

**Utilization of the use of molecular markers as alert tool to determine the presence of antimalaria drug resistance: When and how should be implemented?**

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# What is the role of molecular tools in the surveillance for resistance in the Americas?

- Molecular surveillance is valuable and complementary to other tools
- Highly relevant for population level surveillance
- In areas with limited malaria (where *in vivo* trials cannot be performed)
- Markers are available for some but not all drugs

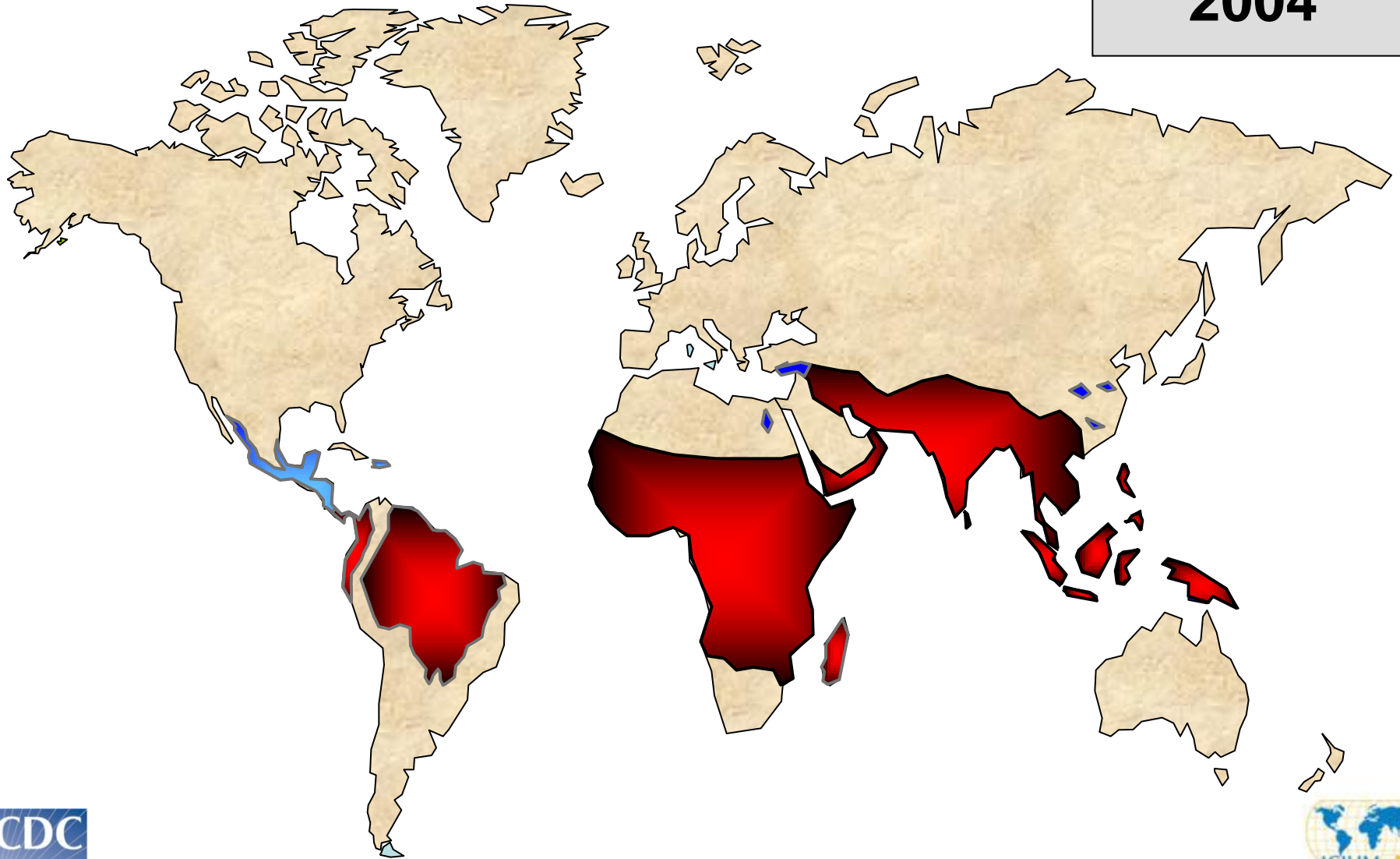
# Molecular Markers

- Chloroquine (CQ)—*Pfcrt*  
(*CVMNK*, *CVMNT*, *SVMNT*, and *CVIET*)
- Sulfadoxine—*Pfdhps* (*437G*, *540E*, *581G*)
- Pyrimethamine—*Pfdhfr*  
(*50R*, *51I*, *108N*, *51I*, *108N*, *164L*)
- Mefloquine (MQ) *Pfmdr-1* (copy number variation)
- Artemisinin—being investigated, regions identified (being reviewed for publication)

 Distribution of Chloroquine-resistant *P. falciparum*

 Distribution of Malaria

2004



# Monitoring Resistance in Central America and Caribbean

- CQ is the primary treatment and SP is the secondary drug
- It is logical to use molecular markers in this region as we have good markers for CQ and SP
- Also helps to know what other imported resistant parasites are present

