



# Artesunate plus Sulfadoxine- Pyrimethamine (SP) deployment and SP Quintuple Mutations - associated or not?

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- dhfr/dhps mutational results in over 4000 samples from 3 African areas with differing malaria endemicities:

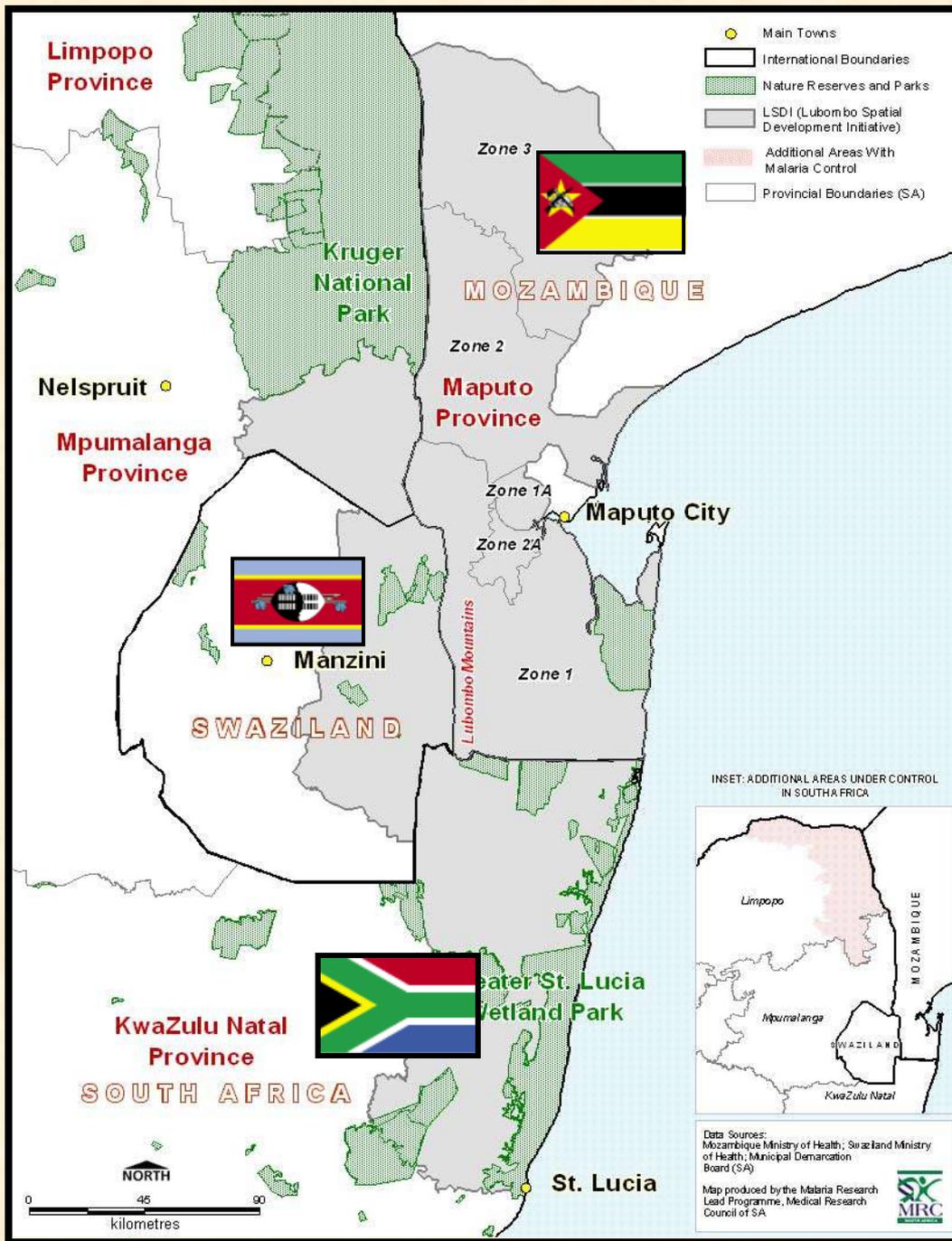
o-Maputo Province (Mozambique) - moderate intensity transmission (EIR = 32)

o-Bioko Island (Equatorial Guinea) - high intensity transmission (EIR = 281 infective bites for *An gambiae* and 787 for *An funestus*) but with an Island effect.

o-Mpumalanga Province (South Africa) - low intensity transmission (EIR <1)

-where artesunate plus SP has replaced either SP or Chloroquine as the first line treatment

