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BRIEF REPORT

Adapting Reactive Case Detection Strategies for *falciparum* Malaria in a Low-Transmission Area in Cambodia

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Reactive case detection around falciparum malaria cases in Cambodia presents a low output. We improved it by including individuals occupationally coexposed with index case patients and using polymerase chain reaction–based diagnosis. The positivity rate increased from 0.16% to 3.9%.

Keywords. malaria elimination; adapted reactive case detection; coexposed screening; polymerase chain reaction (PCR).

With the emergence of multidrug-resistant malaria in Southeast Asia [1], malaria elimination is a high priority in many countries with low endemicity for malaria. A precondition for elimination is a functional surveillance system, allowing detection and real-time notification of each symptomatic case (passive case detection; PCD) [1, 2].

Passive surveillance alone seems to be insufficient to affect malaria transmission sustainably, because the bulk of infection in low-transmission settings occurs in either geographic "hot spots" or demographic "hot pops," which may consist to a large extent of asymptomatic individuals [3]. In Cambodia, one of the low-prevalence countries facing emerging multidrug resistance, the Malaria Elimination Action Framework, aiming to eliminate *Plasmodium falciparum* by 2020, is specifically strengthening active case detection strategies [2]. The Cambodian National Malaria Control Program includes a form of active case detection, reactive case detection (RACD), which targets individuals living in close proximity to case patients identified through PCD by systematically testing household members with rapid diagnostic tests (RDT).

This approach was recently tested in Pailin, a pre-elimination province in western Cambodia, albeit using polymerase chain reaction (PCR)–based household screening [4]. The study showed poor results (a detection rate <1% and no observed differences between index case and control households), challenging the appropriateness of the "classic" household-based RACD approach. This may be particularly pertinent in settings where malaria exposure occurs mainly outside the village, as seems to be true in large parts of Cambodia [5, 6]. Here, RACD might be more efficacious when deployed demographically (in individuals with common risk factors/exposures, such as coworkers) rather than geographically (classic household with or without radius-based screening) [7]. The present study thus aimed to evaluate the added value of expanding the RACD strategy. Specifically, we attempted to quantify the effect of (1) broadening the initial target population of RACD and (2) introducing PCR-based diagnosis in the RACD program.

METHODS

Study Design

This was a retrospective analysis of operational RACD data collected between October 2015 and May 2017 across all villages in the study district.

Study Site

The study was set in Chey Saen district, Preah Vihear province, Cambodia. Chey Saen district has an estimated population of 22 499 in 27 villages [5]. The area is covered by a referral provincial hospital, 3 health centers, and 2 health posts. Malaria transmission is seasonal, with the majority of the infections occurring at the end of the rainy season (August–January), mainly in the forest areas surrounding the district [6]. In 2016, the incidence of symptomatic *P. falciparum* infections in the district was 3.6‰.

Chey Saen has a PCD system delivered through a network of 28 pairs of village malaria workers. Since 2014, this network has been supported and trained by the international nongovernmental organization Médecins Sans Frontières (MSF). Each of the 27 villages is served by village malaria workers, who are able to diagnose malaria infections using RDT and provide antimalarial drug treatment according to national guidelines. Since October 2015, these workers also routinely collect blood from finger-prick spotted on filter paper (dried blood spot) for subsequent PCR diagnosis (performed at the Institut Pasteur du Cambodge).

Reactive Case Detection Program

The RACD program as implemented by the Cambodian National Malaria Control Program screens household members around each index case patient identified during PCD. Index case patients are defined as persons testing positive for *P. falciparum* by malaria RDT (and, in Chey Saen district, confirmed

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by PCR) during PCD activities. For this study, the conventional screening as in the national program (RDT on household members only) was maintained. Additional innovations were then added.

First, "coexposed" individuals, defined as persons supposedly sharing the same risk exposure as the original index patients, because they reported being present at the same presumed time/place of infection were included in the screening, with information gathered through modified World Health Organization–based case investigation forms. Notably, this group included mainly coworkers who worked with the index case patients in settings with a high malaria infection risk, such as forests or plantations. Second, in addition to conventional RDT-based diagnosis, all collected samples were screened for malaria infections by PCR. Investigations based on case investigation forms were conducted by teams of MSF staff.

Polymerase Chain Reaction Detection of *Plasmodium falciparum* Infection

Dried blood spots were transferred to the Institut Pasteur du Cambodge for PCR analysis. Samples were treated as described elsewhere [8] and screened for the presence of *Plasmodium* DNA, using a qualitative real-time PCR assay targeting the *Plasmodium* cytochrome b gene. All positive samples were then analyzed for *Plasmodium* species, using 4 real-time PCR assays specifically amplifying *P. falciparum, P. vivax, P. ovale,* and *P. malariae* [9].

Single-nucleotide polymorphism (SNP) genotyping was performed as described elsewhere [8], with modifications; 12 SNPs were assessed using a PCR–ligase detection reaction–fluorescence microspheres assay to define a molecular barcode for each *P. falciparum* isolate.

Statistical Analysis

All data were double-entered into an Excel database, and each record was cross-checked. Each individual was anonymized using a 10-digit code. Researchers providing qualitative PCR results and assessing malaria genotypes, were blinded as per the origin of the samples (whether the samples were from the malaria index cases or from the secondary cases detected through RACD). Results of the classic and "expanded" RACD were compared using 2-tailed Pearson χ^2 tests, as appropriate.

