

Molecular Surveillance for Drug and Diagnostic Resistance in South and Central America

Kumar V. Udhayakumar, Ph.D
Malaria Branch

Division of Parasitic Diseases
Centers for Disease Control and Prevention,
Atlanta, USA

Talk Outline

- Progress in Peru, Venezuela and Brazil
- Central America
- Training and capacity building
- HRP2 RDT Failures and HRP2/3 genetic deletion

Molecular Markers

- Chloroquine (CQ)—*Pfcrt*
- Mefloquine (MQ) and other ACT—*Pfmdr-1*
 - copy number variation
- Sulfadoxine—*Pfdhps*
- Pyrimethamine—*Pfdhfr*
- Artesunate/Artemisinin—no good markers
- Microsatellite markers
- **P. vivax** Better markers are needed

There are some differences in the molecular patterns of drug resistant markers and population structure of parasites in South and Central America as compared to other regions

Monitoring Resistance in S. America

- Drug resistant parasites are evolving independently in SA
- ACT has been in use since 2001
- Molecular surveillance is valuable and complementary to other tools

Preliminary Results



Peru Studies

- Peruvian National Institute of Health (INS)
 - Nancy Arrospide
 - Wilmer M. Quezada and others
 - Trent Ruebush
- Naval Medical Research Center Detachment
- David Bacon and colleagues

Peru Questions

- What are the molecular pattern of resistant genotypes in different parts of Peru?
- What is the ancestral relationship between the resistant genotypes in different parts of Peru?
- What happened to the resistant genotypes as a result of policy change to introduce ACT?

