Available tools for analyzing reemerging malaria in areas of unstable transmission

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What we want to learn?

Where did the parasite come from?

Is it a single or multiple independent introductions?

What is the genetic background of drug resistance?

Can we find any other information relevant for informing a public health response?

Integrating laboratory tools in epidemiologic investigations

Involve lab scientists to the investigative team

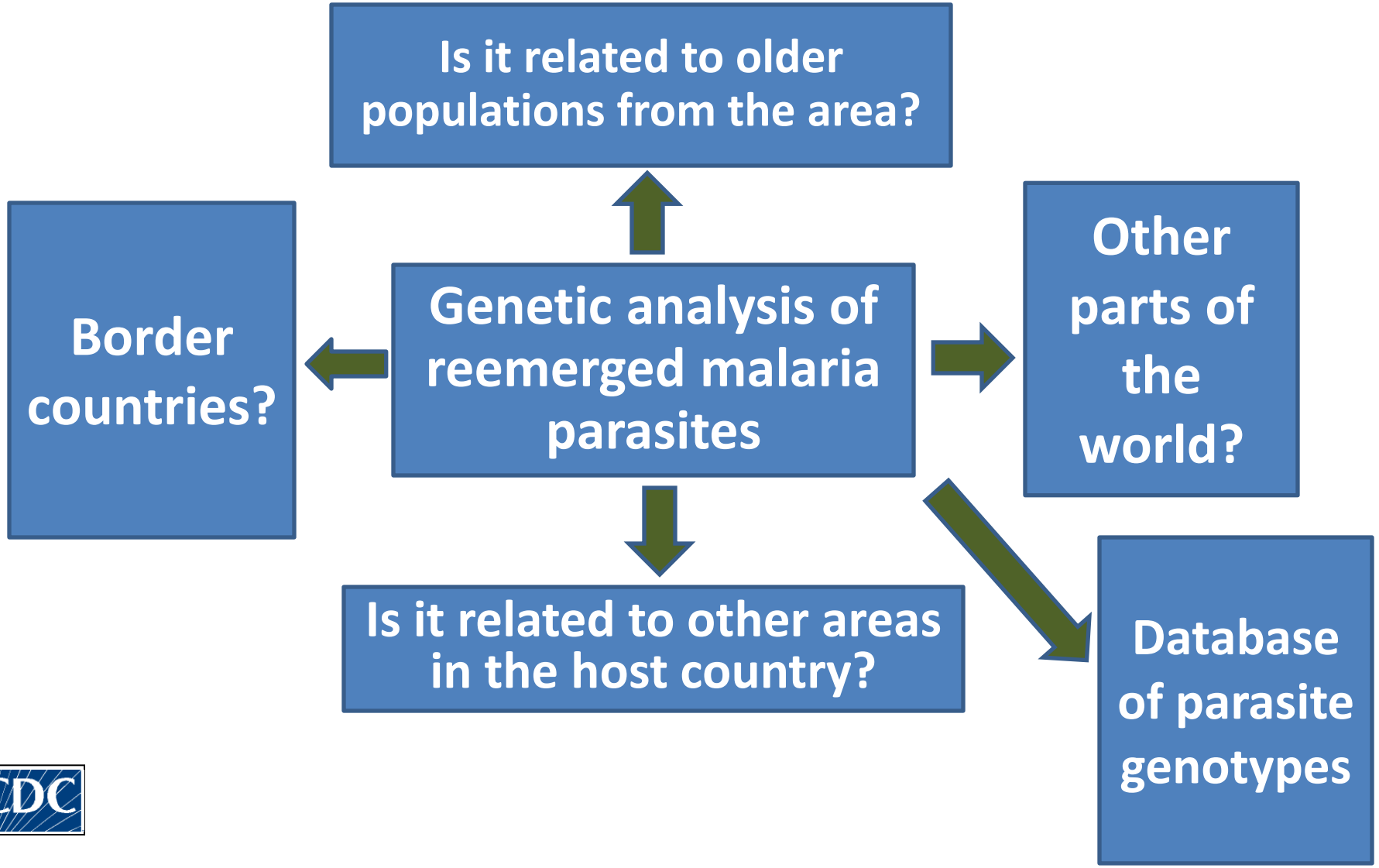
Importance of collecting good specimens for lab investigations from the beginning

Blood specimens for microscopy and storage of blood in filter paper or frozen blood (systematic approach)

Confirm the local microscopic diagnosis with national reference labs (Quality control)

Use molecular tools for confirming species identification and determining origins of parasite population

Molecular Tools



Concepts of using molecular tools

Neutral microsatellite markers

Related to which population?

