THE PROSPECTS OF ELIMINATING MALARIA FROM SUB-SAHARAN AFRICA

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RÉPUBLIQUE DU MALI
UN PEUPLE - UN BUT - UNE FOI

Productive Mentorship 1992-2020

Prof. Donald J. Krogstad

Prof. Ousmane A. Koita

University of Port Harcourt

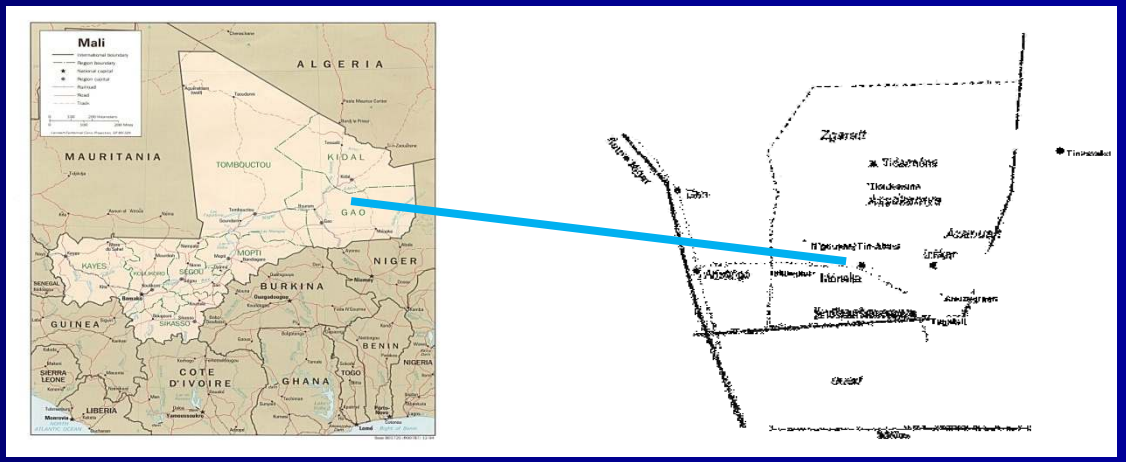
Thanks Prof Aline Noutcha and DVC R&D Prof S. IYEOPU

Focus of this presentation

1. Genetic recombination and Gene Deletion in Malaria parasite

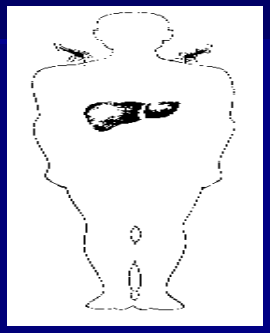
2. Challenges related to Malaria Elimination

Northwest of Mali



Four species of malaria parasites circulate in Mali: *P. falcparum*, *P. malariae*, *P. ovale* and *P. vivax*, Mali Is one of West African countries where the 4 species are present.

SCHEMATIC LIFE CYCLE DIAGRAM FOR THE MALARIA PARASITE



SCHIZOGONY
Haploid asexual intraerythrocytic stage parasites

SCHIZOGONY
Haploid hepatic pre-erythrocytic stage parasites

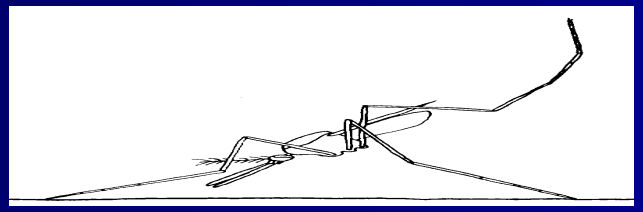
VERTEBRATE (HUMAN) HOST

Haploid gametocytes

ANOPHELINE VECTOR

Haploid gametes

Haploid Sporozoites



FERTILIZATION

MEIOTIC REDUCTION DIVISION (GENETIC RECOMBINATION)