Dual drug policy in Peru

- Coast and West Amazon

1999: CQ resistant, SP sensitive

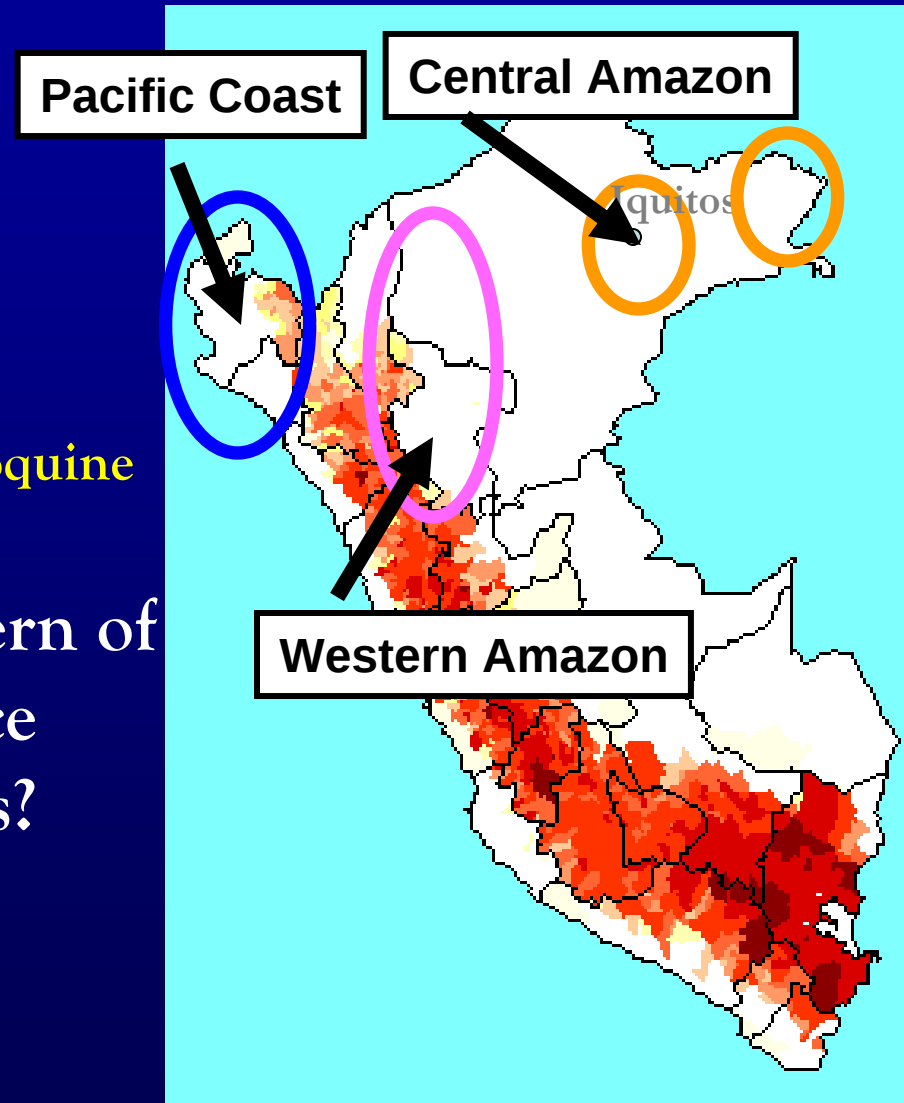
1999-2001: CQ to Artesunate+SP

- Peruvian Cent. Amazon

1999: CQ and SP resistance

1999-2001: SP to Artesunate+Mefloquine

- What is the molecular pattern of CQ , SP and other resistance markers in different regions?



Summary from Peru

- CQ resistant genotype is fixed in all sites tested with two genotypes of different origins.
- SP resistant genotypes were high in the Central Amazon (1999) but declined (2006) after SP removal
- SP resistant genotypes were not found in coast and Western Amazon

Summary from Peru

- Two major Pfmdr1 genotypes found
- No evidence for copy number increase in this gene (marker for MQ)
- Regional difference exist consistent with different drug policy
- Bottled necked population of parasites based on genetic evidence

Evolution of Multi Drug Resistant Parasites in Venezuela



Dr. Ananias Escalante, Arizona State Univ
Dr. Leopoldo Villegas, Venezuela

Drug Resistance in Venezuela

- CQ banned by National Program of Malaria Therapeutics in 1986
- SP stopped in 1998
- MQ monotherapy selectively used till the adoption of ACT from 2002

Investigations on samples from Venezuela

- Bolivar state
- non-immune adults with *P. f.*, 2003-2004 (N=97)
- Genotyped *Pfcr*t, *dhfr* and *dhps* and *mdr1* CNV



Summary From Venezuela

- Multidrug resistant genotypes are accumulating and no decline in CQ and SP resistant genotypes after policy change
- Multiple copy pfmdr1 was found in 14% of samples (2003-2004).
- Further testing for mdr-1 copy number increase in the region (Venezuela, Guyana, Suriname, and Brazil) is warranted



**INSTITUTO
EVANDRO
CHAGAS**



Secretaria de Vigilância em Saúde

INSTITUTO EVANDRO CHAGAS-IEC/SVS/MS

**Molecular Surveillance for
Drug Resistance in Brazil**

MARINETE MARINS POVOA

GISELLE MARIA RACHID VIANA

LABORATÓRIO DE PESQUISAS BÁSICAS EM MALÁRIA

Brazil Study Sites



■ Data reported from 1990s

Summary From Brazil

- CQ resistant genotypes are SVMNT genotypes and fixed
- SP resistant genotypes are fixed
- >85% of parasites with quadruple pfmdr1 mutation
- Copy number increase in pfmdr1 needs to be tested

(M. Pova et al un published)

Monitoring for Chloroquine and SP Resistance in Central America

(CQ is still used for primary treatment)

Dr. Norma Padilla



Betzabe Rodriguez

**Further discussion is needed for
incorporating molecular data for
policy changes especially in the
context of Haiti**

**Partnership and collaboration with
Worldwide Antimalarial Resistance
Network (WWARN)**

Training and Capacity Development

- Mergiory Y Bracho Garrido (Malti Adhin's lab,) Institute of Biomedical Sciences, Suriname (01/13/09 to 02/24/09)
- Betzabe Mara Rodriguez, Molecular Diagnosis Lab, Ministry of Health, Nicaragua (8/24/2009 to 9/25/2009)
- Katherine Jessica Torres-Fajardo, (Dioni's lab) IMTAH, Univ. Cayetano, Peru (7/20/09 to 9/18/2009)

Training and Capacity Development (cont.)

