

Subclinical *Plasmodium falciparum* infections are missed by routine diagnostic methods in school-age Gambian children



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Background:

In the Gambia, decades of malaria control activities – particularly focused on pregnant women and children under 5 who are most at-risk of severe disease – have drastically reduced the burden of malaria. The country aims to be malaria-free by 2025¹.

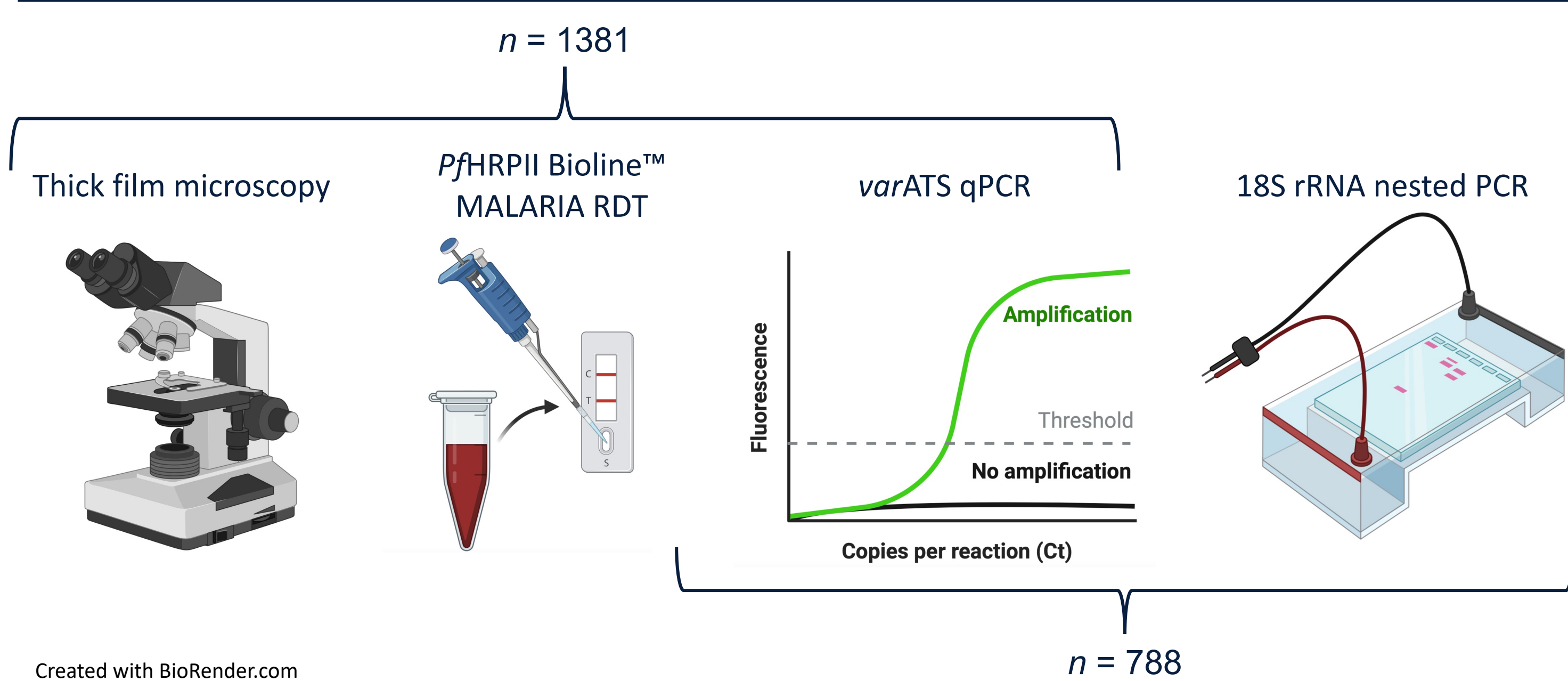
Parasite prevalence decreased >90% between 2011 – 17².

The Gambia is one of few countries to reach the WHO/AFRO milestone of reducing malaria deaths and cases by >40% from 2015–20¹.