Diploid zygote

RECOMBINANT (HYBRID) BLOCK 2 MSP-1 SEQUENCES

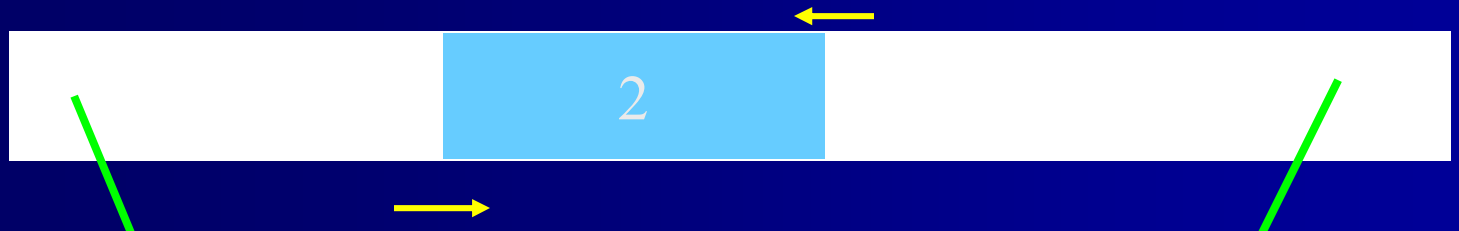
MAD2O

+

RO33

MAD/RO HYBRIDS

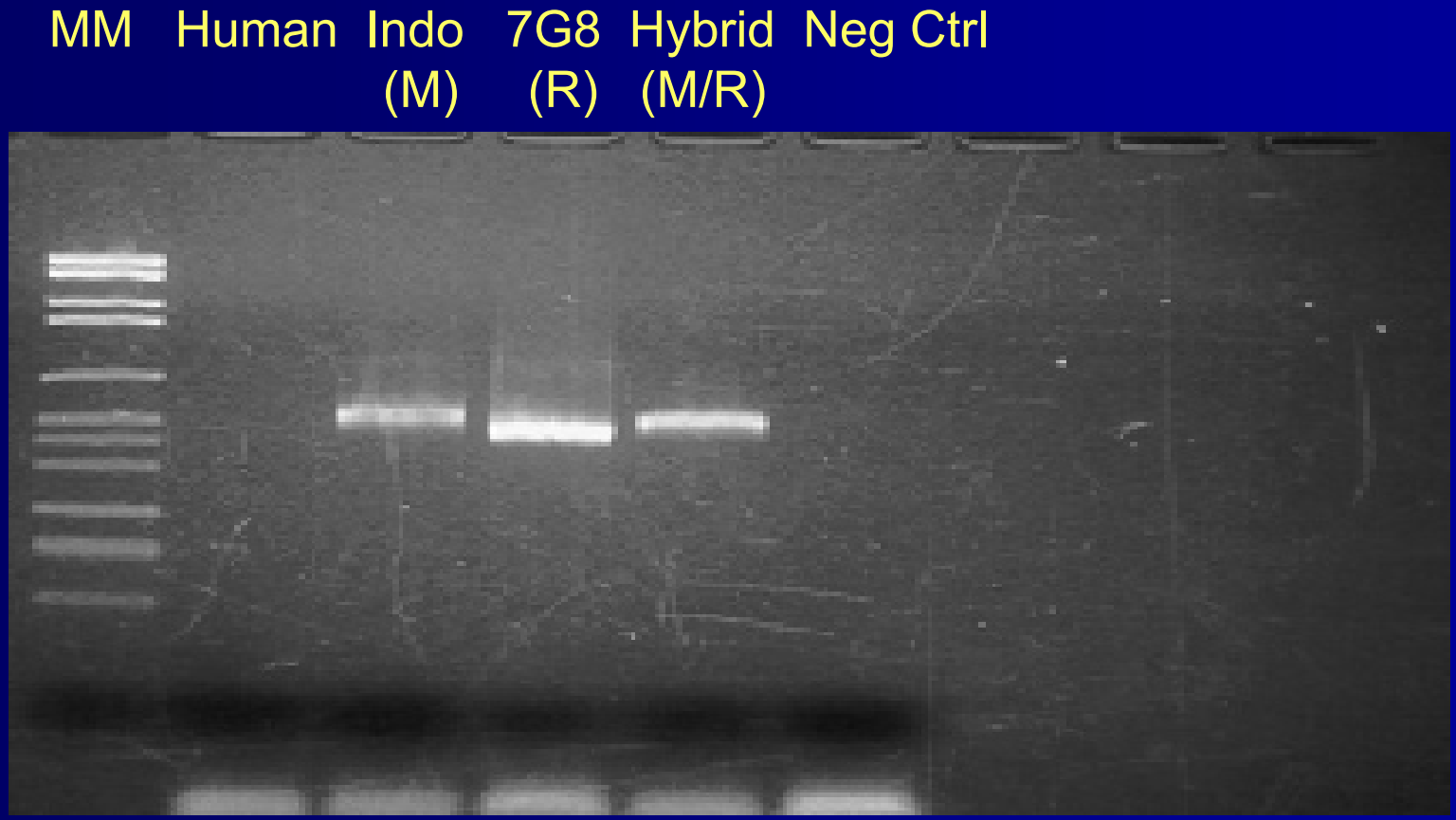
PCR OF BLOCKS 1-3 USING CONSERVED PRIMERS



ACTAGAAGCTTTAGAAGATGCAGTA

TTCGTGCAAATGAATTAGACGTA

AMPLIFICATION OF HYBRID BLOCK 2 SEQUENCE USING CONSERVED PRIMERS



CLONING STRATEGY FOR SEQUENCING

Slight modification of the original PCR conditions (additional template extension at 72ºC for 10 minutes) permits one to add two adenines (independent of coding) at both ends of the amplified product (using *Taq polymerase)*. The PCR product obtained is then suitable for cloning into the TA TOPO vector from Invitrogen which has matching overhanging thymidines at both ends.

UPSTREAM SEQUENCE OF BLOCK 2 IN MSP-1

```
AJMAD20       GTATTAAATGAAGGAACAAGTGGAACAGCTGTTACAACTAGTACA   45
3025MAD20     GTATTAAATGAAGGAACAAGTGGAACAGCTGTTACAACTAGTACA   45
2704MAD20     GTATTAAATGAAGGAACAAGTGGAACAGCTGTTACAACTAGTACA   45
BANCHYB       GTATTAAATGAAGGAACAAGTGGAACAGCTGTTACAACTAGTACA   45
GABHYB        GTATTAAATGAAGGAACAAGTGGAACAGCTGTTACAACTAGTACA   45
SELINHYB      GTATTAAATGAAGGAACAAGTGGAACAGCTGTTACAACTAGTACA   45

AJMAD20       CCTGGTTCAGGTGGTTCAGTTACTTCAGGTGGTTCAGGTGGTTCA   90
3025MAD20     CCTGGTTCAAAGGGTTCAGTTGCTTCAGGTGGTTCAGGTGGCTCA   90
2704MAD20     CCTGGTTCAAAGGGTTCAGTTGCTTCAGGTGGTTCAGGTGGCTCA   90
BANCHYB       CCTGGTTCAAAGGGTTCAGTTACTTCAGGTGGTTCAGGTGGTTCA   90
GABHYB        CCTGGTTCAAAGGGTTCAGTTACTTCAGGTGGTTCAGGTGGTTCA   90
SELINHYB      CCTGGTTCAAAGGGTTCAGTTACTTCAGGTGGTTCAGGTGGTTCA   90

AJMAD20       GTTGCTTCAGTTGCTTCAGGTGGTTCAGGTGGCTCAGTTGCTTCA  135
3025MAD20     GTTGCTTCAGGTGGCTCAGTTGCTTCAGGTGGTTCAGGTAATTCA  135
2704MAD20     GTTGCTTCAGGTGGTTCAGGTGGCTCAGTTGCTTCAGGTGGCTCA  135
BANCHYB       GGTGGTTCAGGTGGTTCA----------------------------  108
GABHYB        GGTGGTTCA-------------------------------------   99
SELINHYB      ----------------------------------------------   90
```