- Claribel Murilo Solano, Cali, Colombia (02/08/10 to 02/26/10)
- Maru and Lucia from Norma Padilla lab, Univ. Guatemala (03/15/10 to 04/15/10)
- Molecular training workshop in Brazil

Future Directions

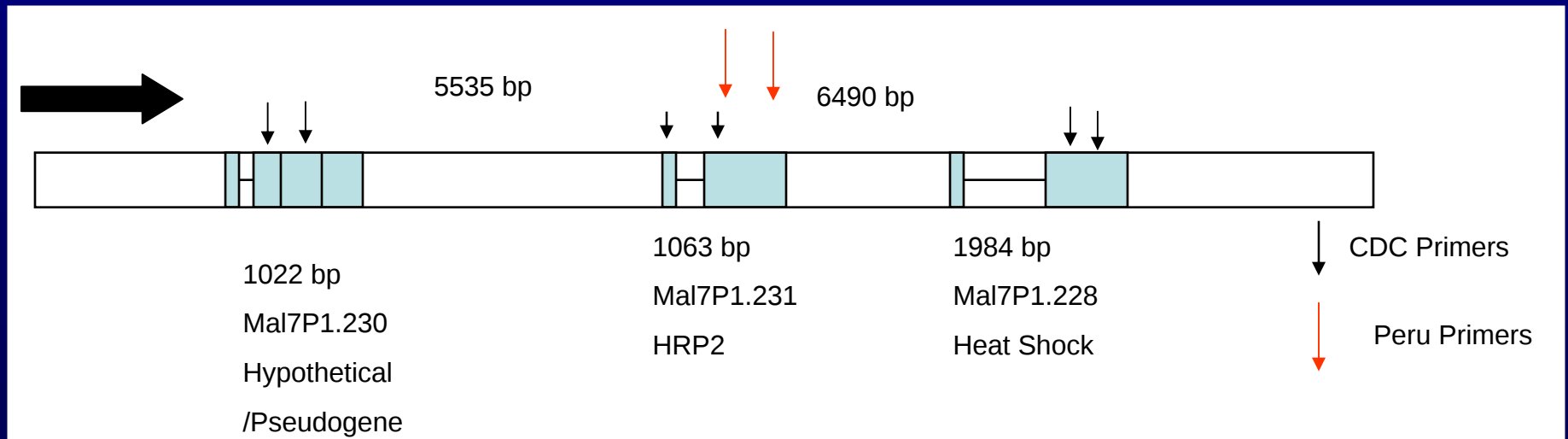
- A comprehensive molecular surveillance strategy is needed for the region
- Alex is working on a protocol

HRP2/3 Gene Deletion Studies

- HRP2 is the antigen detected by most rapid diagnostic tests (RDT)
- HRP2 is present only in *P. falciparum*
- During the recent evaluation of RDTs (WHO/FIND/CDC) it was discovered that some parasites in Peru were missing HRP2 and HRP3 genes
- HRP2 RDTs fail to detect these parasites

