.[19] Surveyors were properly trained in performing and interpreting ICT test results. Supervisors oversaw quality control to ensure that tests were read within 10-15 minutes of collection and properly recorded.

2.4. Surveys

Surveys were conducted in Oriya, the local language. Field assistants administered a baseline household-level questionnaire (in 2007) to adult respondents who underwent the blood testing described above; this included a comprehensive survey of household demographic, socioeconomic, and health characteristics. The questionnaire was not primarily designed to study LF, therefore, it did not include specific questions about LF symptoms, and no clinical examinations were performed. During a return visit in the spring of 2009, a follow-up questionnaire was administered to households that had previously participated in the baseline questionnaire and blood testing described above. As part of this survey, additional questions regarding migration, birthplace, and places of previous residence (for >6 months) were asked.

Individual-level data from the National Family Health Survey II of India (NFHS II; conducted 1998–1999) were used to compare demographic characteristics of the sample population with the general population of Orissa.

2.5. Definition of known LF-endemic districts

Based on a microfilaremia survey which consisted of night-time blood smear collection in select Orissa districts prior to our study (in 2005, sample size 74 500), the following districts were identified as known LF-endemic based on >1% microfilaremia prevalence: Anugul, Baleshwar, Baudh, Cuttack, Debagarh, Dhenkanal, Gajapati, Ganjam, Jajapur, Jharsuguda, Khordha, Nayagarh and Puri (Babu BV and others, unpublished data).

2.6. Statistical analysis

Statistical analysis was conducted using STATA, version 10 (StataCorp LP, College Station, TX, USA).

All reported prevalence rates were adjusted to account for the sampling design, which included population-proportionate sampling of villages at the district level but a fixed number of microfinance-member households sampled within each village. Under this sampling scheme, microfinance-member households in villages with relatively few microfinance-member households would have a greater likelihood of being in the sample. Probability weights were constructed to correct for this bias. Adjusted prevalence rates and tests for statistical significance accounted for stratification using linearized variance estimations with villages as the primary sampling unit, districts as the unit of stratification and probability weights to account for the sampling design. The reported adjusted prevalence rates are therefore representative for the population of microfinance-member households in these five districts.

The Pearson x^2 statistic was used to test for differences in demographics between the sample population and NFHS II and to test for differences in the prevalence of ICT positivity across migration indices or bed net usage.

3. Results

3.1. Study population

Overall, 1848 households completed the baseline questionnaire; of these, 1563 (84.6%) were successfully tested for *W. bancrofti* antigenaemia. Of the 1563 tested individuals included in this analysis, migration data (as determined in the follow-up survey) were complete for 1379 (88.2%) individuals.

The median age of tested subjects was 31 years, and 32.9% (514/1563) were male; the median household size was five persons. Based on data from the 1998-1999 Indian National Family Health Survey II, our sample was older ($P < 0.001$), more educated ($P = 0.001$) and contained more females ($P < 0.001$) than the overall population of Orissa state.

3.2. Prevalence of W. bancrofti infection

Overall, *W. bancrofti* antigenaemia was present in 354 (adjusted prevalence 20.9%; 95% Cl 16.7–25.0%) of the 1563 subjects across all five districts, ranging from 15.3% (95% Cl 8.5–22.1%; 111/556) adjusted prevalence in Bargarh district to 32.2% (95% Cl 24.2–40.2%; 84/260) adjusted prevalence in Sambalpur district (Table 1). There was no significant correlation between age and the prevalence of antigenaemia across the entire sample (data not presented). However, our age-restricted sample did not allow us to test for potential correlations below age fifteen.

3.3. Migration

The population showed little short-term migration, as just 5.7% (adjusted prevalence; 75/1559) relocated between 2007 and 2009. Only 2.7% (adjusted prevalence; 35/1340) of the population reported ever living for more than six months in a known LF-endemic district in Orissa. Similarly, only 1.9% (adjusted prevalence; 32/1355)

Table 1
Prevalence of *Wuchereria bancrofti* antigenaemia (ICT positive) by district

District	ICT positive %[a] (95% CI)	*n*
Sambalpur	32.2 (24.2–40.2)	84/260
Balangir	26.7 (20.8–32.5)	85/339
Bargarth	15.3 (8.5–22.1)	111/556
Kendujhar	15.6 (10.0–21.3)	60/325
Kandhamal	15.5 (4.5–26.6)	14/83
Overall	20.9 (16.7–25.0)	354/1563