# Molecular surveillance for chloroquine resistance in Central America and Caribbean



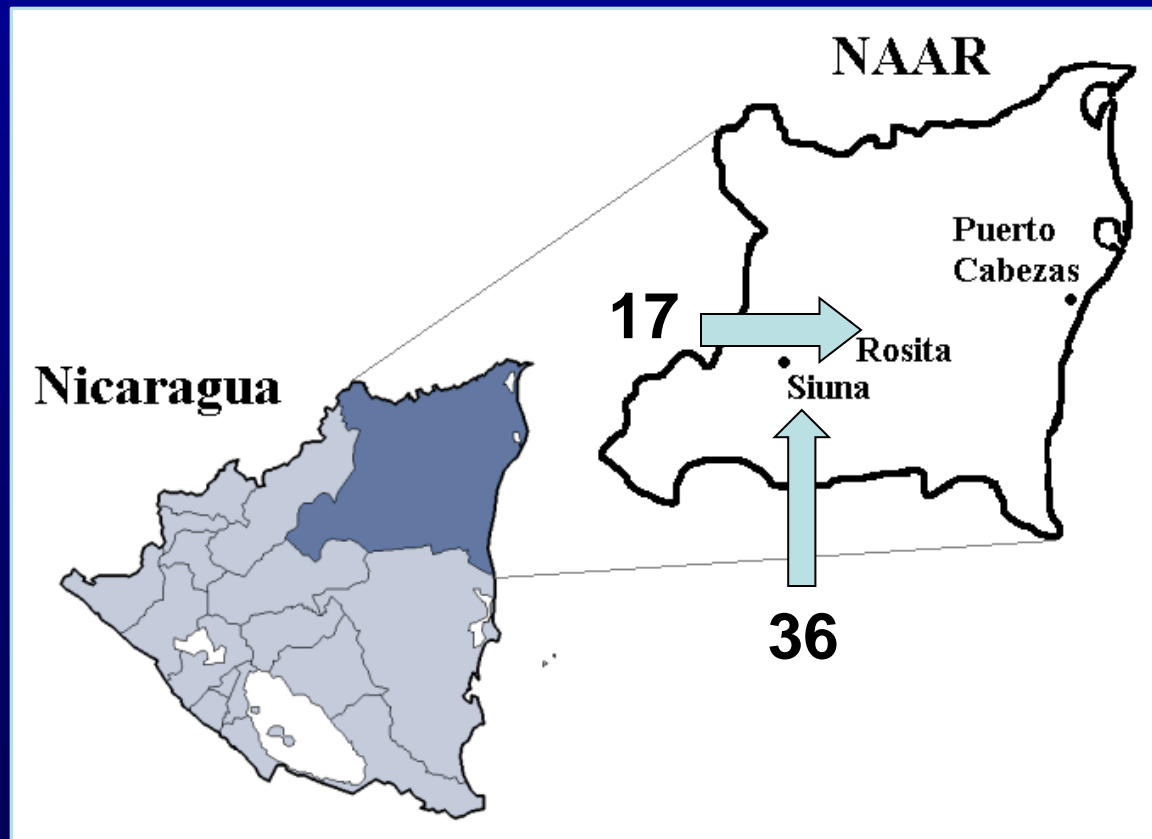
# Guatemala

Imported cases of  
malaria –military  
personnel visiting  
Africa

Norma Padilla  
Maru and Lucia



# CQ and SP resistance genotypes in Nicaragua-Preliminary findings





# Nicaragua-CQ and SP resistance genotypes (2005)

## North Atlantic Autonomous Region (NAAR)

<u>Molecular marker</u>	<u># samples</u>
<b>CQ resistance</b>	
WT (CVMNK )	51 (96.2%)
CVIET	2 (3.8%)
<b>SP resistance</b>	
<i>dhfr</i> WT	50(96.2%)
108N mutant	1(1.9%)
51N,59R,108N	1(1.9%)
<i>dhps</i> WT	48 (97.9%)
437G	1 (2.1%)



# **Nicaragua-CQ and SP resistance genotypes (2010-2011)**

**North Atlantic Autonomous Region (NAAR)**

**Over 50 samples being tested and no evidence for CQ resistant parasites so far**

**Betzabe Mara Rodriguez, Molecular Diagnosis Lab, Ministry of Health, Nicaragua (ongoing)**



# Honduras-CQ resistance molecular survey

68 samples from an in vivo trial conducted in 2009

Eastern Hondura region  
Gracias a Dios

**100% CQ sensitive**

**Survey from 2011 collection is ongoing**



Dr. Rosa Elena Mejia and Dr. Tamara Mancero

Dr. Gustavo A Fontecha, National Autonomous University of Honduras

