suggested, we admit that it is the way science perpetuates itself. Given the present situation and information, we do not see any justification to reinterpret our data.

We sincerely hope that our responses reassure the readership of the validity and robustness of the evidence regarding artemisinin resistance.

### Note

***Potential conflicts of interest.*** All authors: No reported conflicts of interest. 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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### References

1. Das S, Saha B, Hati AK, Roy S. Evidence of artemisinin-resistant *Plasmodium falciparum* malaria in Eastern India. N Engl J Med **2018**; 379:1962–4.
2. Global Malaria Programme. Artemisinin and artemisinin-based combination therapy resistance. (WHO) Status report, October 2016. Available at: https://apps.who.int/iris/bitstream/handle/10665/250294/WHO-HTM-GMP-2016.11-eng.pdf;jsessionid=2F5FD02CBA6BDD0D55E11F252B58691B?sequence=1
3. WWARN K13 Genotype-Phenotype Study Group. Association of mutations in the Plasmodium falciparum Kelch13 gene (Pf3D7_1343700) with parasite clearance rates after artemisinin-based treatments a WWARN individual patient data meta-analysis. BMC Med **2019**; 17:1. doi:10.1186/s12916-018-1207-3
4. World Health Organization. Artemisinin resistance and artemisinin-based combination therapy efficacy: Status report, August 2018. Geneva: World Health Organization, **2018**. Available at: http://apps.who.int/iris/bitstream/handle/10665/274362/WHO-CDS-GMP-2018.18-eng.pdf?ua=1.
5. Das S, Manna S, Saha B, Hati AK, Roy S. Novel *pfkelch13* gene polymorphism associates with artemisinin resistance in eastern India. Clin Infect Dis **2018**. doi: 10.1093/cid/ciy1038.
6. Phyo AP, Nkhoma S, Stepniewska K, et al. Emergence of artemisinin-resistant malaria on the western border of Thailand: a longitudinal study. Lancet **2012**; 379:1960–6.
7. Ahmed A, Lumb V, Das MK, Dev V, Wajihullah, Sharma YD. Prevalence of mutations associated with higher levels of sulfadoxine-pyrimethamine resistance in *Plasmodium falciparum* isolates from Car Nicobar Island and Assam, India. Antimicrob Agents Chemother **2006**; 50:3934–8.
8. Das S, Chakraborty SP, Tripathy S, Hati A, Roy S. Novel quadruple mutations in dihydropteroate synthase genes of *Plasmodium falciparum* in West Bengal, India. Trop Med Int Health **2012**; 17:1329–34.
9. Das MK, Lumb V, Mittra P, Singh SS, Dash AP, Sharma YD. High chloroquine treatment failure rates and predominance of mutant genotypes associated with chloroquine and antifolate resistance among falciparum malaria patients from the island of Car Nicobar, India. J Antimicrob Chemother **2010**; 65:1258–61.

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## Ultralow-density *Plasmodium falciparum* Infections in African Settings

To the Editor—As countries accelerate towards elimination, an increasing proportion of infections may be of low parasite densities. In a recent report, Girma and colleagues [1] deployed ultrasensitive diagnostics to characterize asymptomatic infections in Ethiopia. The *Plasmodium falciparum* prevalence was 1.3% by microscopy, 3.6% by conventional rapid diagnostic tests (RDT), 8.5% by ultrasensitive Alere RDT, 22.2% by loop-mediated isothermal amplification and 21.5% by ultrasensitive quantitative reverse transcription-polymerase chain reaction (qRT-PCR). These findings are in line with a growing body of evidence demonstrating the superiority of ultrasensitive diagnostics in detecting low-density infections, when compared to microscopy and standard RDTs [2]. The reported qRT-PCR prevalence is considerably higher than prevalence estimates from a meta-analysis tool that relates microscopy and PCR prevalence data from population surveys [3]. Based on the meta-analysis, one would expect a *P. falciparum* PCR prevalence in the range of 2.9% to 10.6%. The higher prevalence in the study by Girma and colleagues [1] may be explained by their approach to targeting highly abundant RNA targets instead of DNA targets. Their finding thus suggests that there may be a reservoir of infections that is too low to be detected by conventional diagnostics or even conventional PCR [4]. Our own findings, from cross-sectional surveys in pre-elimination settings of South Africa, are in line with the findings of Girma and colleagues [1], in the sense that we also detected infections with ultralow parasite densities, below the limit of detection of conventional PCR. Our study observed no RDT-positive infections or 18S nested-PCR–positive infections among 1475 individuals, whilst 3.9% of the study population was positive for *P. falciparum* parasites by sensitive, telomere-associated repetitive element 2–based quantitative PCR (qPCR), sometimes with genetically complex infections (Table 1).

The real challenge of the study by Girma and colleagues [1], as well as of our own work, lies in the interpretation of such parasite survey data in relation to transmission patterns, particularly in low-transmission settings. Ethiopia and South Africa have both set targets for malaria elimination. It is unclear to what extent the presence of ultralow-density infections may challenge these ambitions. The authors correctly point out the

**Table 1. *Plasmodium falciparum* Infection and Multiplicity of Infection Outcomes in South Africa**

| | Local Subjects | Migrant Subjects |
|---|---|---|
| RDTs (First Response Malaria) | 0 (0/933) | 0 (0/542) |
| 18S rRNA PCR | 0 (0/933) | 0 (0/542) |
| TARE-2 qPCR % (n/N) | 2.6% (24/993) | 6.1% (33/542) |
| Mean multiplicity of infections (range) | 1.8 (1–3) | 2.8 (1–5) |