DOWNSTREAM SEQUENCE OF BLOCK 2 IN MSP-1

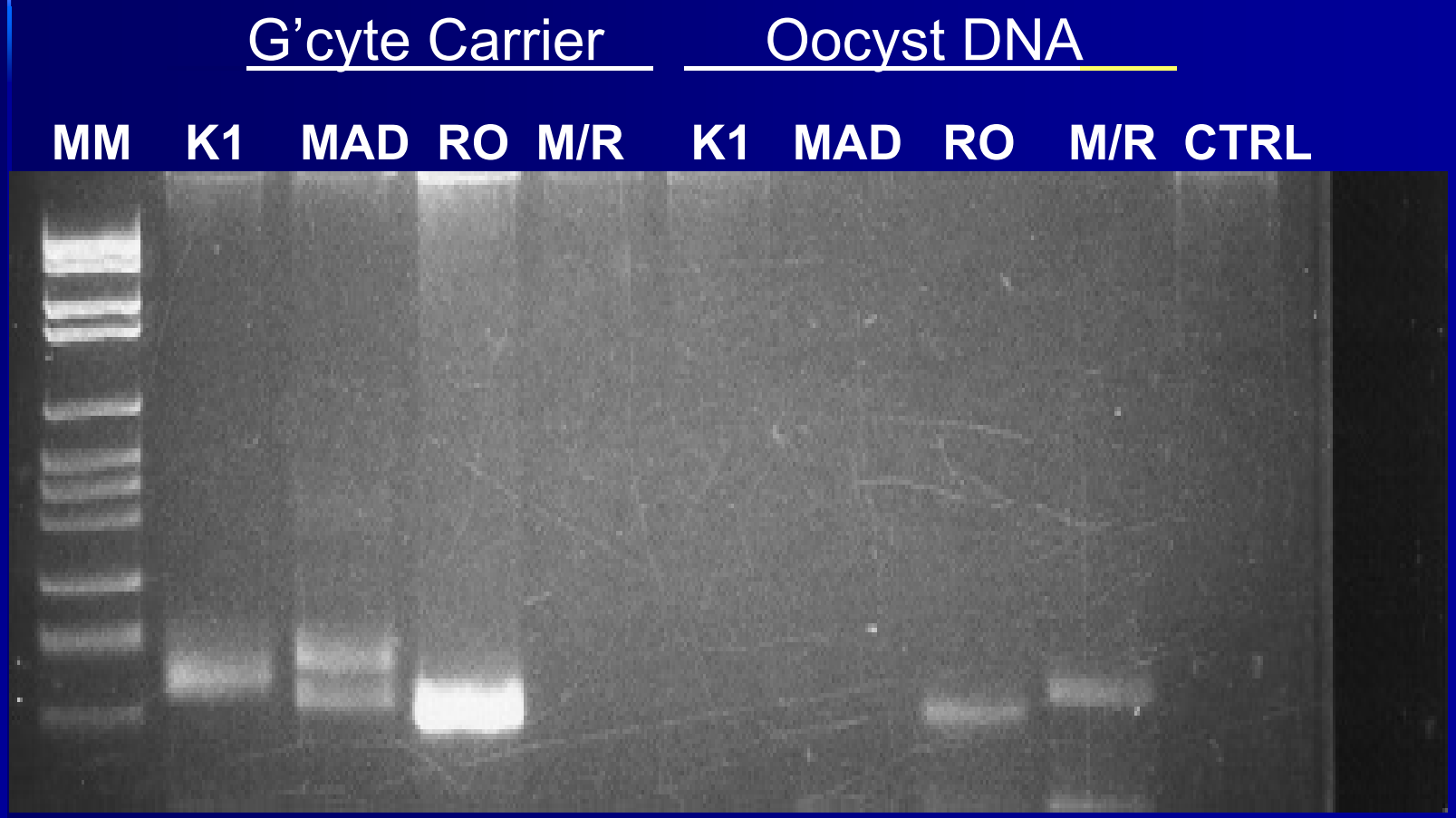
```
BANCHYB      TCAGGTGCTACAGTACCTTCAGGTACTGCAAGTACTAAAGGTGCT  42
GABHYB       TCAGGTGCTACAGTACCTTCAGGTACTGCAAGTACTAAAGGTGCT  42
SELINHYB     TCAGGTGCTACAGTACCTTCAGGTACTGCAAGTACTAAAGGTGCT  42
RO33B5T10    TCAGGTGCTACAGTACCTTCAGGTACTGCAAGTACTAAAGGTGCT  42
RO33B5T8     TCAGGTGCTACAGTACCTTCAGGTACTGCAAGTACTAAAGGTGCT  42
RO33GAB      TCAGGTGCTACAGTACCTTCAGGTACTGCAAGTACTAAAGGTGCT  42
RO33GHANA    TCAGGTGCTACAGTACCTTCAGGTACTGCAAGTACTAAAGGTGCT  42
RO33SNGAL    TCAGGTGCTACAGTACCTTCAGGTACTGCAAGTACTAAAGGTGCT  42
RO3B36T6     TCAGGTGCTACAGTACCTTCAGGTACTGCAAGTACTAAAGGTGCT  42

BANCHYB      ATAAGATCTCCAGGTGCTGCAAATCCTT---------- 70
GABHYB       ATAAGATCTCCAGGTGCTGCAAATCCTT----------70
SELINHYB     ATAAGATCTCCAGGTGCTGCAAATCCTT----------70
RO33B5T10    ATAAGATCTCCAGGTGCTGCAAATCCTT----------70
RO33B5T8     ATAAGATCTCCAGGTGCTGCAAATCCTT----------70
RO33GAB      ATAAGATCTCCAGGTGCTGCAAATCCTT----------70
RO33GHANA    ATAAGATCTCCAGGTGCTGCAAATCCTT----------70
RO33SNGAL    ATAAGATCTCCAGGTGCTGCAAATCCTT----------70
RO3B36T6     ATAAGATCTCCAGGTGCTGCAAATCCTT----------70
```

DETECTION OF CROSS- OVERS DURING MEIOSIS

- Screening for gametocyte carriers (children from 4 to 18 years of age),
- Transportation of subjects from Bancoumana to Bamako for the feeding experiment,
- Exposure to 50-60 F1 *An. gambiae* mosquitoes one generation removed from Bancoumana,
- Fed mosquitoes held 7-8 days and are then dissected for oocysts.

HYBRID SEQUENCES IN MOSQUITO OOCYSTS



RECOMBINATION IN THE ANOPHELINE MOSQUITO

MAD20	GTATTAAATGAAGGAACAAGTGGAACAGCTGTTACA	36
Hybrid	GTATTAAATGAAGGAACAAGTGGAACAGCTGTTACA	36
MAD20	ACTAGTACACCTGGTTCAAAGGGTTCAGTTACTTCA	72
Hybrid	ACTAGTACACCTGGTTCAAAGGGTTCAGTTACTTCA	72
MAD20	GGTGGTTCAGGTGGTTCAGGTGGTTCA	99
Hybrid	GGTGGTTCAGGTGGTTCAGGTGGTTCAGGTGCTACA	108
RO33	CAGGTGCTACA	11
Hybrid	GTACCTTCAGGTACTGCAAGTACTAAAGGTGCTATA	144
RO33	GTACCTTCAGGTACTGCAAGTACTAAAGGTGCTATA	47
Hybrid	AGATCTCCAGGTGCTGCAAATCCTTCAGA	173
RO33	AGATCTCCAGGTGCTGCAAATCCTTCAGA	76

HYBRID BLOCK 2 SEQUENCES: SIZE POLYMORPHISMS

ISOLATES	SIZES (bp)
Sélingué 5	164
Gabriel Touré	173
Sélingué 7	173
GamBancou 2134	173
Bancou45 oocyst	173
Bancou34T4	182

RDTs and *hrp2* Gene Deletions

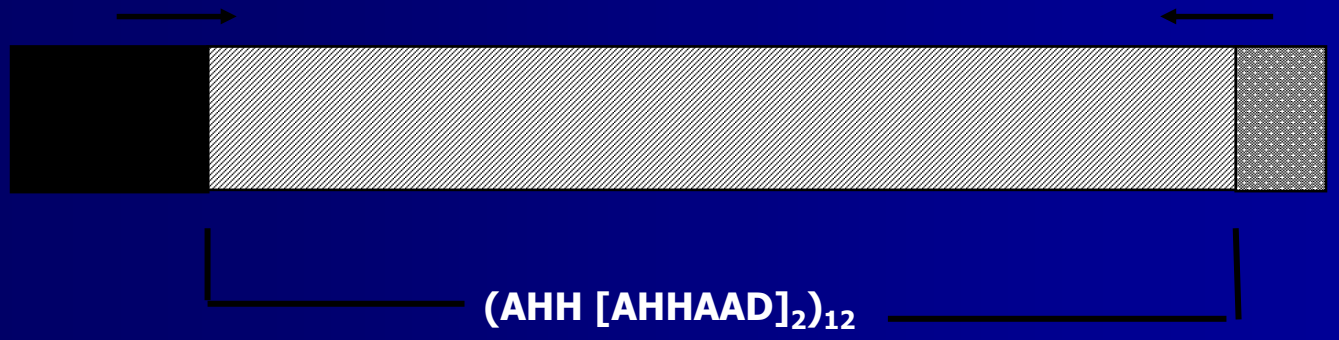
- Twenty-six (26) of 480 smear-positive specimens had false-negative RDTs using an HRP2-based RDT.
- PCR amplification for the histidine-rich repeat region of *hrp2* was negative in half (10/22) of the false-negatives, consistent with spontaneous deletion of *hrp2* or its central region.