ICT: immunochromatographic card test
[a] Adjusted for sampling design to generalize measurements to the sampled population; reported percentages therefore differ from raw percentages calculated from *n*

reported that their birthplace was in an LF-endemic district. Only 14.7% (adjusted prevalence; 173/1379) of the population had lived in any district other than their current residence for more than six months, most (94.0% adjusted prevalence; 167/1379) in only one other district. Of these respondents, 84.6% (adjusted prevalence; 139/167) were also born in another district, suggesting that 97.0% (adjusted prevalence; 1345/1379) of the population had never lived in another district, aside from their current residence or birthplace.

There were no significant correlations between any of the migration indices and *W. bancrofti* antigenaemia (Table 2). Adjusted prevalence of *W. bancrofti* antigenaemia did not differ significantly between individuals who reported living in another district for more than six months and those who had never lived in another district (37/173; adjusted prevalence 24.6% vs. 281/1206; adjusted prevalence 19.8%, $P = 0.46$).

3.4. Bed net usage and Wuchereria bancrofti antigenaemia

At the time of testing, 66.0% (adjusted prevalence; 1022/1547) of households owned at least one bed net, most of which were untreated. Similarly, 55.8% (adjusted prevalence; 859/1545) of respondents reported that they usually slept under a bed net during seasons when many mosquitoes were present, which suggested regular, long-term usage of bed nets prior to this study. Individuals who reported sleeping under a bed net (insecticide-treated or untreated) the night before the survey tended to have lower rates of *W. bancrofti* antigenaemia compared to individuals who had not slept under a bed net (41/226; adjusted prevalence 16.7% vs. 312/1321; adjusted prevalence 21.9%, $P = 0.14$).

3.5. Antifilarial medication usage

Among the 1563 subjects in the cohort, 22 (1.5% adjusted prevalence) reported that a household member had consumed an antifilarial medication during the previous year. This consumption was distributed sparsely across villages; on average, only 2.4% (adjusted prevalence; on average, 0.3/12 households) of interviewed households in any village reported antifilarial medication usage, confirming the absence of LF mass drug administration control programs. The most frequently reported medication source was a government health care center.

4. Discussion

Our data demonstrate that these five land-locked districts of western Orissa state represent a filariasis-endemic region not addressed by current MDA programs nor appropriate surveillance. Among our sampled population (persons 15–60 years of age), 20.9% (adjusted prevalence; 354/1563) were antigenaemic for *W. bancrofti,* and the prevalence in all five districts was 15.3% (adjusted prevalence; 111/556) or higher. This is comparable to the antigenaemia rates reported for other nearby districts in which MDA programs have recently been active, such as Ganjam, Puri and Cuttack districts.[11,14,20] At the same time, the low rate of self-reported LF medication usage confirms that MDA programs are not ongoing in our study districts.

ICT card tests detect antigens released by adult *W. bancrofti* worms and generally yield antigenaemia rates three to five times higher than the microfilaremia prevalence, which is a more direct marker of LF transmission.[21] However, even if we assume that only one-fifth of the ICT positive population is microfilaremic, we still conclude that over 4% of adults in our population are microfilaremic, which is well above the threshold at which MDA should be initiated.[6] Since ICT card tests are one of the diagnostic tools of choice for mapping LF, our methodology allows for comparisons to other LF programs. ICT card tests have expanded the range of feasible field testing because they do not require highly trained staff, laboratory facilities or

Table 2
Prevalence of *Wuchereria bancrofti* antigenaemia (ICT positive) in subpopulations by migration history

	% ICT positive[a] (ICT + / all)		*P*-value[b]
	Yes	No	
Ever lived in another district for > 6 months[c]	24.6% (37/173)	19.8% (281/1206)	0.46
Ever lived in a known LF-endemic[d] district in Orissa	10.3% (6/35)	20.6% (301/1305)	0.16
Born in a known LF-endemic[d] district in Orissa	9.1% (5/32)	21.0% (308/1323)	0.11
Relocated between 2007 and 2009	16.9% (13/75)	21.2% (341/1484)	0.51