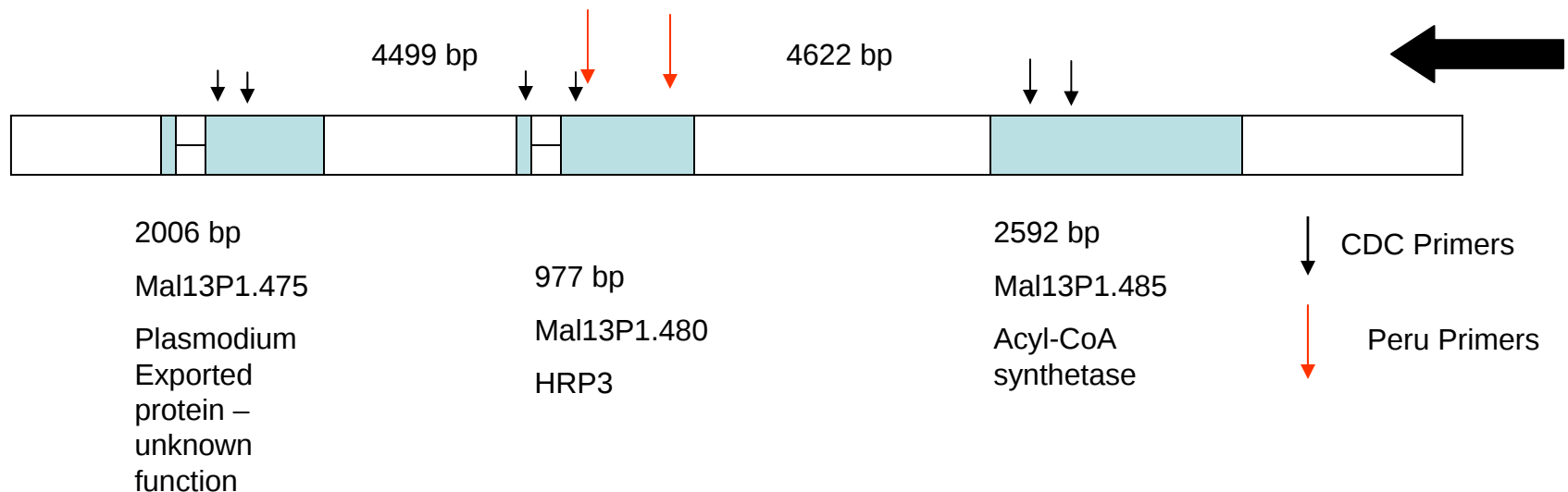
Genes of Interest (HRP2)

- HRP2:
 - Located on chromosome 7 (same chromosome as *Pfcr*t gene)
 - Contains numerous histidine repeats (key trait exploited by RDTs for detection)
 - Mal7P1_230 and Mal7P1_228 are the immediate upstream and downstream genes respectively.