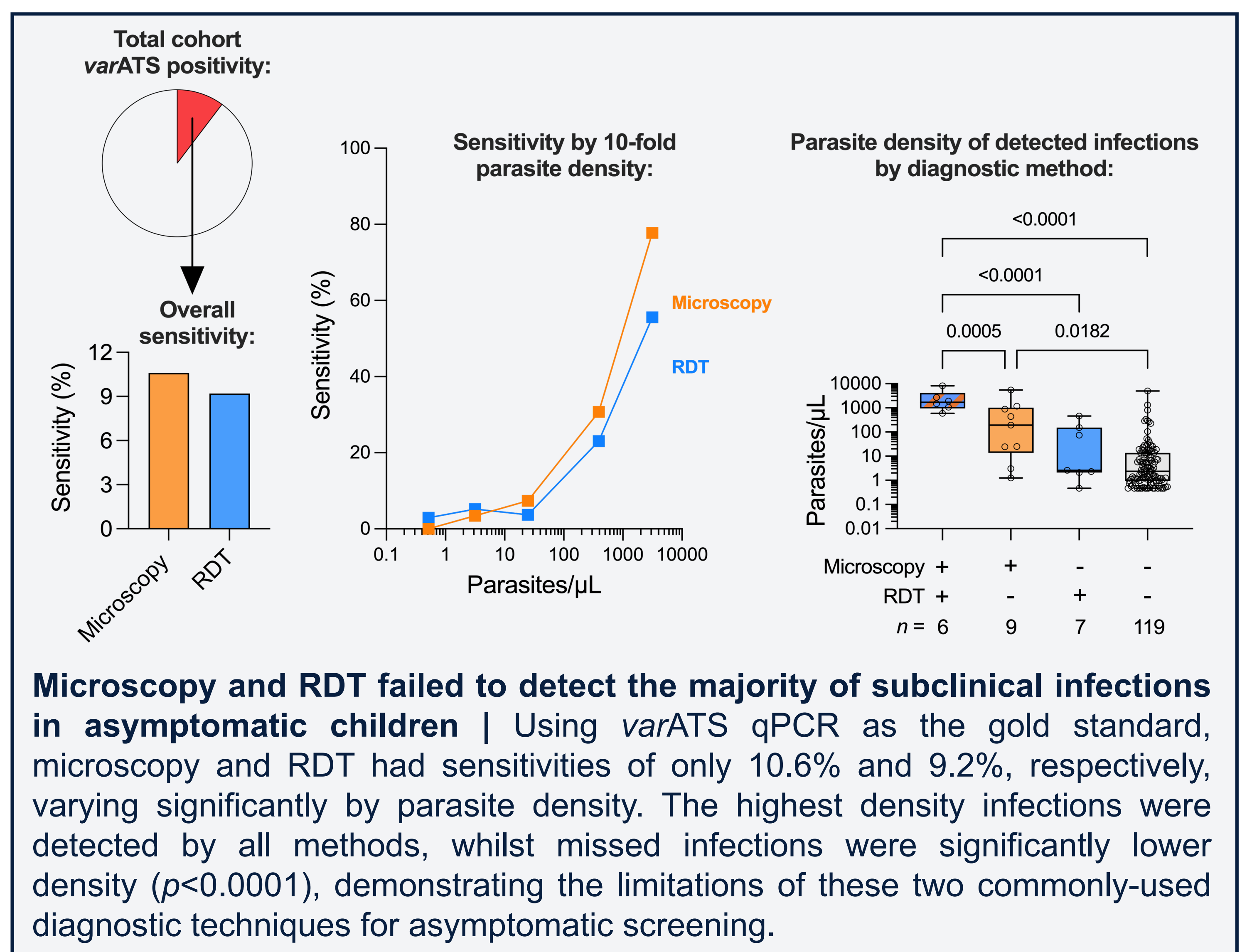
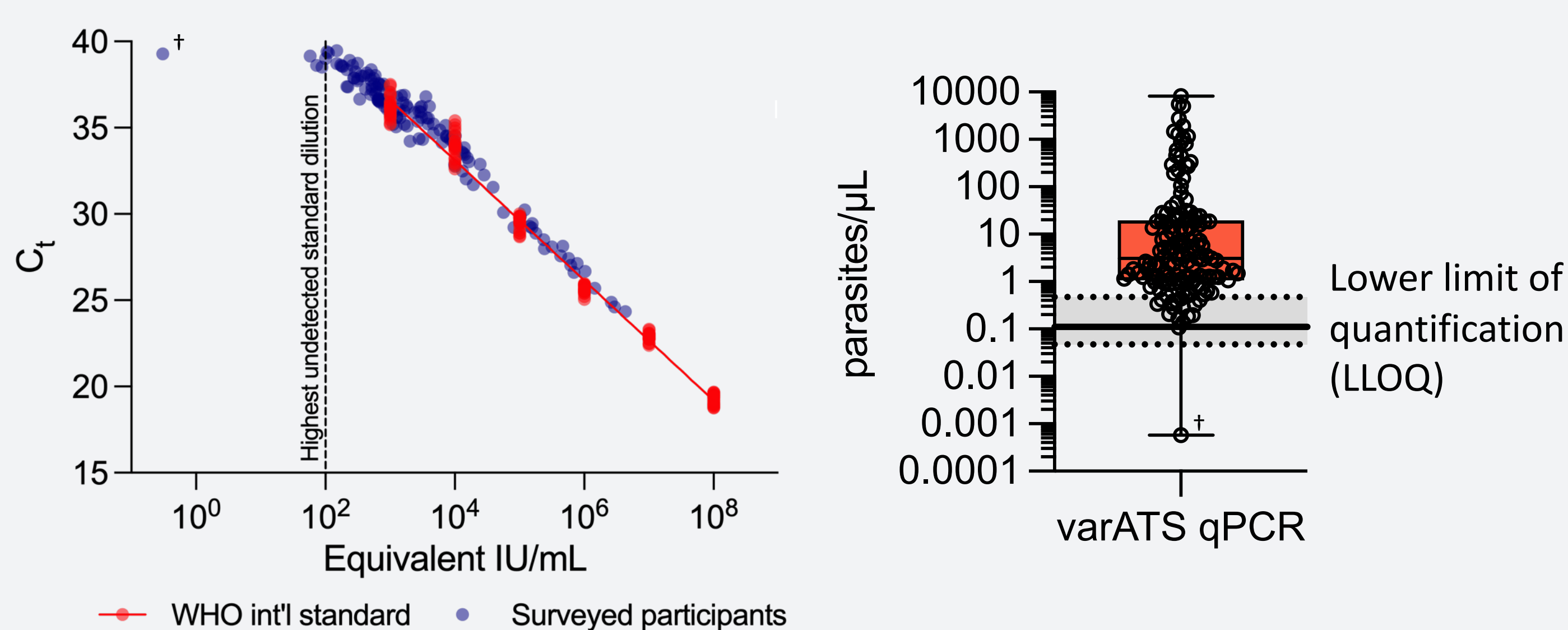
Transmission is highly seasonal, with distinct rainy and dry seasons.