Meisy Mendoza, National Malaria Laboratory

# Haiti- CQ and SP resistance genotypes 2010 samples

(preliminary analysis, Barnwell J et al unpublished)

<u>Molecular marker</u>	<u># samples</u>
<b>CQ resistance</b>	
WT (CVMNK )	105 (98.13%)
CVIET	2 (1.87%)
<b>SP resistance</b>	
<i>dhfr</i> WT	42(60.86%)
108N mutant	27(39.13%)
<b>High resist. triple mutant</b>	<b>0 (0%)</b>

(Total 345 blood spots, 118 +ve for 18S gene amplification)



# Monitoring Resistance in S. America

- Drug resistant parasites are evolving independently in SA
- ACT has been in use since 2001
- What molecular markers?
- Validate new markers being discovered (let us not forget the history of CQ and SP rest.)



# Chloroquine resistance is fixed



Two haplotypes  
SVMNT/CVMNT-A  
CVMNT/CVMET-B

CQ is used for *P. vivax* treatment

# SP resistance is fixed in most of the Amazon but not in the coast



**Dhfr**  
51I,108N,164L

**dhfr**  
50R, 51I, 108N

**dhps**  
437G, 540E, 581G

**Fixed In the Amazon  
(except in Peru and  
Colombian region?)**

**Low /no resistance  
In the coast  
But now we have SP  
rest moved to coast**

# Pfmdr1

(N86Y, Y184F, S1034C, N1042D, D1246Y)



Quadruple/triple mutants-fixed

Two haplotypes (alpha and beta)

Copy number increase in Venezuela  
Suriname?

Brazil?