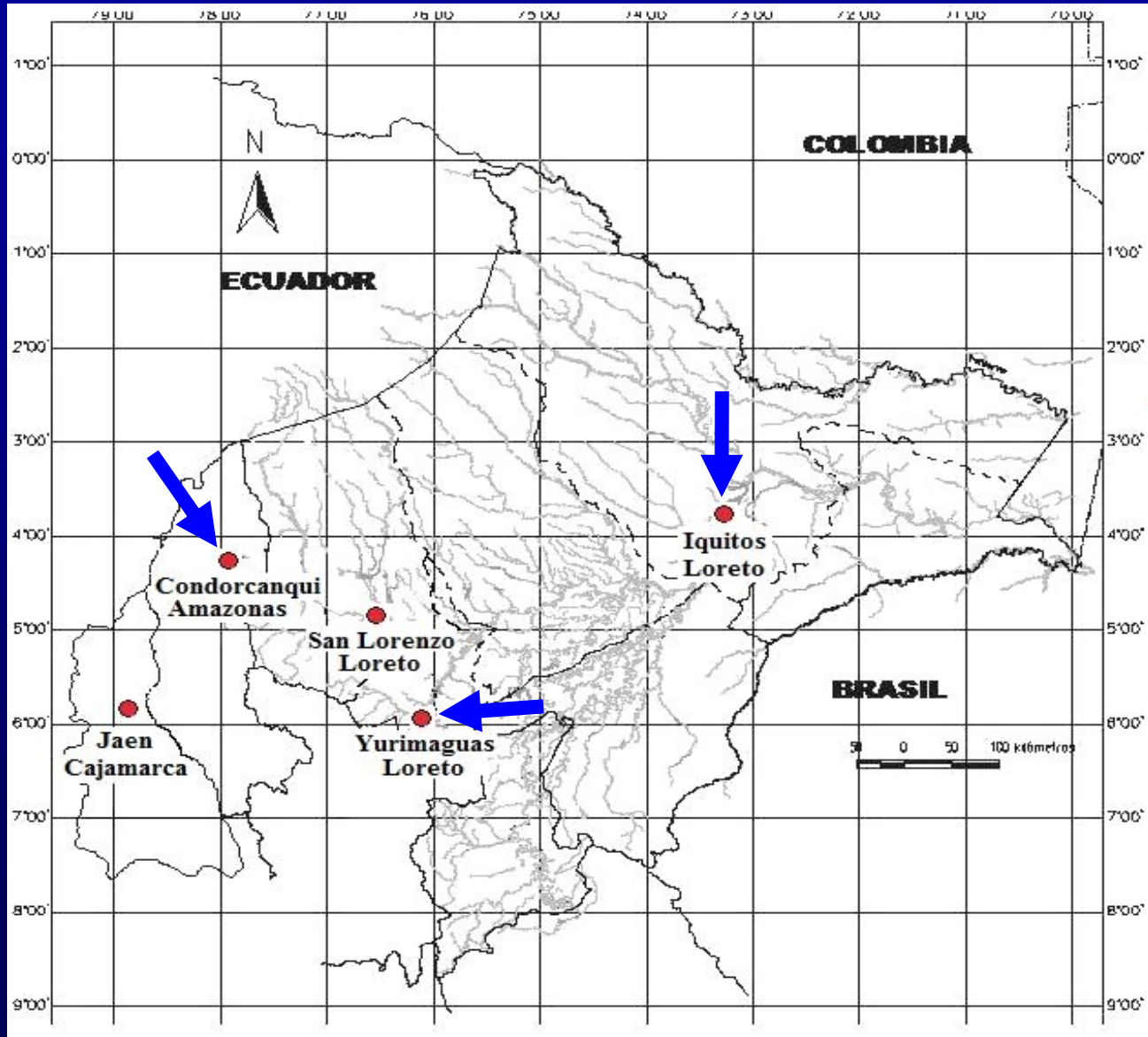


Genes of Interest (HRP3)

- HRP3:
 - Located on chromosome 13 near the telomeric region.
 - Also contains numerous histidine repeats.
 - Mal13P1_485 and Mal13P1_475 are the immediate upstream and downstream genes respectively.