Am. J. Trop. Med. Hyg., 86(2), 2012, pp. 194–198
doi:10.4269/ajtmh.2012.10-0665

False-Negative Rapid Diagnostic Tests for Malaria and Deletion of the Histidine-Rich Repeat Region of the *hrp2* Gene†

Ousmane A. Koita, Ogobara K. Doumbo, Amed Ouattara, Lalla K. Tall, Aoua Konaré, Mahamadou Diakité, Mouctar Diallo, Issaka Sagara, Godfred L. Masinde, Safiatou N. Doumbo, Amagana Dolo, Anatole Tounkara, Issa Traoré, and Donald J. Krogstad*

HRP-2 GENE DELETION IN MALARIA PARASITE



Expected size of the 923 bp PCR product

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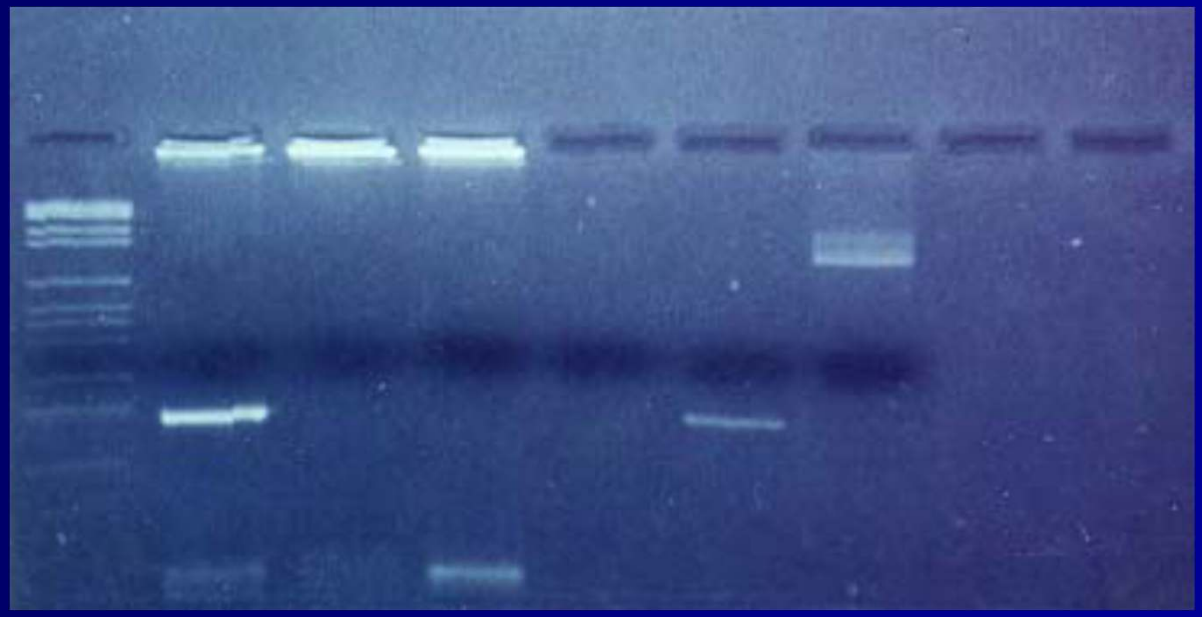
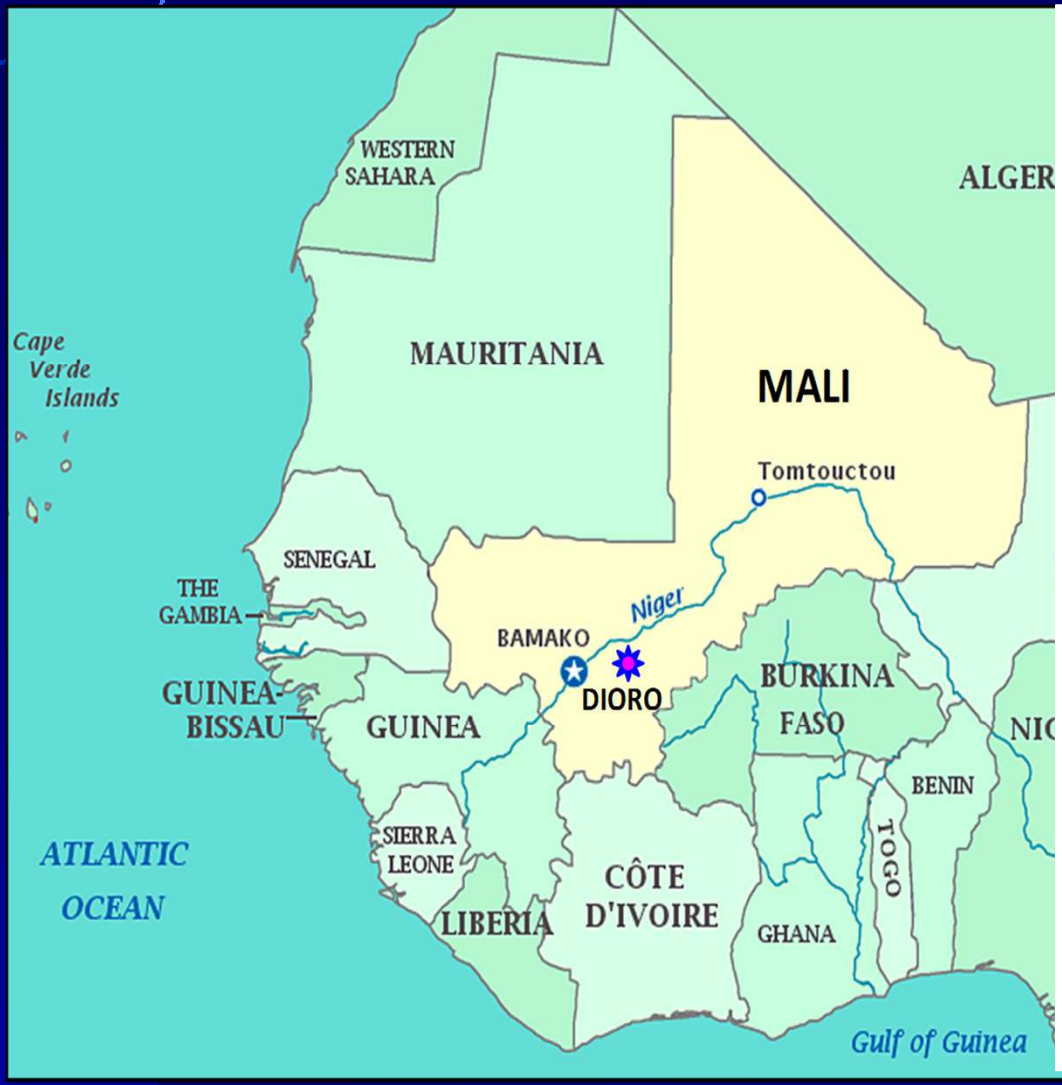
hrp2 PCR in Subjects with and without *hrp2* Deletions