-assess effect of artesunate plus SP deployment on SP quintuple mutation prevalence/frequency



-Lubombo Spatial Development Initiative (LSDI) was established in 1999

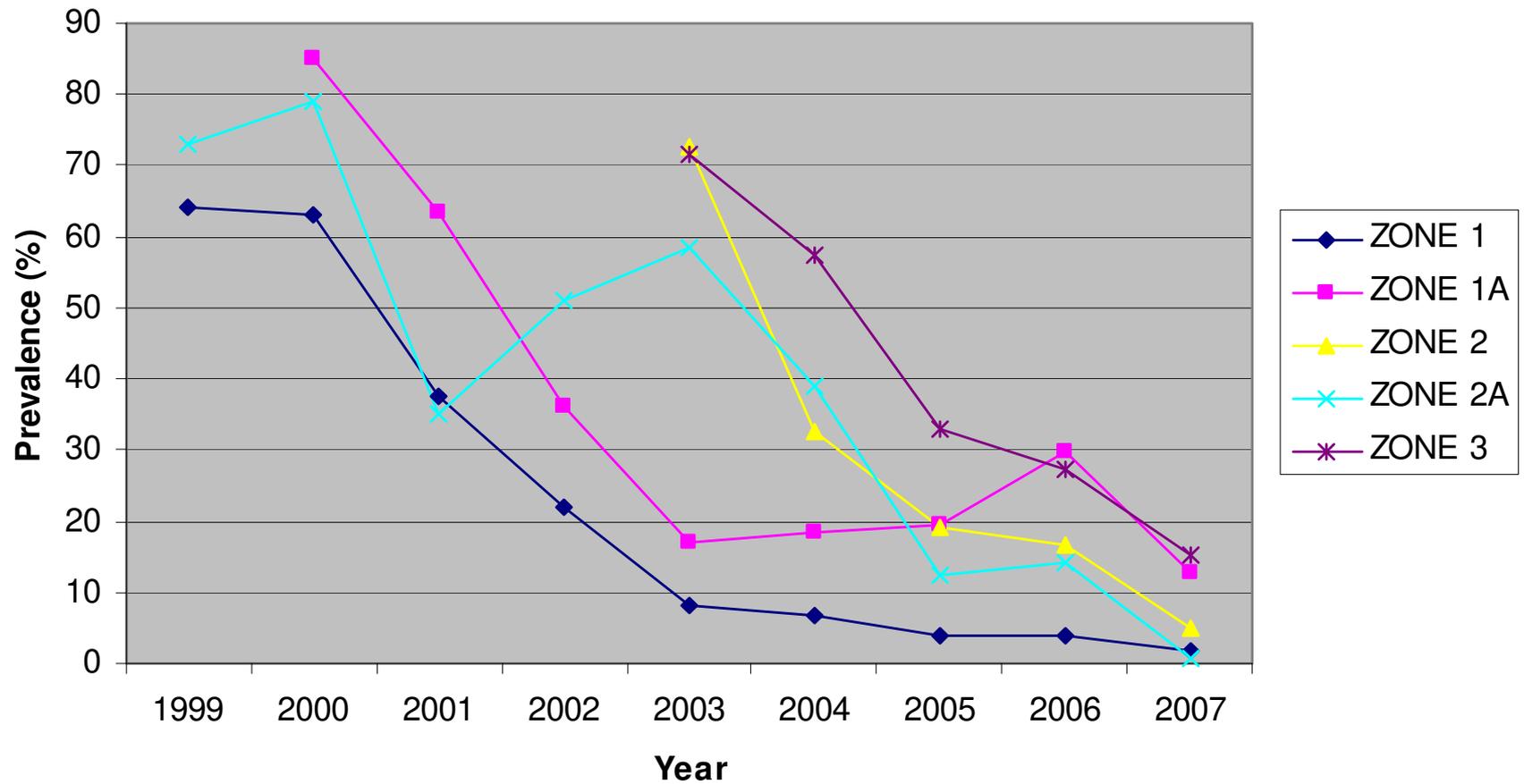
-a cross border collaboration

-develop the Lubombo region into an economically viable region:

- infrastructure development
- malaria control



## Malaria prevalence in children aged between 2 & 15 years in Maputo Province Mozambique, (1999-2007)



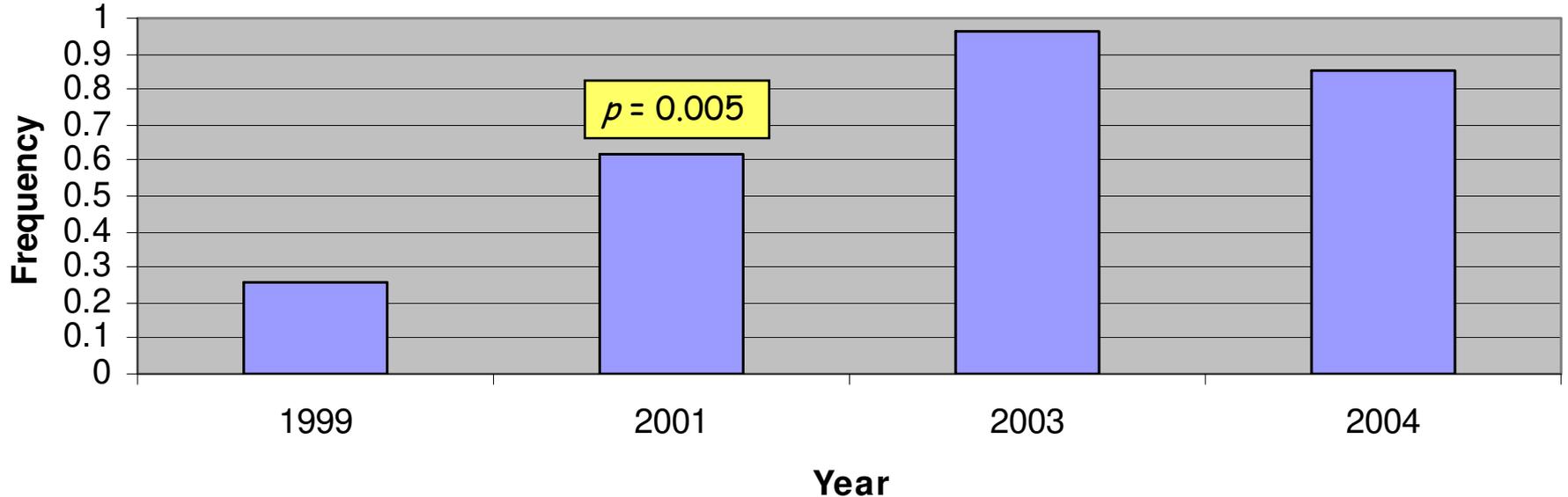


-Samples (finger prick blood spots blotted onto 3M Whatman filter paper strips) were collected as part of annual parasite prevalence surveys from 1999 to 2004

-2 175 samples were rapid test positive (35%), of which 1 215 were subjected to mutational analysis.

-Codons 51, 59, 108, 164 of the *dhfr* gene and 436, 437, 540 and 581 of the *dhps* gene were assessed for presence of mutant alleles using PCR and endonuclease restriction cleavage

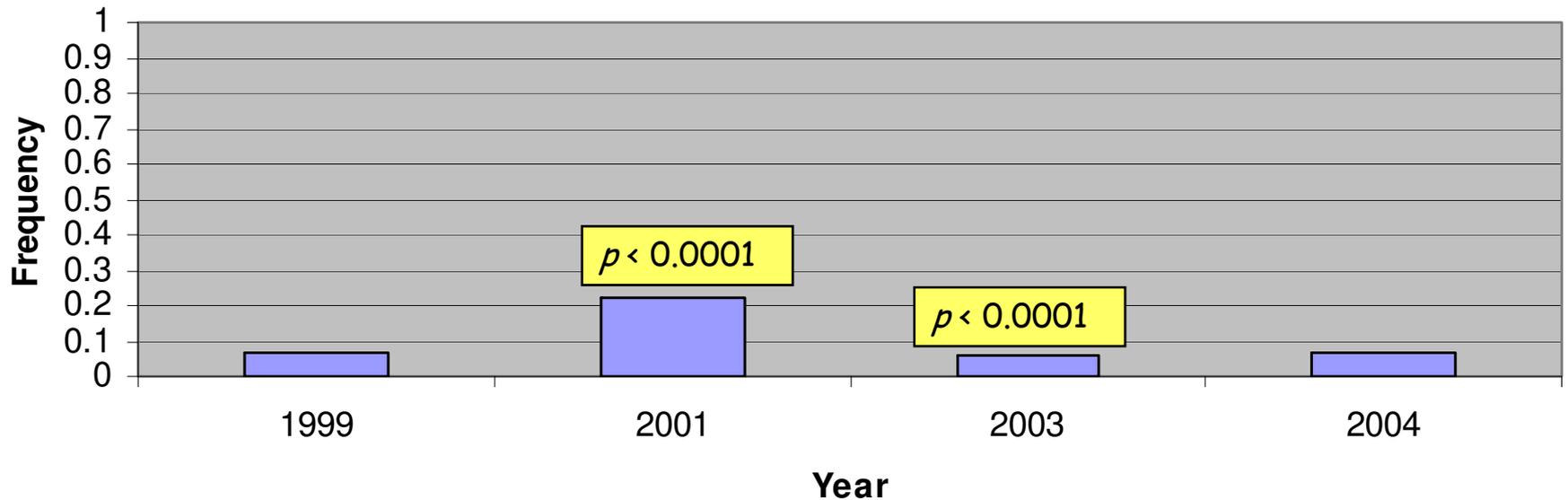
### *dhfr* triple mutation frequency in Maputo Province, Mozambique (1999-2004)