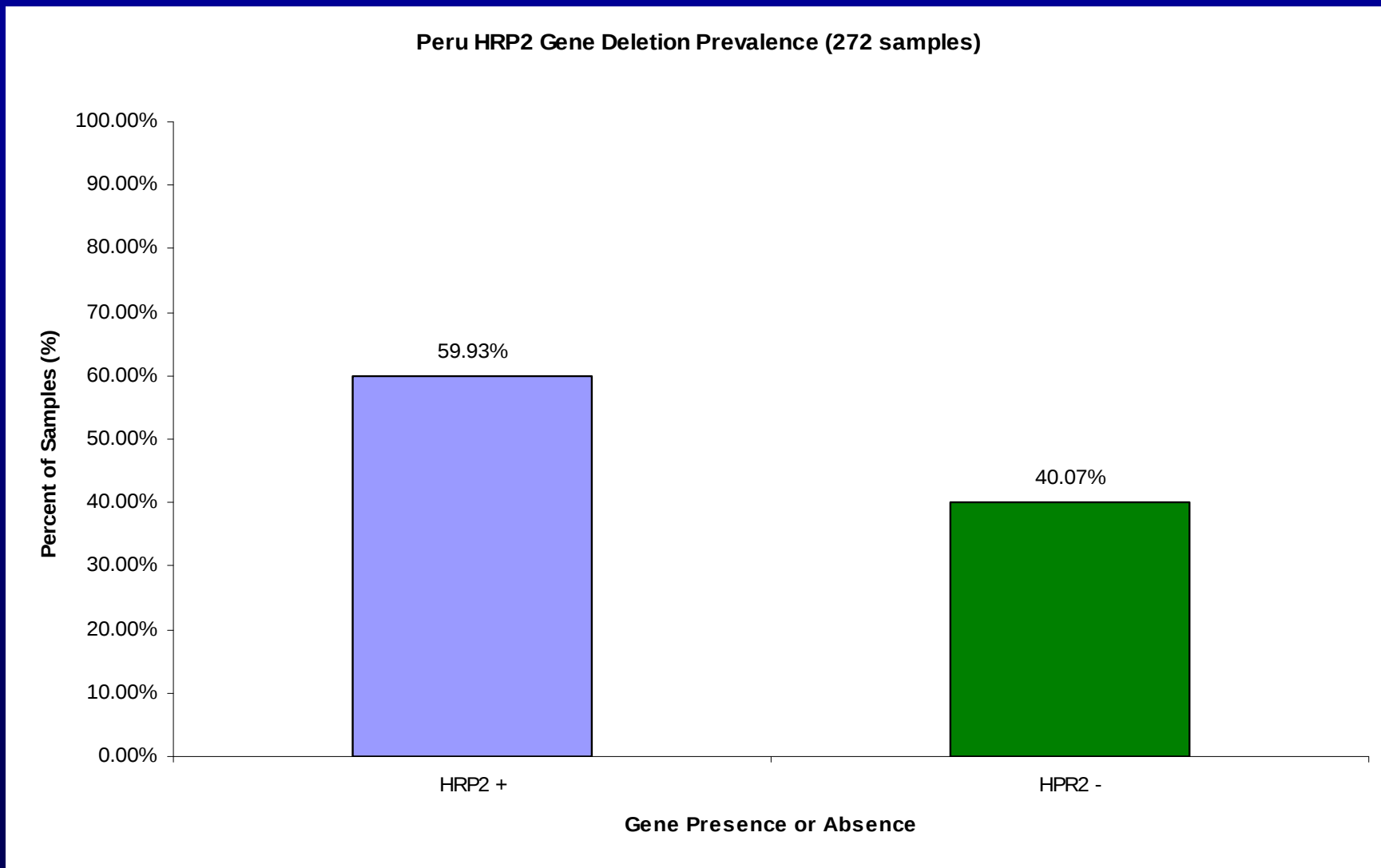


Genotype Profiling

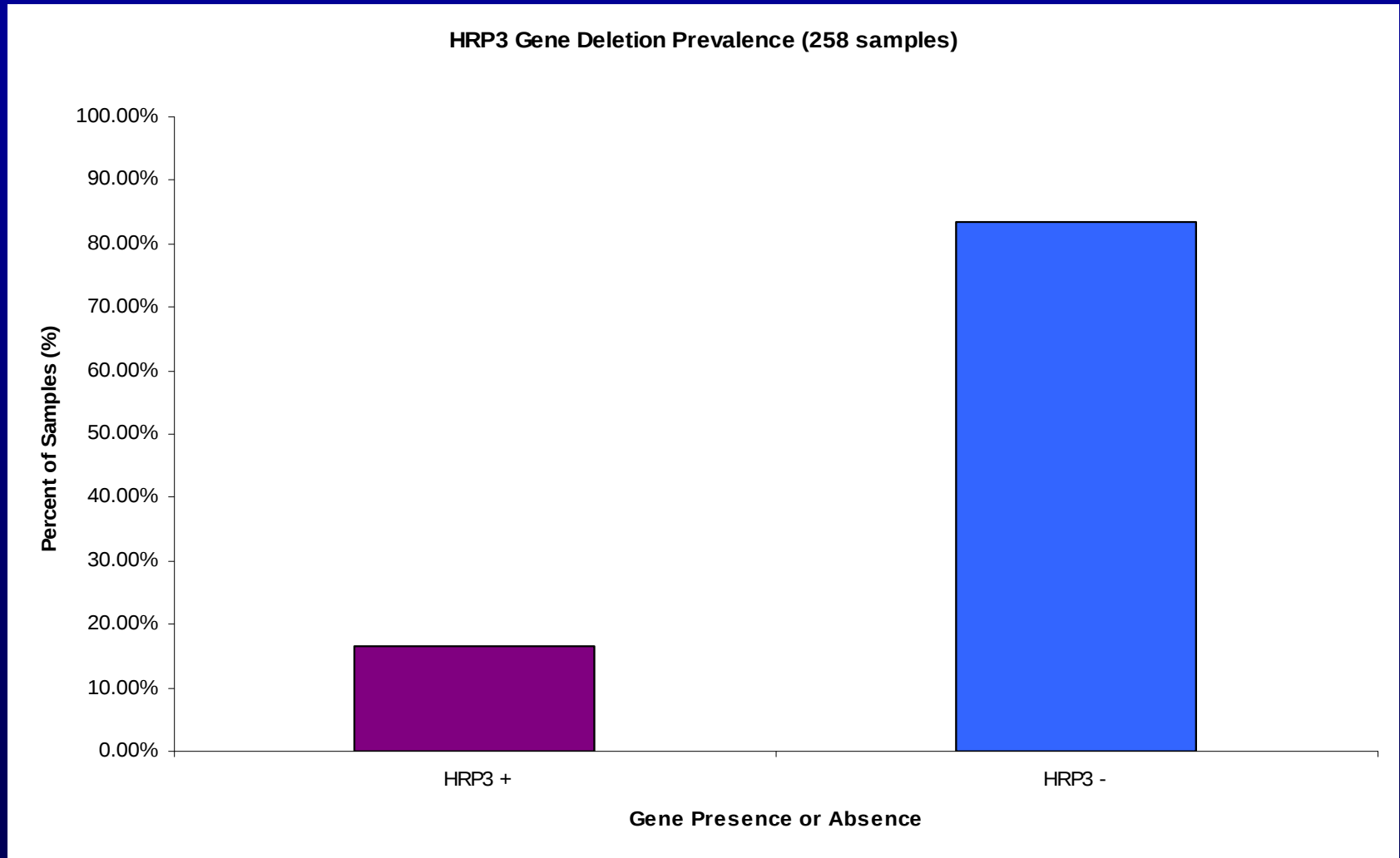


Sample
Collection
Sites

Peru HRP2 Gene Deletion Prevalence



Peru HRP3 Gene Deletion Prevalence



HRP2/3 Gene Deletion Studies

- Preliminary data shows 5-10% of parasites in Brazil lack HRP2 gene (Para and Rondonia)
- Colombian Amazon region has high level of deletion and other sites are being investigated
- Other countries?

HRP2/3 Gene Deletion Studies

- Suriname-Malti Adhin
- Guyana-Nicolas Ceron
- Bolivia-Arletta Anez
- Ecuador-Raul Veloz Perez

Acknowledgements

- Nancy Arrospide/INS
- David Bacon/US Navy Peru
- Leopoldo Villegas
- Ananias Escalante
- Trent Ruebush
- Wilmer M. Quezada
- Malti Adhin
- Mergiory Bracho
- Marinete Marin Povoá
- Giselle M. Rachid Viana
- Cesar Bedoya
- Betzabe Rodriguez
- Norma Padila
- Jaime Chang
- Keith Carter
- Alex Macedo de Oliveira
- Many others

Acknowledgements

Sean Griffing (NSF)

Tonya Mixson

Luke Syphard

Ira Goldman

Pauline Abdallah

John Barnwell

Andrea McCollum

Sankar Sridaran

Amanda Poe

Cecilia Nelson

Naomi Lucchi

Larry Slutsker

- USAID, AMI, RAVREDA, PAHO
- CDC Antimicrobial Resistance Working Group
- CDC ASM and EID Fellowships