Malaria burden has now shifted to older, school-age children who often maintain subclinical infections that go untreated.

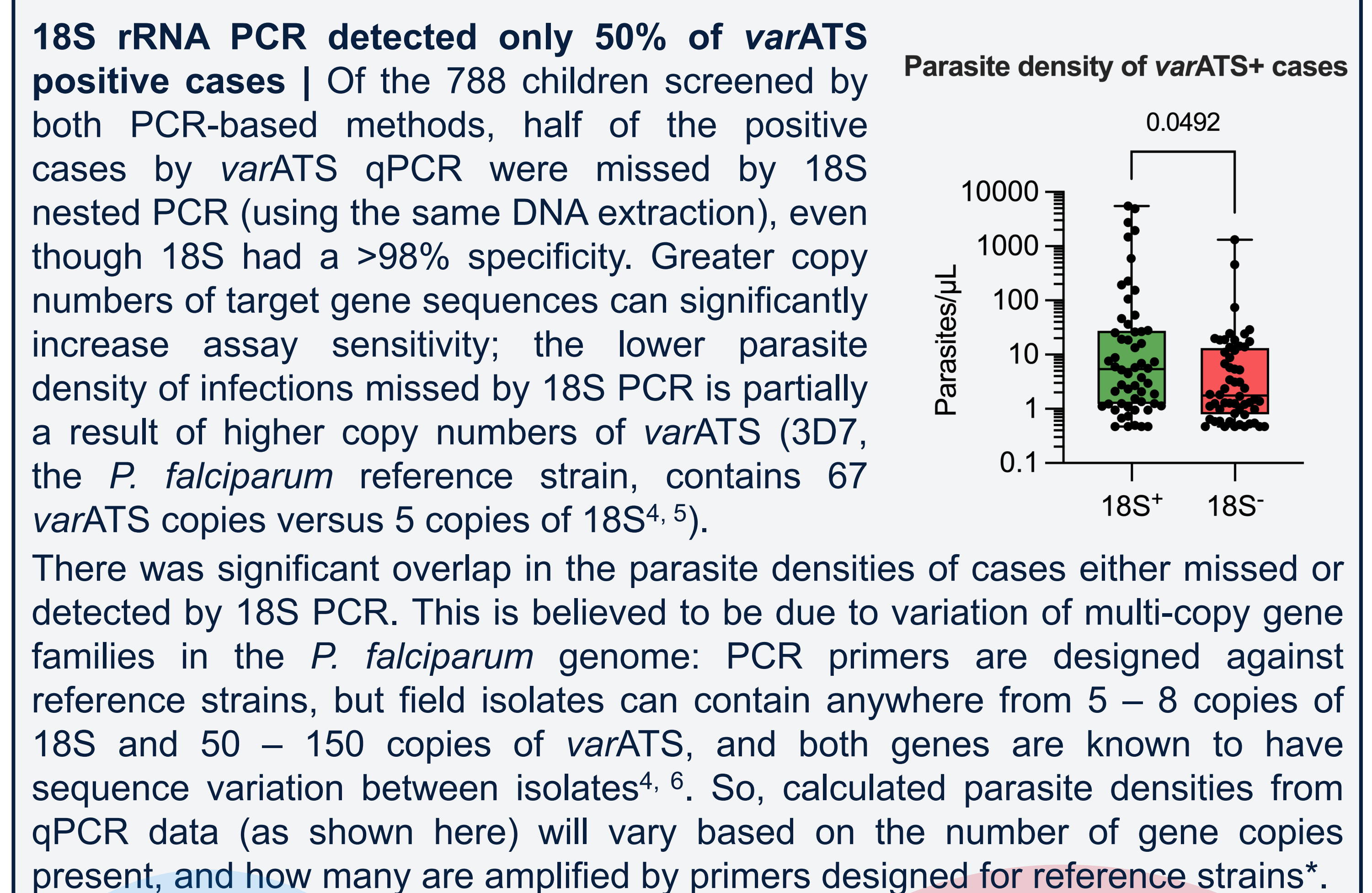
Study methods | During the December – January dry season, 1,650 children ages 8–15 were surveyed from 29 villages in the Upper River Region of The Gambia, the region of the country with the highest prevalence of *P. falciparum*³. Children were in good general health, and were afebrile. Blood was collected via finger prick and tested for *P. falciparum* by RDT and microscopy; retrospectively, all samples were also screened using *varATS* qPCR. In total, 1381/1650 children were screened by all of these methods. Additionally, 18S rRNA nested PCR results were also available for the first 788 children screened. These data were used to assess levels of subclinical *P. falciparum* infections circulating during the dry season. Additionally, diagnostic methods could be compared for their ability to accurately detect and diagnose these subclinical infections.



Subclinical *P. falciparum* infections are widespread in afebrile school-age children in the Upper River Region of The Gambia during the dry season | Based on the most sensitive of our diagnostics, *varATS* qPCR, 10.2% of children had detectable *P. falciparum* DNA, although there was significant variation by village. Parasite density in parasites/μL was estimated using a 10-fold dilution standard curve of the NIBSC/WHO International Standard (NIBSC code 04/176). Average parasite density detected in the cohort was low (median 3.2 parasites/μL, IQR 1.1 – 19.1).



Microscopy and RDT failed to detect the majority of subclinical infections in asymptomatic children | Using *varATS* qPCR as the gold standard, microscopy and RDT had sensitivities of only 10.6% and 9.2%, respectively, varying significantly by parasite density. The highest density infections were detected by all methods, whilst missed infections were significantly lower density ($p < 0.0001$), demonstrating the limitations of these two commonly-used diagnostic techniques for asymptomatic screening.



~10% of school-age, otherwise healthy Gambian children maintained *P. falciparum* infections into the dry season.

