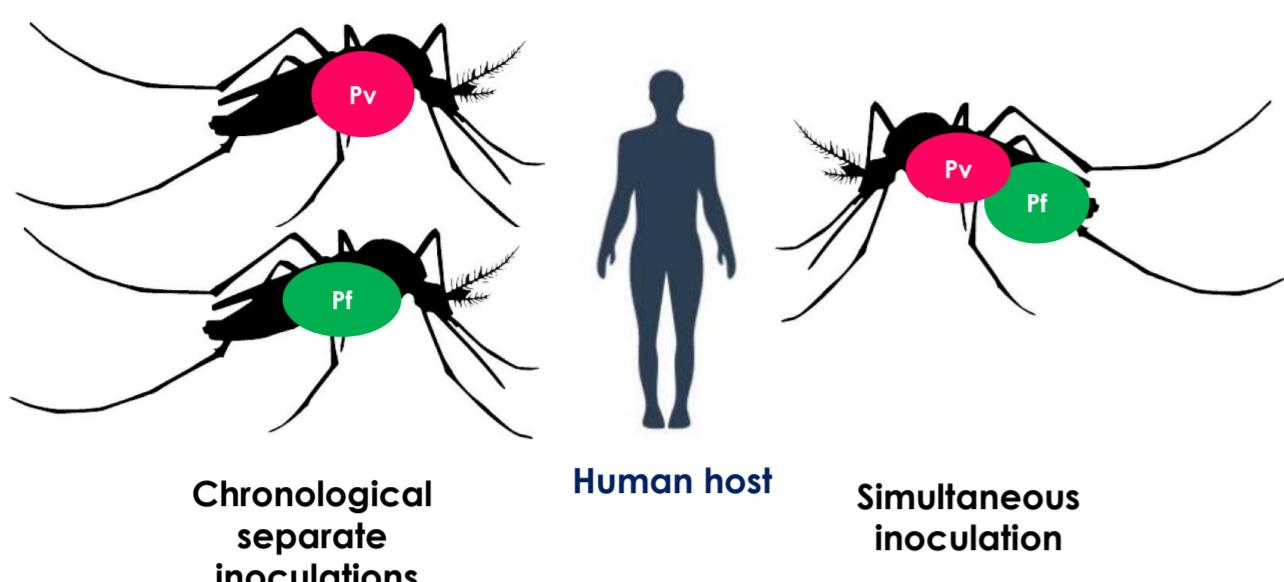


Clinical epidemiology and biology of mixed *P. falciparum* and *P. vivax* malaria in central India