Other countries?  
Not in Peru 2006

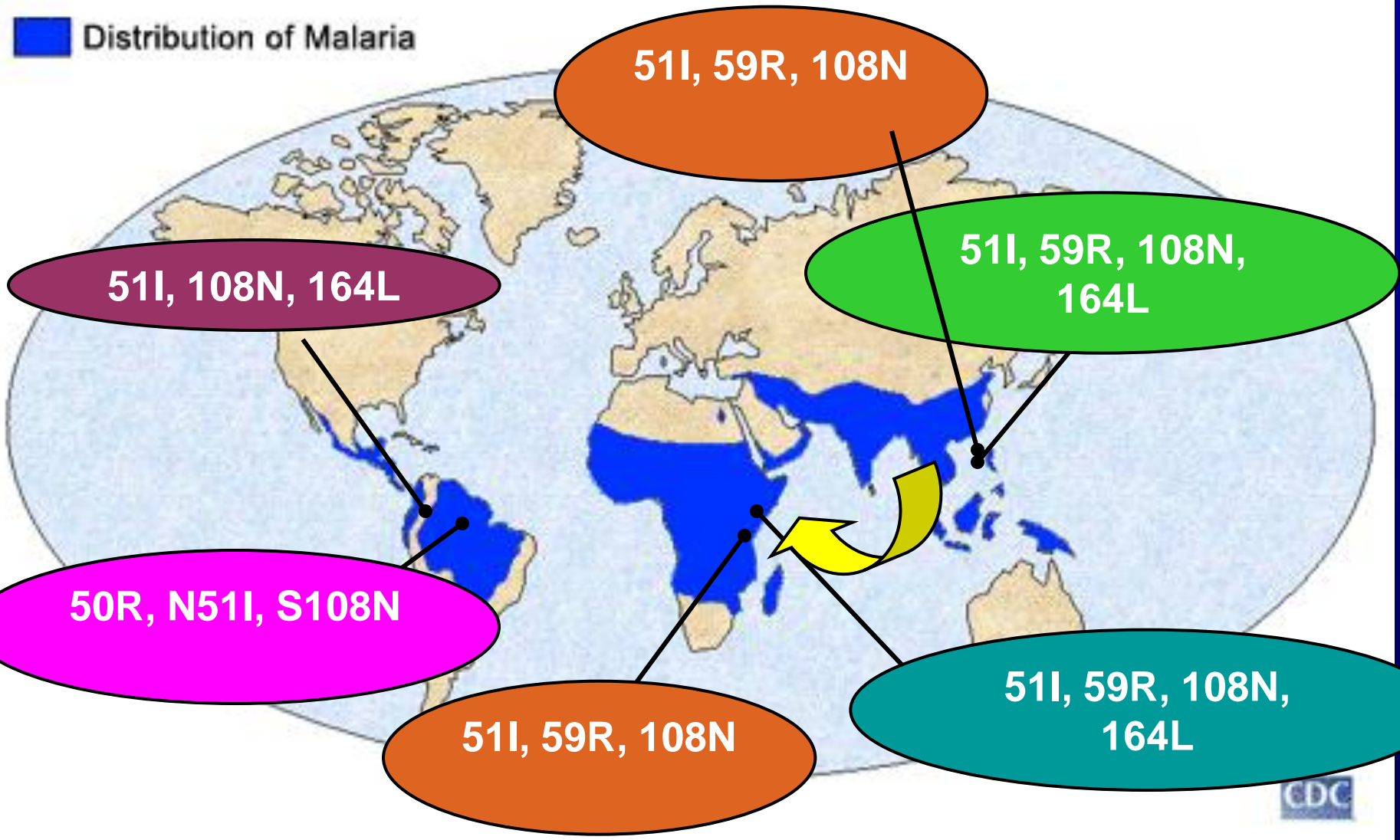
MQ and LU resistance?





# Origins and Spread of Highly Pyrimethamine Resistant Dhfr Genotypes

 Distribution of Malaria



# Future Directions

A comprehensive molecular surveillance strategy is needed for the region (including for speciation)

Central America and Caribbean regions can do annual molecular surveillance for CQ resistance marker and if possible SP resistance markers

This can be integrated with their ongoing passive and active surveillance

Can be integrated for HRP2 surveillance as well



# Future Directions

Link all in vivo clinical trials with molecular marker analysis  
(WWARN collaboration)

Imported cases of malaria (travel history)

MDR1 copy number –South America (MQ and LU)

Validate new markers for artemisinin resistance

Simple field usable techniques for field use



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