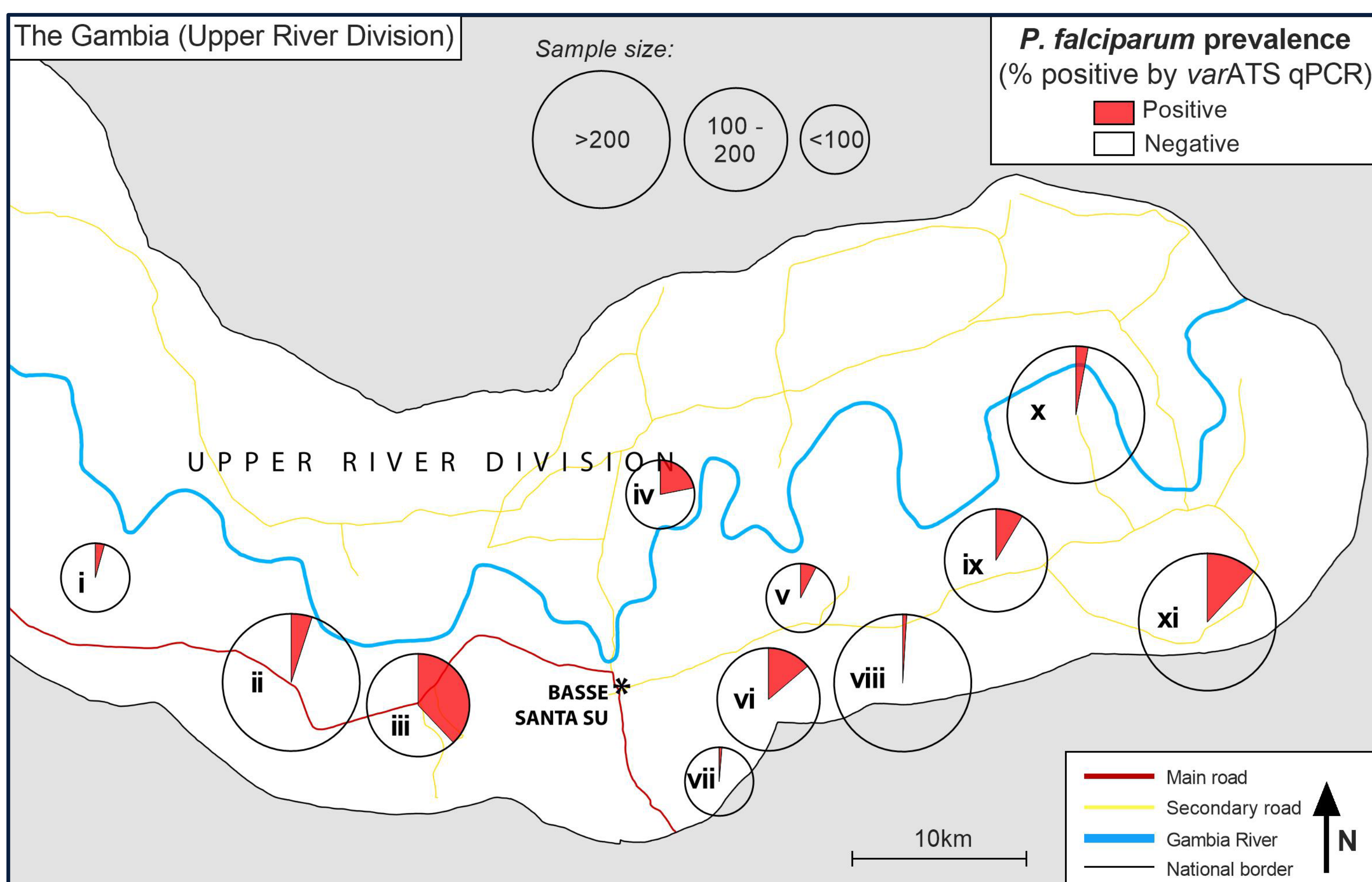
Low-density infections were frequently missed by PfHRP2-based RDTs and experienced microscopists.

18S rRNA nested PCR missed 50% of *varATS* positive cases.

Subclinical malaria is linked to anaemia, progression to symptomatic disease and bacterial coinfections⁷, as well as contributing to onward transmission across dry seasons.

Treating these infections is crucial to reaching malaria elimination status.

Better international standards and universal diagnostic protocols are needed for detecting low-density *P. falciparum* infections.



References:
¹ <https://www.afro.who.int/news/progress-towards-malaria-elimination-gambia>
² <https://www.afro.who.int/news/gambia-draws-line-against-malaria-gears-eliminate-it>
³ Mooney J.P. et al. (2022). *Frontiers in Immunology*, 13.
⁴ Otto, T. et al. (2018). *Wellcome Open Research*, 3(52).
⁵ Mercereau-Pujalon et al. (2002). *International Journal for Parasitology*, 32(11).
⁶ Hofmann, N. et al. (2015). *PLoS Medicine*, 12(3).
⁷ Chen, I. et al. (2016). *PLoS Medicine*, 13(1).

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