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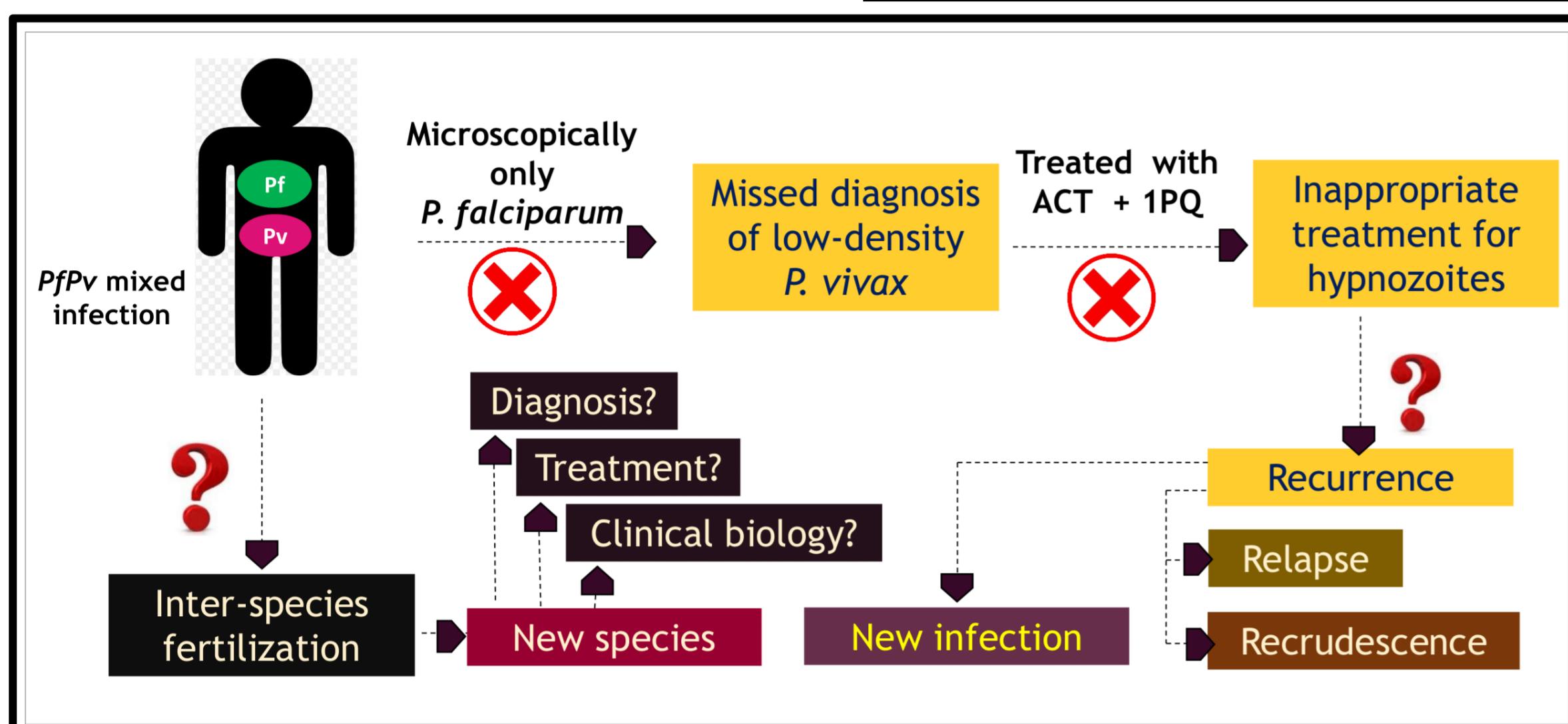
Mixed Plasmodium infections may occur either from:



- Mixed infections could involve any combination of *Plasmodium* species, *P. falciparum* (Pf) & *P. vivax* (Pv) mixed infections demand greater attention
- Burden of malaria due to mixed infections is underreported (not reported under govt malaria surveillance programs)
- Prevalence reported by individual research studies: 4-46%
- No study reported to focus on its biology & implications
- It is unclear how individual species behave & interact with other species during mixed infections
- Co-existence of >1 species provides ample opportunities to sexually interact inside the mosquito (already reported in rodent parasites)

