Evaluation of ELISA Protocols for *Plasmodium Falciparum* Circumsporozoite Protein Detection in Malaria Vector Mosquitoes

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Introduction

- Determination of malaria sporozoite rates is essential for estimating the number of infective mosquitoes.
- The ELISA-CSP method is used for detecting circumsporozoite proteins, but is prone to false-positives.

Aims

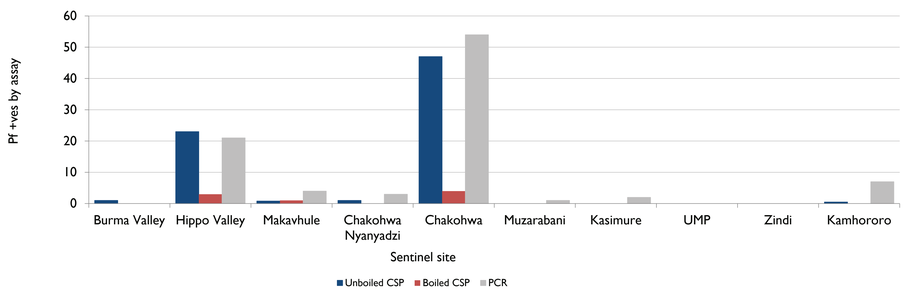
- The study compared the performance of the ELISA-CSP with and without boiling in detecting *Plasmodium falciparum* in the salivary glands of wild *Anopheles* mosquitoes, using PCR as the reference.

Methods

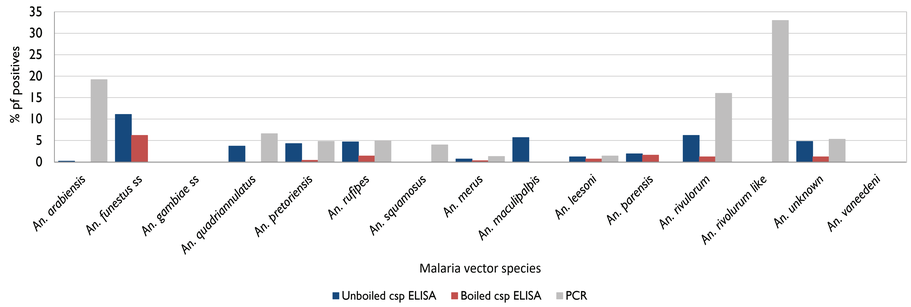
- Female *Anopheles* mosquitoes (n=2144) collected from 12 designated national malaria vector sentinel surveillance sites.
- *Anopheles* mosquitoes were dissected to separate head and thorax from abdominal segments.
- The head and thorax sections were crushed into homogenate and subjected to ELISA-CSP following the Robert A Wirtz 2016 protocol.
- The homogenate was then further boiled for 10 minutes for removal of false positives.
- Part of the same homogenate for each mosquito sample was subjected to PCR for detection of *Plasmodium falciparum* infection.

Results

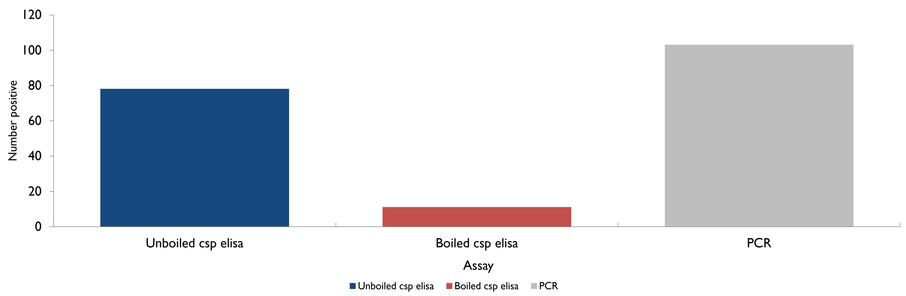
Plasmodium falciparum infected *Anopheles* by assay method at 10 sentinel sites, Zimbabwe (2017-2018)



Pf-positive *Anopheles* species by assay method



Number of sporozoite-positive samples by assay method



Diagnostic performance

Parameter	Unboiled CSP ELISA	Boiled CSP ELISA
Sensitivity	8.3%	2.5%
Specificity	96.4%	99.5%
ROC 95% CI	0.52	0.61 (0.43, 0.70)

Both boiled and unboiled ELISA-CSP are very conservative in informing *Plasmodium falciparum* infective rates in mosquito homogenates.

Discussion

- ELISA-CSP had high specificity and a false sporozoite positive rate of 3.6% without boiling and **0.5%** with boiling step, which is instrumental for reliable incrimination of emerging vectors.
- Several studies indicate the possibility of overestimation of sporozoite rate using ELISA-CSP and boiling the ELISA lysate can reduce false positivity.
- Although PCR is more sensitive, its disadvantage is that it will detect the presence of all Plasmodium DNA and is not sporozoite stage specific
- The positive PCR result does not necessarily indicate the species to a vector(i.e infectious) but does point to this possibility it could be infectious, as analysis of the head and thorax would not pick up parasite DNA from infected midguts.

Conclusions

- PCR could be used as initial step to identify potential malaria vectors
- A number of *Anopheles* species historically not considered as vectors were identified as sporozoite positive. This is the first step in identifying new vector species and the roles of these in malaria transmission should be further investigated

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