Lane 1: DNA molecular weight markers VI (Roche, Indianapolis, IN).
Lanes 2-6: results of PCR with DNA from a thick smear-positive subject with a negative ParaSight F test using allotype-specific primers for the Block 2 region of *msp1* (Lanes 2-4 demonstrate one K1 amplicon and no MAD20 or RO33 amplicons), forward and reverse primers for *hrp2* (Lane 5 demonstrates the absence of *hrp2* amplicons) and species-specific primers for *P. falciparum* ribosomal DNA (Lane 6, amplicon of the expected size, ~206 bp). In contrast, Lane 7 provides a positive control for the *hrp2* PCR based on DNA from a thick smear-positive subject with a positive ParaSight F test (same forward and reverse primers).

DIORO Mali ICEMR Site



Population : 11,395 inhabitants
Prevalence of Malaria infection: ~70%
EIR: 4.77 infectious bites per month
Control measures:
1] Prompt free treatment of uncomplicated *P. falciparum* malaria with ACTs,
2] Universal coverage with long-lasting insecticidal nets (LLINs),
3] Free care for malaria since 2006 (MDGs, MVP, Ségou).

Study Site : DIORO

The Dioro irrigation scheme is based on manual opening gravity-fed wooden flood gates on the Niger River behind the Markala Dam.

•The primary flood gates are opened between August 17th and 30th each year and flood 7,500 ha for rice cultivation from September to December.

•In December, the water in this initially flooded area is drained (to permit harvesting of the first rice crop) into a second area to permit a second rice crop between December and April or May from the flooding of an additional 7,500 ha (by opening a second series of wooden flood gates).

• The result is that there is an enormous flooded area of stagnant water in the Commune of Dioro for mosquito breeding from August to April or May each year (≥ 7,500 hectares).

Methods

- Sample: all subjects with fever seeking care at the Dioro Clinic from May 2012 to June 2013,
- Consent was obtained for fingerprick blood samples for thick smears, RDTs and filter paper blots. Assent obtained for subjects less 18 years of age.
- Thick smears, RDTs (Paracheck, First Response and Bioline) and filter paper blots were obtained from each subject.

Dioro Health Center

Basic Demographic Data

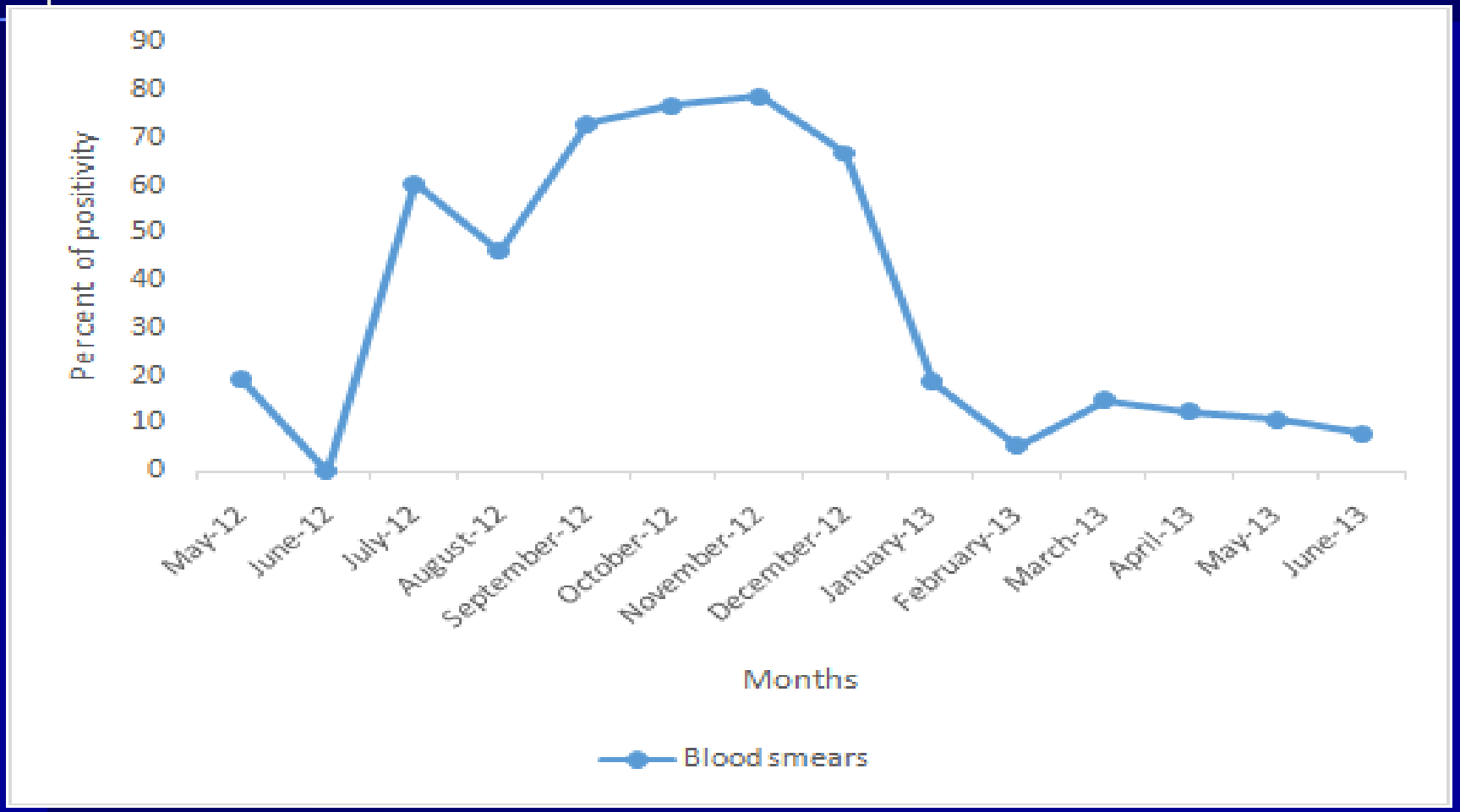
Age group (years)	Number	Percent (%)
1-4	**189**	**27.4**
5-9	**262**	**37.9**
10-16	**240**	**34.7**
Total	**691**	**100**
Gender		
Male	**346**	**50.1**
Female	**345**	**49.9**
Total	**691**	**100**

Rapid Diagnostic Test and Thick Smear

Rapid Diagnostic Test	Thick smear		Total
	Negative	Positive	
Negative	112	277	389
Positive	15	287	302
Total	127	564	691