ICT: immunochromatographic card test
[a] Adjusted for sampling design to generalize measurements to the sampled population; reported percentages therefore differ from raw percentages calculated from *n*.
[b] P-values from Pearson x^2 statistic corrected for sampling design.
[c] Includes districts in any Indian state.
[d] Known LF-endemic districts in Orissa are defined having a microfilaremia rate > 1% based on a 2005 Orissa survey (Babu BV et al., unpublished data). These districts are: Anugul, Baleshwar, Baudh, Cuttack, Debagarh, Dhenkanal, Gajapati, Ganjam, Jajapur, Jharsuguda, Khordha, Nayagarh and Puri.

night-time blood collection.[4] Although these indirect cost-saving features have not been well documented, they are thought to offset the higher material cost of the ICT card test when compared to night-time blood smears.[22]

The migration patterns of our sample suggest that the observed prevalence of *W. bancrofti* antigenaemia in our study districts is not due to an influx of LF-infected individuals from regions in Orissa previously known to be LF-endemic, but rather due to autochthonous transmission. Since LF transmission generally requires prolonged exposure to an endemic area, we collected detailed migration histories.[2] Only 14.7% (adjusted prevalence; 173/1379) of our sample had ever lived in another district for more than six months, and only 2.7% (adjusted prevalence; 35/1340) reported previous residence in the known LF-endemic districts of Orissa. Importantly, rates of antigenaemia did not differ significantly between individuals with and without migration histories.

We found a negative correlation between self-reported bed net usage and *W. bancrofti* antigenaemia, although our study was not powered to detect a significant association. This is consistent with previously published studies on the potentially protective effect of bed nets on LF transmission and supports initiatives to coordinate eradication efforts between malaria and LF.[23–25]

Our study has several limitations. First, our sample consisted of microfinance organization members and therefore was not conducted among a representative sample of the population; this is reflected in the demographic differences between our study cohort and the general population of Orissa state. Consequently, our results cannot be generalized to the entire population. Second, the restriction of our ICT testing to persons aged 15-60 biased the age distribution in our sample towards older individuals. Because filariasis prevalence increases with age in most populations, the age distribution in our sample probably resulted in an overestimate of *W. bancrofti* antigenaemia compared to a population-based sampling methodology.[20,26–29] However, even if we were to assume the unlikely possibility that all persons under 15 years of age who live in the surveyed households were ICT negative, the overall antigenaemia prevalence would still have been 14.9%, with an estimated microfilaremia prevalence of at least 3%. Thus, even when we account for the age distribution of our sample, the estimated prevalence remains high and supports the need for additional attention to LF mapping and control programs in these districts. Finally, although unlikely, we cannot discount the possibility of false positives from cross-reactivity to other parasitic infections or false results (positive or negative) from undetected defective ICT cards.

Although the age distribution of our sample likely resulted in an overestimate of the population-based LF prevalence, several other factors could have resulted in underestimation. First, use of ICT cards restricted our measurement to *W. bancrofti* infections.[4] While bancroftian filariasis is the predominant form of lymphatic filariasis in both India and in Orissa, small foci of *B. malayi* transmission do exist in Orissa.[20,30] Second, our sample consisted predominantly of women (67.1%) and families with higher levels of education, both of which have been associated with lower rates of LF antigenaemia.[1,31] Therefore, our measured prevalence may have underestimated the true prevalence of LF antigenaemia in the population.

In summary, our study demonstrates that *W. bancrofti* is highly endemic in five western Orissa districts previously thought to be non-endemic. The observed prevalence rates in our study districts are well above the threshold for initiation of MDA based on current guidelines,[6] and indeed are above those in many nearby districts in India in which MDA is currently ongoing.[11,14,20] These rates are not explained by migration into our study districts from nearby, previously known LF-endemic districts, and are therefore likely due to ongoing autochthonous transmission in these areas. These findings support calls for a population-based determination of antigenaemia and microfilaremia prevalence of the entire region to better determine the need for MDA,[9] and for subsequent consideration of implementing an MDA program in our study districts to interrupt transmission of filariasis.

Authors' contributions: BGB, AM, and AT generated the research question. PKF analyzed the data and wrote the manuscript, which was edited by BGB. BGB, AT, AM and JY designed the underlying study. DK and LK oversaw data collection. All authors participated in revision of the manuscript and read and approved the final version. PKF and BGB are guarantors of this paper.

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Conflicts of interest: None declared.

Ethical approval: The study was reviewed and approved by the Human Subjects Review Boards at Stanford University and Duke University. All study participants gave oral informed consent after explanation of the procedures in the local language.

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