Different clonal populations in Peru

Central America (CVMNK)

Drug resistance markers

Which region? Eg: pfcrt

South America (Amazon) (SVMNT)

South America (West/Coast) (CVMNT)

Other markers

Further refining of locations

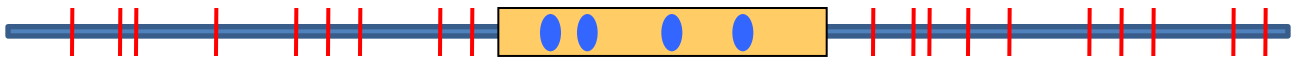
Africa/SE Asia (CVIET)

Use of molecular tools for investigation of resurgent malaria in Peru

- ***P. falciparum*** **was nearly eliminated for a decade on the Peruvian coast**
- **Reappeared in 2010-continues..**
- **Investigation and analysis**

Methods

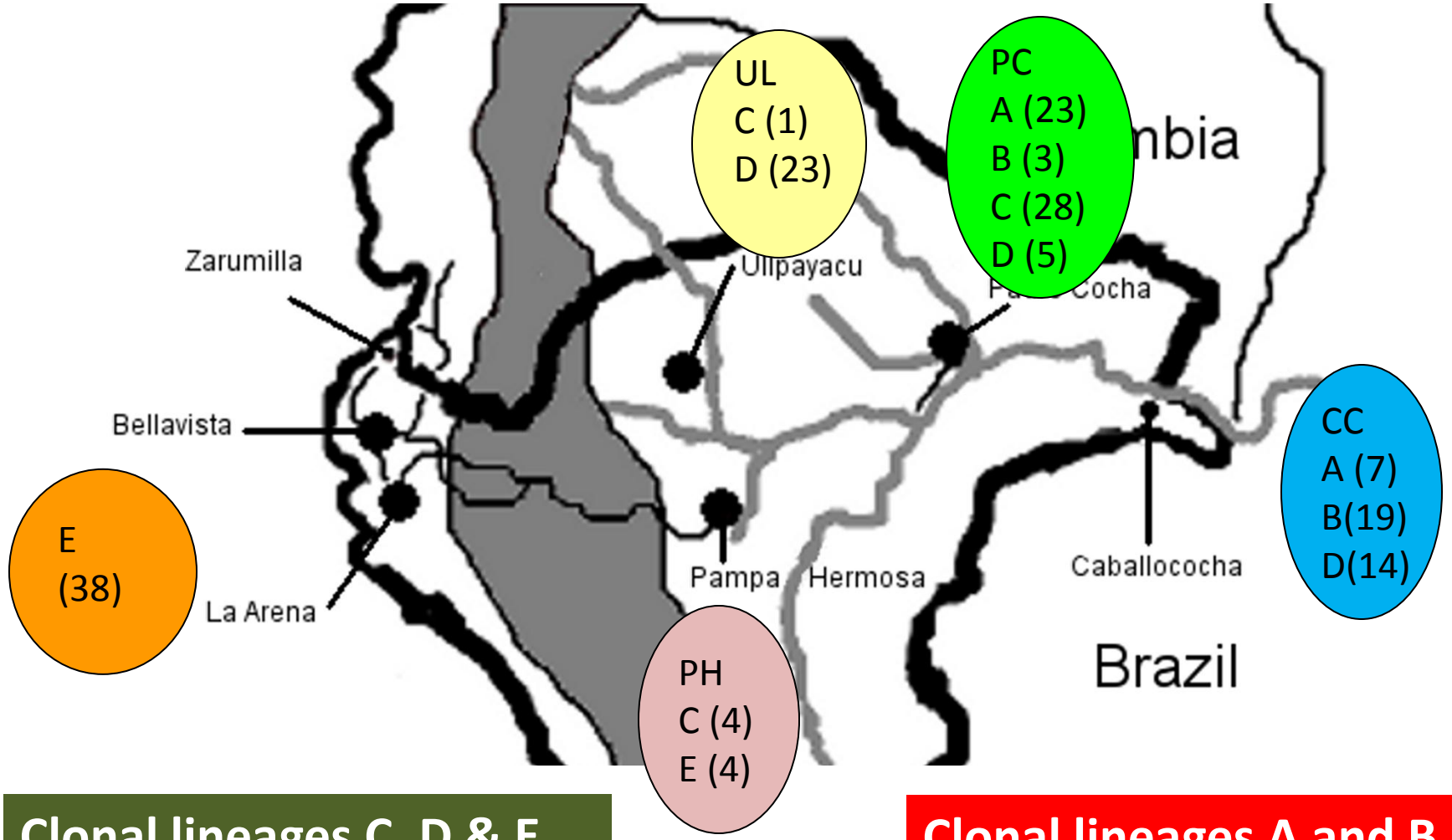
- 20 filter paper samples from INS, Peru
- DNA isolation and PCR for species
- Microsatellite markers (random repeats of 2-6 bp of nucleotides scattered in the genome)
- Eg: ATATATATATATAT



Methods (cont.)