Parameter	Raw Data	Percent	95% Confidence
Sensitivity	287/309	50.9%	46.7-55.1%
Specificity	112/127	88.2%	81.3-93.2%
Positive Predictive Value	287/302	95.0%	91.9-97.2%
Negative Predictive Value	112/389	28.8%	

Seasonal Changes in Positive Smears

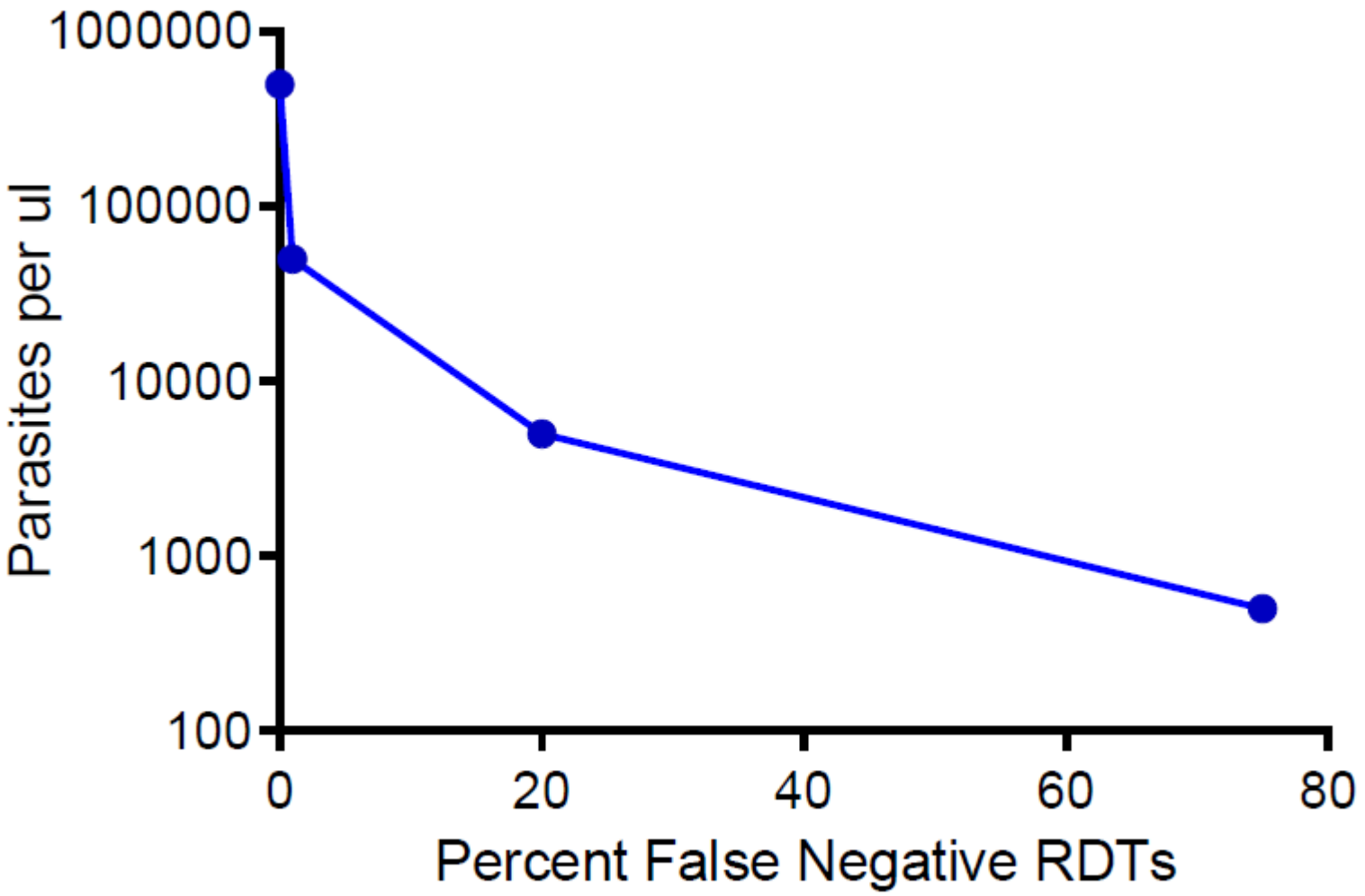


Seasonality of False-Negative RDTs

Malaria Season	YES	NON	TOTAL
LOW	258	190	448
HIGH	19	224	243
TOTAL	277	414	691

P value < 0.0001

Parasitemia and False negative RDTs



Clonality (Multiplicity of Infection) and Frequent False-Negative RDTs

- Previous studies have shown that the multiplicity (complexity) of infection is lower in specimens with false-negative RDTs than in specimens with true-positive RDTs:

RDT Test Results and the Number of Parasite Genotypes in Specimens with Thick Smears positive for *P. falciparum*

RDT Result	Mean Number Genotypes
False Negatives	1.00 ± 0.00 (n=12)
True Positives	2.42 ± 0.84 (n=19)

Am J Trop Med Hyg 2012; 86(2): 194-198.

Explaining the Increased Frequency of False-Negative RDTs in the Dry Season

Three or more factors may explain/contribute to seasonal variation in the frequency of false-negative RDTs:

- 1] spontaneous HRP2 deletions,
- 2] lower MOIs during the dry season which potentially also increase false-negative RDTs and
- 3] less frequent acquisition of new infections because of less intense transmission during the dry season.

Implications of these results for malaria control

- Regardless of the mechanism(s) involved, false-negative RDTs pose a challenge for malaria control,
- Improved malaria control paradoxically decreases the sensitivity of the RDT because it reduces both parasite densities and multiplicity of infection.
- As a result, sensitivity of the RDT may progressively worsen as malaria control improves,
- This issue (false-negative RDTs) is a priority for country-specific malaria control programs in endemic areas as well as international groups (PMI, WHO).

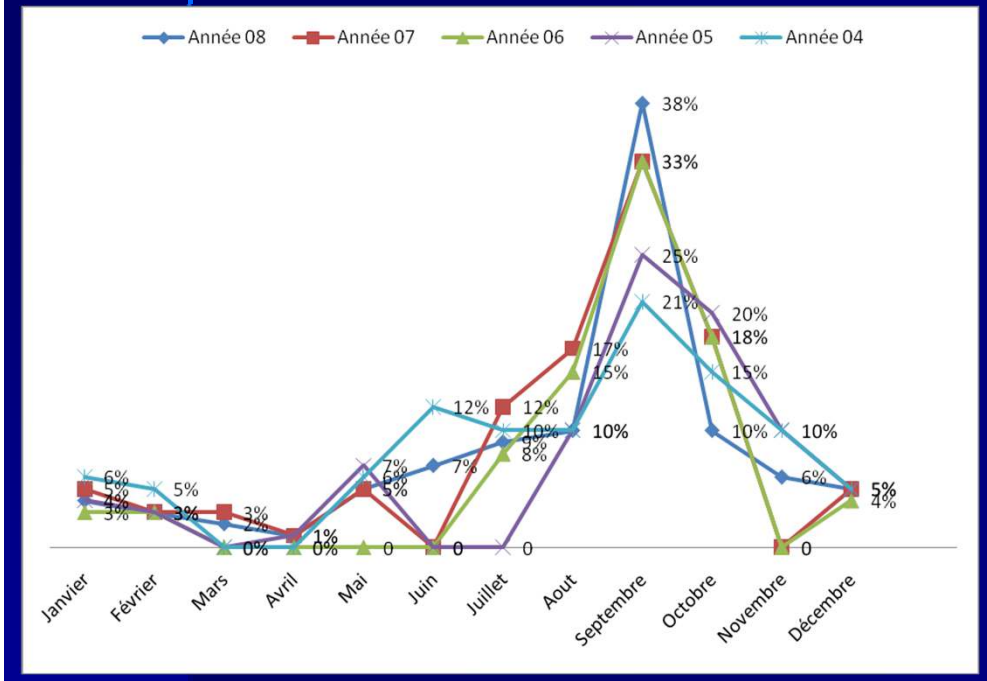
CONCLUSION (1)

