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Response to Woodrow and Fanello

To the Editor—We thank Woodrow and Fanello [1] for their letter regarding our cross-sectional survey of *pfhrp2*-deleted *Plasmodium falciparum* in the Democratic Republic of Congo (DRC). We agree that polymerase chain reaction (PCR) methods are imperfect for proving the absence of a gene, especially in the setting of low DNA concentrations. However, we dispute their conclusion that the *pfhrp2* deletion calls in our study are largely artifactual.

Woodrow and Fanello [1] point out that subjects included in the study initially had *P. falciparum* diagnosed by means of real-time PCR targeting the *pfldh* gene. They fail to note, however, that we used a relatively insensitive PCR assay with a limit of detection of approximately 100 parasites per microliter. In our hands, the typical limits of detection of the downstream *pfhrp2* exon 1/2 and exon 2 assays are approximately 0.4 and 4 parasite genomes per microliter (10^{-5} and 10^{-4} ng/μL), respectively. Thus, the influence of stochastic PCR failure due to low DNA concentration is expected to be limited.

In addition, Woodrow and Fanello [1] highlight patterns of differential amplification of *pfhrp2* exon 1/2 and exon 2 as evidence of borderline DNA concentrations. They also imply that published reports do not predict a high frequency of partial gene deletions. In fact, robust evidence supports the existence of partial *pfhrp2* deletions due to chromosomal breakage in the subtelomere of chromosome 8 [2, 3]. Recent genomic analyses also confirm that the patterns and frequency of copy number variation in this region of the chromosome vary geographically, a finding supported by the heterogeneity of *pfhrp2* deletion patterns reported in large surveys from different sites [4–8]. Therefore, deletion patterns that are distinct to a particular location are not unexpected. Nonetheless, we agree that the pattern and frequency of partial deletions we observed differ from findings in other large surveys and bear further study. Initial investigation suggests that assays for exon 1/2, but not exon 2, may amplify paralogous genes, producing a result that gives the appearance of a partial deletion when a complete deletion is actually present.

Furthermore, we agree that the testing algorithm can be improved. First, future studies should involve thorough analyses of both *pfhrp2* and *pfhrp3*. Because we originally designed the study to focus on *pfhrp2*, we did not systematically evaluate the prevalence of *pfhrp3* deletions in this survey, which we acknowledge could lead to an underestimation of their prevalence. Second, streamlined algorithms that assess both genes in parallel and incorporate simplified controls would permit high-throughput testing. We have since implemented these measures in ongoing studies of *pfhrp2* and *pfhrp3* deletions.

As with artemisinin resistance, the potential emergence of *pfhrp2* and/or *pfhrp3* (*pfhrp2/3*) gene deletions in Africa cannot be ignored. High rates of rapid diagnostic test (RDT) failure among symptomatic patients infected with *pfhrp2/3*-deleted *P. falciparum* in Eritrea are particularly concerning [9, 10]. In response, the World Health Organization has made it a priority to evaluate and address reports of false-negative RDT results [11].

We share and support the letter writers' efforts to promote the thoughtful implementation of malaria diagnostic testing policies. Prematurely replacing HRP2-based RDTs with less sensitive, less heat-stable alternatives in the DRC would be akin to "throwing the baby out with the bathwater." Our study findings indicate that these deletions are common among asymptomatic children in the DRC, and ongoing studies of their impact on symptomatic subjects of all ages are crucial for policy development.

Notes

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Pfhrp2 Deletions in the Democratic Republic of Congo: Evidence of Absence, or Absence of Evidence?

To the Editor—Parr et al report that *Plasmodium falciparum* parasites with deletion of the *pfhrp2* gene can be found in children across all provinces of the Democratic Republic of Congo [1]. It is possible to differentiate gene deletion from other causes of a negative *P. falciparum* histidine-rich protein 2 (PfHRP2)–based rapid diagnostic test via a series of investigations [2, 3], starting with positive microscopic identification of *P. falciparum*, a straightforward way of minimizing at the outset the chance that the gene cannot be detected simply because of low levels of DNA [4]. Parr et al used many of these recommended steps, and the work was clearly undertaken to a high technical standard, but a distinct feature of the study was that the diagnosis of *P. falciparum* was determined by real-time polymerase chain reaction (PCR) detection of *pfldh*, a relatively sensitive approach designed to capture all *pfhrp2*-deleted parasites. The article comes to a confident conclusion that the *pfhrp2*-negative PCR results represent gene deletion rather than insufficient DNA.

Was this confidence justified? The answer, in our view, is a clear "no." Unsurprisingly, a large proportion (90%) of the "*pfhrp2*-deleted" samples were negative on microscopy. In a set of samples where parasitemia is generally below the level of microscopic detection, successful PCR amplification of a control gene does not guarantee that another gene will be robustly amplified from the same sample. Borderline DNA concentrations will cause stochastic failure of individual PCR reactions, so samples negative by PCR for *pfhrp2* but positive for other genes and, hence, fulfilling the study's criteria for "*pfhrp2* deletion," will be inevitable simply because of the number of samples studied.