Ethics Approval

The study was approved by the Ethics Review Board of MSF and by the Cambodian National Ethics Committee on Health Research (No. 0094NECHR).

RESULTS

Over the study period, a total of 194 index cases of *P. falciparum* malaria infection were identified through PCD. Around them, 623 household members were screened with malaria RDT and PCR. Only 1 case (0.16%) was detected in the classic RACD (defined as simple RDT-positive screening of household members), whereas the broadening of the target population to coexposed individuals required 162 additional RDTs to be performed and yielded 6 additional new cases (RDT detection rate among coexposed persons, 3.7%). Refining classic RACD by introducing PCR resulted in 19 additional *P. falciparum* cases among the household members (3.2%). Combining both approaches in an expanded RACD (broadened target population and introduction of PCR) produced a final gain from 1 to 31 cases (overall positivity rate, 3.9%; 31 of 785), with the highest positivity rate among the coexposed (6.8%; 11 of 162) (Table 1).

Overall, the PCR detection rates from screening of coexposed individuals (6.8%) were significantly higher than those from household screening only (3.2%) ($P = .03$; Pearson χ^2 test). Finally, the result of the expanded RACD yielded a final detection rate of 3.9%. The difference between that and the detection rate of classic RACD (0.16%) was significant ($P < .001$)

For 4 of the 11 positive samples from coexposed persons, the SNP molecular markers of the *P. falciparum* isolates were available for both the index patient and coexposed individual. Three of 4 samples (75%) from coexposed individuals showed the same genotype as the sample from the index patient. There was not enough DNA to analyze genotypes of other coexposed individuals and household members. On the other hand, all the 20 secondary case patients identified through household screening with PCR reported a positive history of high-risk exposure; 5 were also defined as coexposed.

DISCUSSION

To our knowledge, this is the first study evaluating the expansion of malaria RACD activities in the context of a PCD program, by broadening the inclusion criteria toward coexposed individuals and introducing PCR-based diagnostics. The rationale supporting the study is built around the malaria transmission dynamics in Cambodia, where most of cases of *P. falciparum* infection are linked to occupational exposure and mobility—that is, to discrete populations whose social structure is composed of an underlying network of members (eg, people occupationally exposed to forest

Table 1. Absolute Number of *falciparum* Malaria Cases and Positivity Rates for Both RDT and PCR, for Each Component of the "Expanded" RACD Activity in Chey Saen District, Based on 194 Index Cases (October 2015 to May 2017)

RACD Components	Tests Done, No.	RDT-Positive Cases, No. (%)	PCR-Positive Cases, No. (%)
Household screening	623	1 (0.16)	20 (3.2)
Screening of coexposed individuals	162	6 (3.7)	11 (6.8)
All RACD screening	785	7 (0.9)	31 (3.9)

Abbreviations: PCR, polymerase chain reaction; RACD, reactive case detection; RDT, rapid diagnostic test.

environment, loggers, miners, and migrant laborers). It has been postulated that unveiling this network could serve to intercept more asymptomatic cases and thus help deplete the reservoir of submicroscopic carriers [3, 10].

Overall, the expanded RACD approach seemed promising, with a detection rate of 3.9% across all categories, compared with 0%–2% in the current international literature on RACD in low-transmission settings [11]. Several additional messages emerge from the study. First, the very high gain (from 1 to 31 cases) provided by the use of PCR supports the use of more sensitive diagnostic methods, such as hypersensitive RDTs in RACD for pre-elimination and elimination contexts [12]. Although hypersensitive RDTs remain less sensitive than PCR, they may be highly useful in the frame of expanded RACD, owing to their ease of use and improved detection capacity compared with classic RDTs. Second, one of the main added values of the expanded RACD seemed to be the demographic expansion (toward nonhousehold, coexposed persons), which led to a 1.5-fold increase of PCR-based detection of *P. falciparum* infections (from 20 to 31). In addition, some of household members reported being coexposed. This illustrates the likely importance of a deeper investigation aimed at identifying networks of persons sharing the same exposure threat at the index patient.

Third, the high RDT positivity rate among coworkers contrasts with the RDT positivity rate of 0.16% among household members. This may indicate that coworkers are more likely to have infections with higher parasitemia, linked to the same risk exposure as the index patients. Results of the 12-SNP genotyping analysis of coexposed individuals support this hypothesis.

In conclusion, in Cambodia, where malaria transmission is very low and does not usually occur at village level, we demonstrated that the classic RACD approach, relying on the use of RDT and screening based on geographic criteria, is likely insufficient to substantially affect *P. falciparum* transmission. The added value of the expanded RACD, which produced a higher yield than the classic strategy, confirmed that RACD strategies, to be fruitful and meaningful, must be adapted to the local *P. falciparum* epidemiological landscape [3, 9], characterized both by the acquisition of malaria infection outside the village and by the important role played by subpatent infections in maintaining disease endemicity [10]. We encourage our work to be replicated in different settings, to further assess its relevance across contexts with different transmission dynamics and support its incorporation into general policy. Importantly, the logistic and financial constraints concerning the use of PCR should be addressed; this should include investigations into the use, cost, and diagnostic yield of future hypersensitive RDTs and other highly sensitive point-of-care tests [13].

Note

Potential conflicts of interest. All authors: No reported conflicts. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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