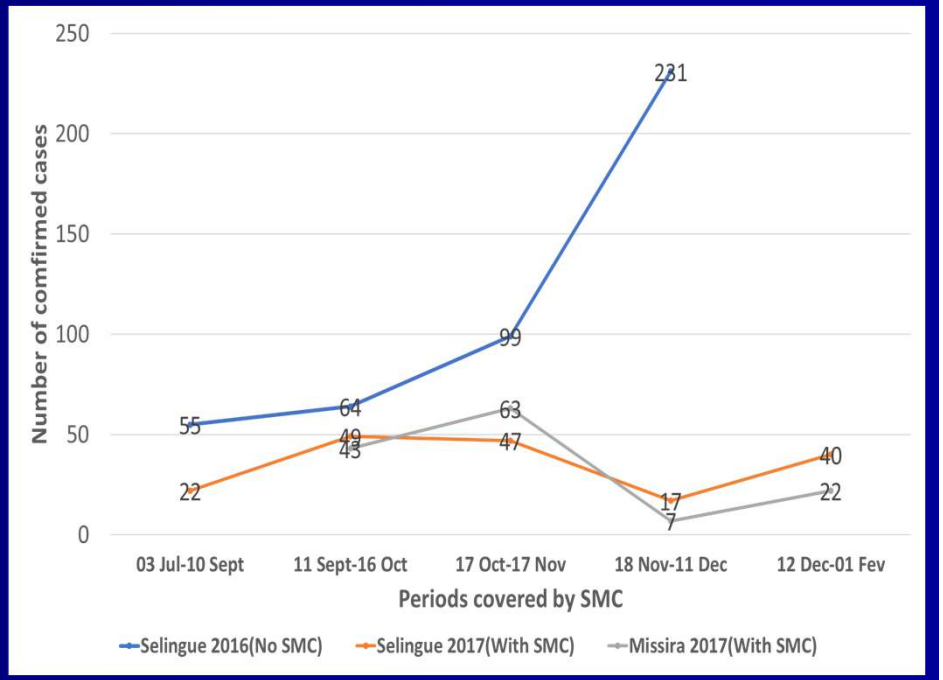
- This recombinant parasite is produced during the meiotic reduction division in the mosquito, The size polymorphisms observed in these sequences are consistent with crossover events at 9 bp intervals based on repeating TCA trinucleotides in Block 2.

CONCLUSION (2)

Subteleomeric deletion occurs in malaria parasite. The hrp2/3 situated at the end of the chromosome can be deleted and therefore Rapid Diagnostic Test (RDT) may produce false negative, currently treatment by ACT is based on this test. About, 2.5% of *P. falciparum* parasites circulating in Mali lack of hrp2 gene.