- Chose 7 neutral markers for analyzing population structure-PCR and analysis in a sequencer
- Previous studies indicated clonal populations (eg: coast had single clonal lineage-E)
- Drug resistant markers (pfcrt, dhfr, dhps)-sequencing
- Previous studies have shown distinct drug resistant genotypes in Peru

Clonal lineages in Peru (1999-2000)



Clonal lineages C, D & E
CQ rest but SP sensitive

Clonal lineages A and B
CQ and SP resistant

CDC

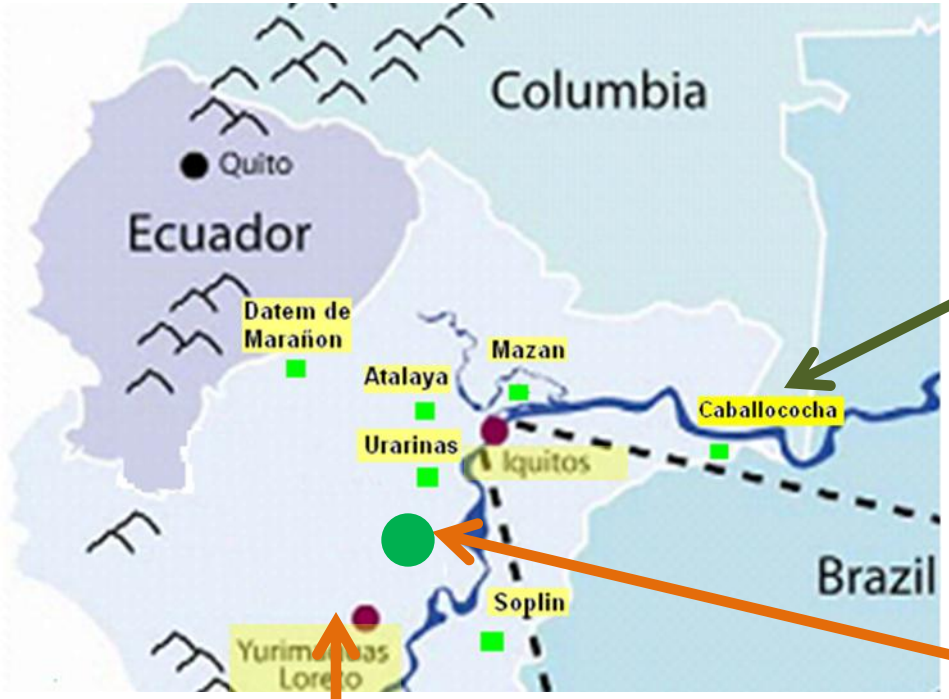
Griffing S et al., PLoS One, 2011

Microsatellites identified a single clonal population

- 18/18 samples showed an identical microsatellite marker pattern (clonal lineage called B/PR)
- 2 samples did not amplify

Ch6 TA1	Ch4 Polya	Ch12 PfPK2	Ch6 TA109	Ch10 2490	Ch2	Ch3
172	182	172	163	83	232	134

Peruvian Amazon has a similar clonal population (2009-2010)



Caballacocha

B/PR 1/1

Requena

B/PR 100% (12)

Yurimaguas

B/PR 1/12

CQ and SP resistant lineage

- **CQ resistant pfcrt genotype SVMNT**
- **Sulphadoxine resistant 437G,540E,581G**
- **Pyrimethamine resistant 50R,51I,108N**
- **This genotype is different from common genotype 51I,108N,164L found in Peru**
- **Documented the introduction of this new genotype in Peru in 2006 (Bacon et al 2006)**

Another surprise

- **All the isolates found in Requena had the HRP2 gene (RDTs detect HRP2) deletion**
- **We hypothesized reemergent population in the coast may have HRP2 deletion**
- **Testing showed deletion of HRP2 in all 18 isolates from Tumbes**

Conclusions

- **All the 18 samples (2 did not amplify) we tested have come from a single clonal lineage and likely a single introduction.**
- **These clonal populations are identical to a population found in at least 3 sites in Loretta (may be a source population)**
- **They are CQ and SP resistant and have deleted the HRP2 gene (AS+SP treatment may not be suitable and HRP2 rapid tests will not work).**

Conclusions (cont.)

- **Molecular tools are useful in the investigation of malaria reemergence**
- **Continued collection of molecular data especially in populations targeted for elimination will be crucial for future surveillance and investigation of reemergence**
- **AMI efforts to collect molecular data has helped to answer important questions**

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Peruvian Amazon has a similar clonal population