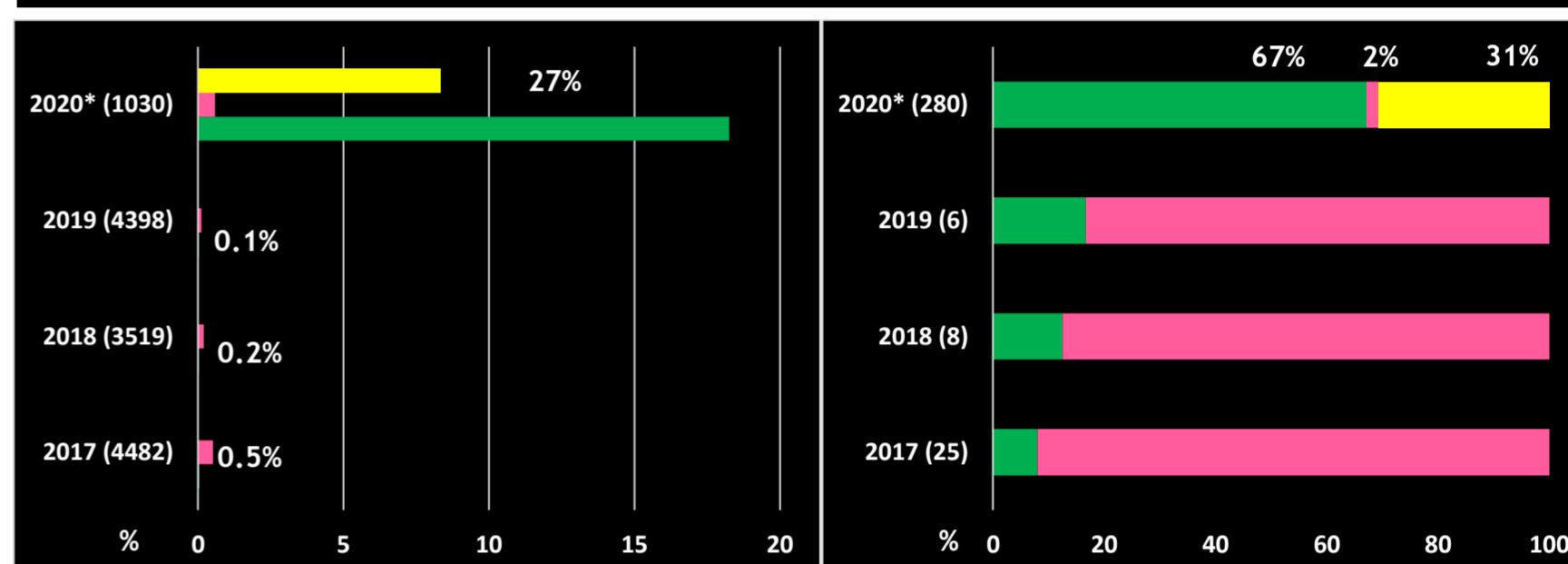
Objectives

- To estimate the prevalence & study the clinical profile and complications of *P. falciparum*-vivax mixed infections
- To develop a method to separate each species from mixed *P. falciparum*-vivax culture/infection
- To investigate hetero-species interactions (fertilization) between *P. falciparum* and *P. vivax* gametes