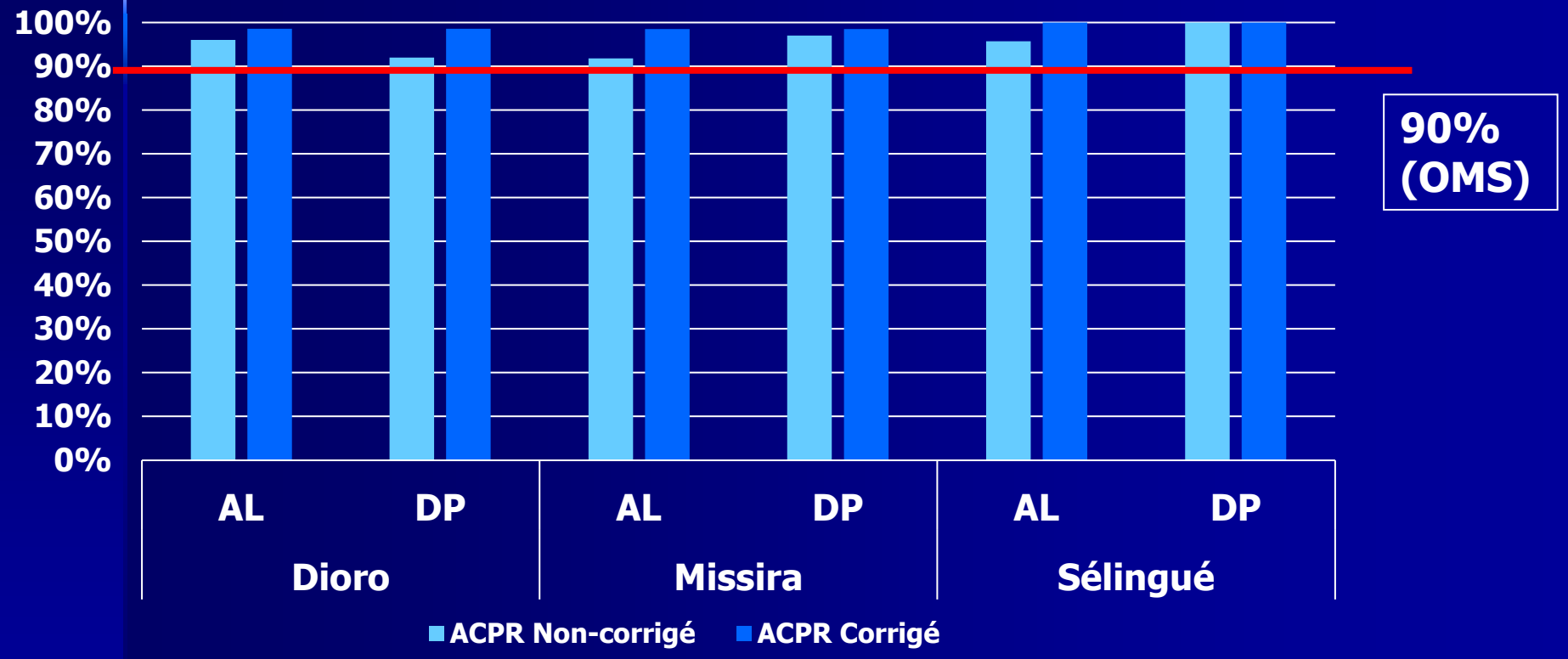
IMPACT OF SMC ON MALARIA CASES IN MALI



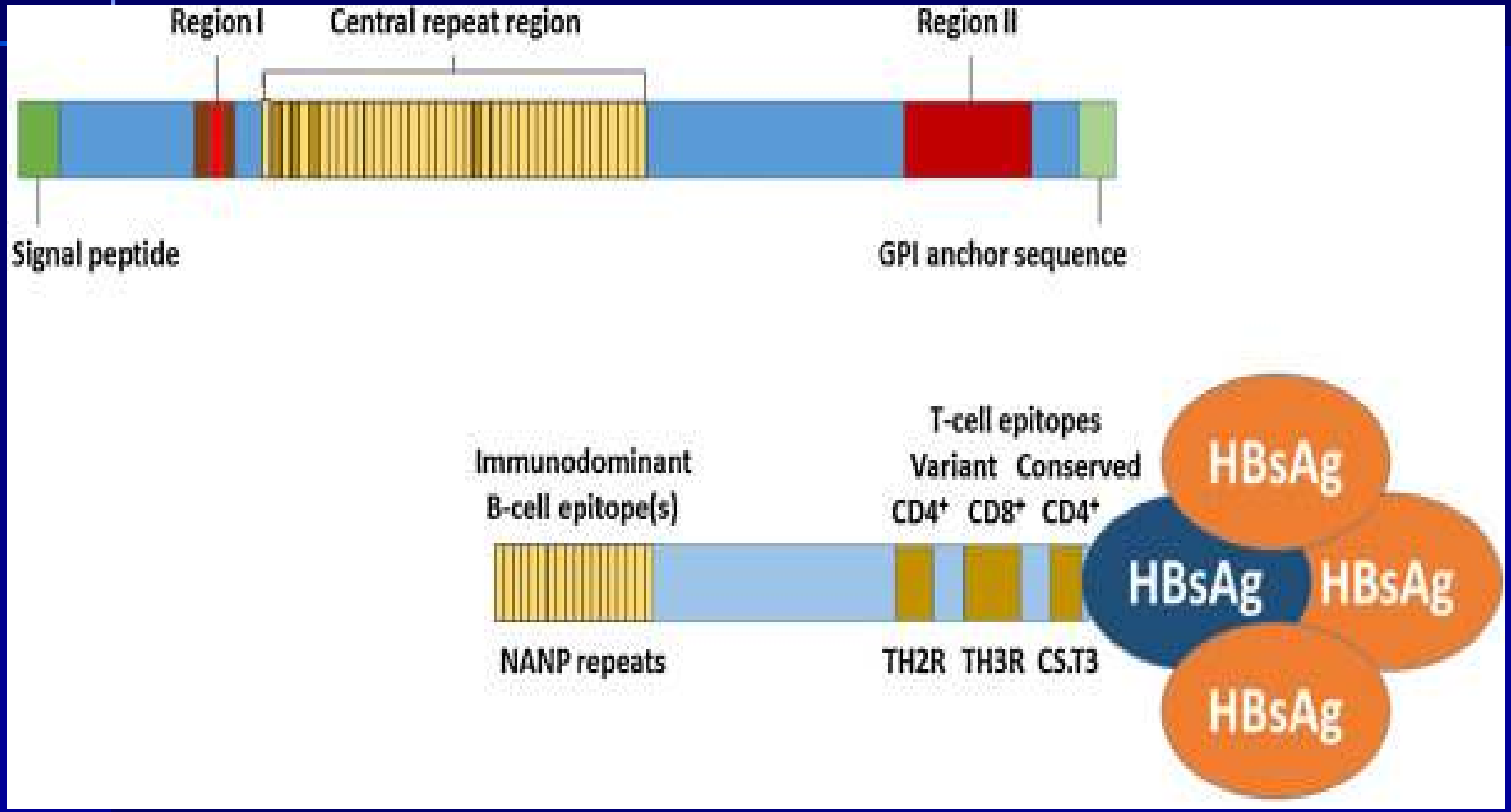


Mamadou Bah, PhD, thesis, 2013

THERAPEUTIC EFFICACY STUDY (PMI/USAID)



CONCEPTION OF RTS,S/AS01 VACCINE



EFFICACY OF PHASE 3 OF RTS,S/AS01 VACCINE

Age Group	6-12 weeks of age (n = 6537)	5-17 months of age (n = 8922)
Vaccine Efficacy against clinical malaria, 3-dose group (95% CI)	18.3% (11.7 to 24.4)	28.3% (23.3 to 32.9)
Vaccine efficacy against clinical malaria, 4-dose group (95% CI)	25.9% (19.9 to 31.5)	**36.3% (31.8 to 40.5)**
Vaccine Efficacy against severe malaria, 3-dose group (95% CI)	10.3% (–17.9 to 31.8)	1.1% (–23.0 to 20.5)
Vaccine efficacy against severe malaria, 4-dose group (95% CI)	17.3% (–9.4 to 37.5)	32.2% (13.7 to 46.9)

CONCLUSION

- **Scientifique:**

  Modest efficacy of 30%, in taking into account the gene polymorphisme of candidat vaccine and high plasticity of the parasite genome,

  Selection of resistant strains to RTS,S as consequence of massive immunization

CONCLUSION

- **Financial**:

8,68 US$ per dose when incorpaorated in the Immunization program targetting 3.905.460 children less than 59 months.

The financial burden **81.358.542.720 FCFA**

CONCLUSION

- **Ethics:**

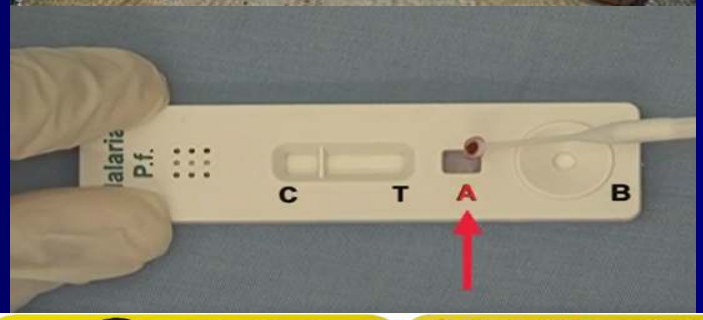
  A vaccine must be tested on adults first before children;

- **Society**:

  More suspicion towards immunization program

NEW APPROACHES IN MALARIA TREATMENT

-] Household care



NEW APPROACHES IN MALARIA TREATMENT

- **In the community,**

Strengthen the capacity of the health community center Mini blood bank

CHALLENGES

Malaria elimination remains a challenge, such as genetic diversity of the parasite, the financial burden, population mobility and environmental context.

Lack of political commitment: U$25 millions year 2022[5].

Research within our universities should look at endogenous solutions

Acknowledgments

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  Aliou Sissako
- Tulane University, School of Public Health and Tropical Medicine
  Don Krogstad and Fran Krogstad

University of Port Harcourt

Thanks Prof Aline Noutcha and DVC R&D Prof S. IYEOPU

Life, a Challenge

View of Bamako from my office