Subjects were recruited in 2 community-wide, cross-sectional surveys among asymptomatic participants in 2014 and 2015. The 18S rRNA PCR [5], TARE-2 qPCR [6], and multiplicity of infections [7] were based on established protocols, using 4.2 μL of blood from filter paper bloodspots. Abbreviation: PCR, polymerase chain reaction; RDT, rapid diagnostic tests; rRNA, ribosomal RNA; TARE-2 qPCR, telomere-associated repetitive element-2 quantitative PCR.

limitations of cross-sectional surveys for answering such questions, since these fail to take into account parasite dynamics that may fluctuate on a daily basis [8]. The authors also did not perform any assessment of gametocyte carriage or transmissibility to mosquitoes, whilst longitudinal surveys that accurately measure parasite kinetics, gametocyte production, and onward transmission potential are probably needed to truly determine the relevance of low-density infections for onward transmission. This contribution to transmission not only depends on their infectivity to mosquitoes, but also on real-life mosquito exposure [9]. In areas with low vector densities, inefficient vectors, or effective vector control, the transmission potential of low-density or ultralow-density infections is likely to be very limited. In other settings, such infections may plausibly form a stumbling block for elimination [10]. The study by Girma and colleagues [1] thereby forms a relevant starting point to examine these important questions, which urgently need addressing to inform malaria policy.

## Note

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## References

1. Girma S, Cheaveau J, Mohon AN, et al. Prevalence and epidemiological characteristics of asymptomatic malaria based on ultrasensitive diagnostics: a cross-sectional study. Clin Infect Dis **2018**.
2. Galatas B, Martí-Soler H, Nhamussua L, et al. Dynamics of afebrile *Plasmodium falciparum* infections in Mozambican men. Clin Infect Dis **2018**; 67:1045–52.
3. Okell LC, Bousema T, Griffin JT, Ouédraogo AL, Ghani AC, Drakeley CJ. Factors determining the occurrence of submicroscopic malaria infections and their relevance for control. Nat Commun **2012**; 3:1237.
4. Bousema T, Okell L, Felger I, Drakeley C. Asymptomatic malaria infections: detectability, transmissibility and public health relevance. Nat Rev Microbiol **2014**; 12:833–40.
5. Snounou G, Viriyakosol S, Zhu XP, et al. High sensitivity of detection of human malaria parasites by the use of nested polymerase chain reaction. Mol Biochem Parasitol **1993**; 61:315–20.
6. Hofmann N, Mwingira F, Shekalaghe S, Robinson LJ, Mueller I, Felger I. Ultra-sensitive detection of *Plasmodium falciparum* by amplification of multi-copy subtelomeric targets. PLOS Med **2015**; 12:e1001788.
7. Ranford-Cartwright LC, Taylor J, Umasunthar T, et al. Molecular analysis of recrudescent parasites in a *Plasmodium falciparum* drug efficacy trial in Gabon. Trans R Soc Trop Med Hyg **1997**; 91:719–24.
8. Farnert A, Snounou G, Rooth I, Bjorkman A. Daily dynamics of *Plasmodium falciparum* subpopulations in asymptomatic children in a holoendemic area. Am J Trop Med Hyg **1997**; 56:538–47.
9. Gonçalves BP, Kapulu MC, Sawa P, et al. Examining the human infectious reservoir for *Plasmodium falciparum* malaria in areas of differing transmission intensity. Nat Commun **2017**; 8:1133.
10. Tadesse FG, Slater HC, Chali W, et al. The relative contribution of symptomatic and asymptomatic *Plasmodium vivax* and *Plasmodium falciparum* infections to the infectious reservoir in a low-endemic setting in Ethiopia. Clin Infect Dis **2018**; 66:1883–91.

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## Reply to Awandu et al

To the Editor—We thank Awandu and colleagues for their insightful comments on our recent article describing ultrasensitive diagnostic tests for detection of asymptomatic malaria in a highly endemic region of Gambella, Ethiopia [1]. The higher prevalence noted in Gambella

**Table 1. Nested Polymerase Chain Reaction (PCR) Results of Samples Positive for *Plasmodium falciparum* by Quantitative Reverse Transcriptase PCR (n = 48) From the Gambella Study**

| Type | No. (%) | Median Parasite Count/mL | Range (First Quartile –Third Quartile) |
|---|---|---|---|
| Nested PCR positive | 29 (60.42) | 11 390 | 1902.5–42 875 |
| Nested PCR negative | 19 (39.58) | 95.8 | 27.5–439.9 |

Abbreviation: PCR, polymerase chain reaction.

**Table 2. Variation in the Limit of Detections of the Different Molecular Tools for Diagnosing *Plasmodium falciparum* (modified from [12])**

| Method | Target Gene | LOD (parasites/mL) |
|---|---|---|
| Nested PCR | 18S rRNA, dhfr-ts, 28S rRNA, stevor | 100–10 000 |
| PCR | mitochondrial DNA | 500 |
| qPCR | 18S rRNA, cox1, cytb, TARE-2 | 20–3000 |
| qRT-PCR RNA extraction [1] | 18S rRNA | 2–20 |
| LAMP | 18S rRNA, mitochondrial DNA | 1000–100 000 |
| US-LAMP RNA extraction [1] | 18S rRNA, exp1 | 0.8–50 |
| NASBA | 18S rRNA | 20 |

Abbreviations: LOD, limit of detection; NASBA, nucleic acid sequence-based amplification; PCR, polymerase chain reaction; qPCR, quantitative polymerase chain reaction; qRT-PCR, quantitative reverse transcriptase polymerase chain reaction; rRNA, ribosomal RNA; RT, reverse transcriptase; TARE-2, telomere-associated repetitive element 2; US-LAMP, ultrasensitive loop mediated amplification.