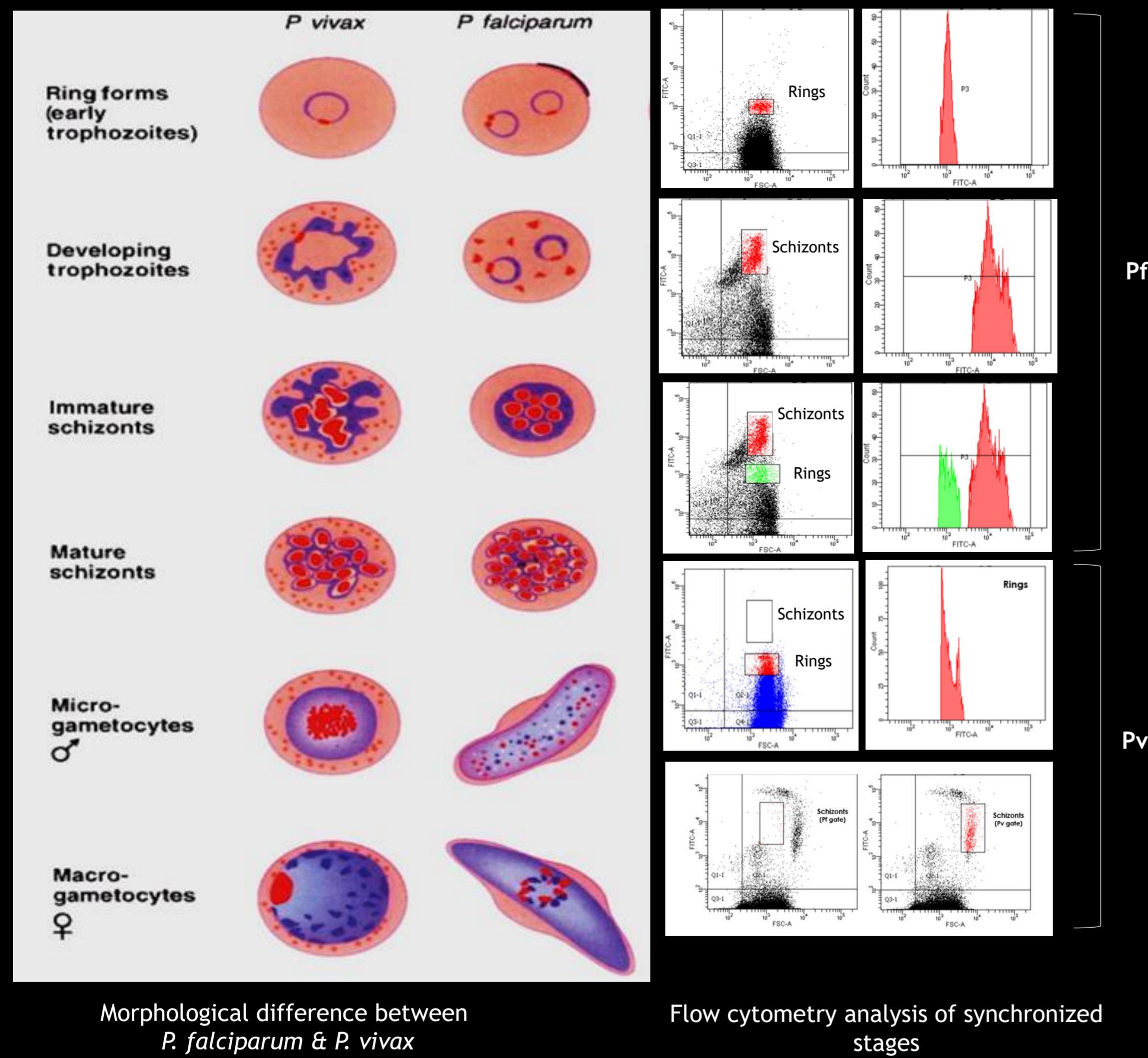
Prevalence of *P. falciparum* & *P. vivax* mixed infections

- A total of 1030 blood samples collected from June-Nov 2020 from 4 different government hospitals in central India
- Samples were screened by microscopy, RDT & PCR
- All patients with mixed PfPv infection followed telephonically till 12 months to identify associated complications & recurrences, if any

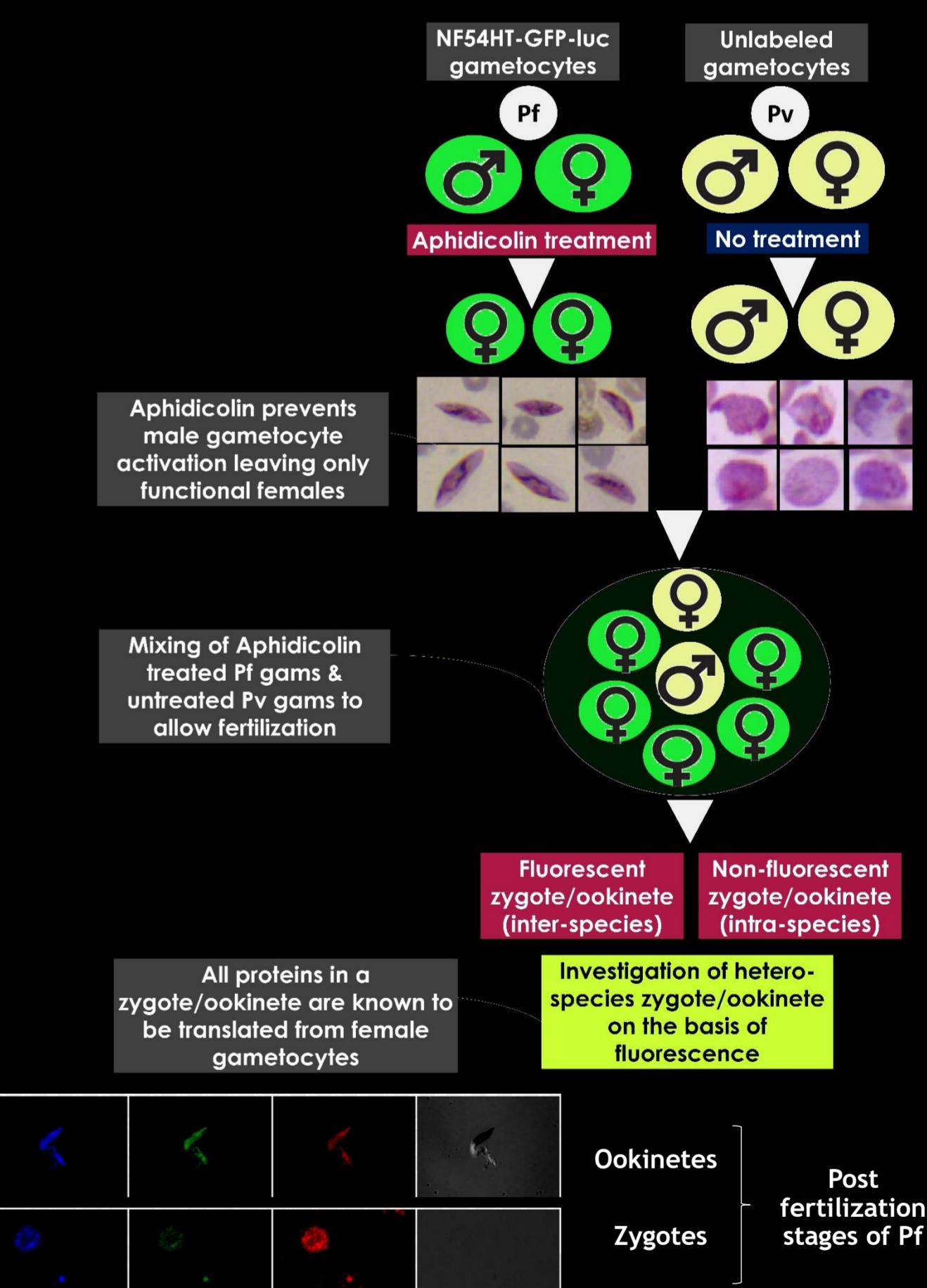


■ Pf Years 2017-2019: Data retrieved from the government surveillance program (Microscopy)
 ■ Pv Year 2020*: Current study outcomes (PCR)
 ■ PfPv • Light microscopy & RDT fail to detect all 27% (280/1030) mono & mixed infections
 • Current study reported 18% (188/1030) mixed PfPv infections in the year 2020
 • Zero mixed cases reported by government surveillance program in 2017-2019
 • During follow up, no clinical complications or recurrence observed

Separation of each species from mixed *P. falciparum*-vivax infections



Investigation of interspecies fertilization between Pf & Pv



- Different blood stages of both *P. falciparum* & *P. vivax* were synchronized with sorbitol (early stages) and MACS (late stages)
- Flowcytometry analysis of synchronized population was done to make stage-specific gates
- P. vivax* stages were analyzed on the same gates made for *P. falciparum* in order to see the difference

Outcome

P. falciparum & *P. vivax* were separable at the schizont-stage from mixed infections

Implications

The developed method would allow us to separate Pf & Pv from mixed infections in order to investigate parasite biology and infection dynamics of each species during mixed infections in terms of growth, development, response to antimalarials etc.

Outcome

- A platform was established to investigate fertilization between *P. falciparum* & *P. vivax*
- No zygotes/ookinetes observed in *P. vivax* (control; untreated) as well as in mixture of *P. falciparum* (females) & *P. vivax* (both male & female)

Major Challenge

- Lack of *P. vivax* *in vitro* culture
- Clinical isolates are unable to complete even one asexual cycle outside the host

Implications

Interspecies sexual interactions have the potential to influence the dynamics of malaria transmission & the established method could be further improved to investigate the same