-in 1999 in neighbouring KwaZulu-Natal - *dhfr* triple mutation frequency was:

- 0.38 in population
- 0.62 in patients

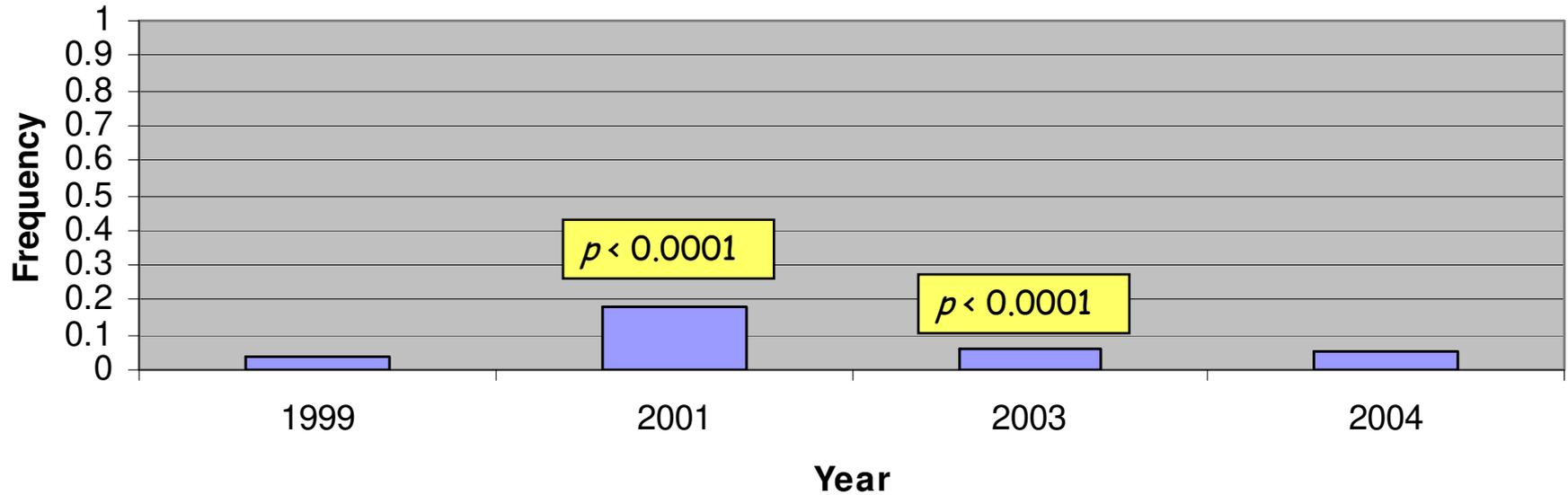
## *dhps* double mutation frequency in Maputo Province, Mozambique (1999-2004)



-in 1999 in neighbouring KwaZulu-Natal - *dhps* double mutation frequency was:

- 0.15 in population
- 0.47 in patients

## Quintuple mutation frequency in Maputo Province (1999-2004)



-mirrored the *dhps* double frequency trend

## CONCLUSIONS

--mutation frequency increased by 2001 - most likely a result of increased SP drug pressure associated with the SP resistant malaria epidemic in neighbouring KwaZulu-Natal

--from 2001 to 2004 there was a decrease in the frequency of *dhps* double and quintuple mutations to baseline levels; this is most likely associated with decrease in SP pressure as KwaZulu-Natal replaced SP with artemether-lumefantrine in 2001

--high frequency of the *dhfr* triple mutation, may imply limited therapeutic life of SP

--this study highlights the regional impact of the drug policy in neighbouring countries

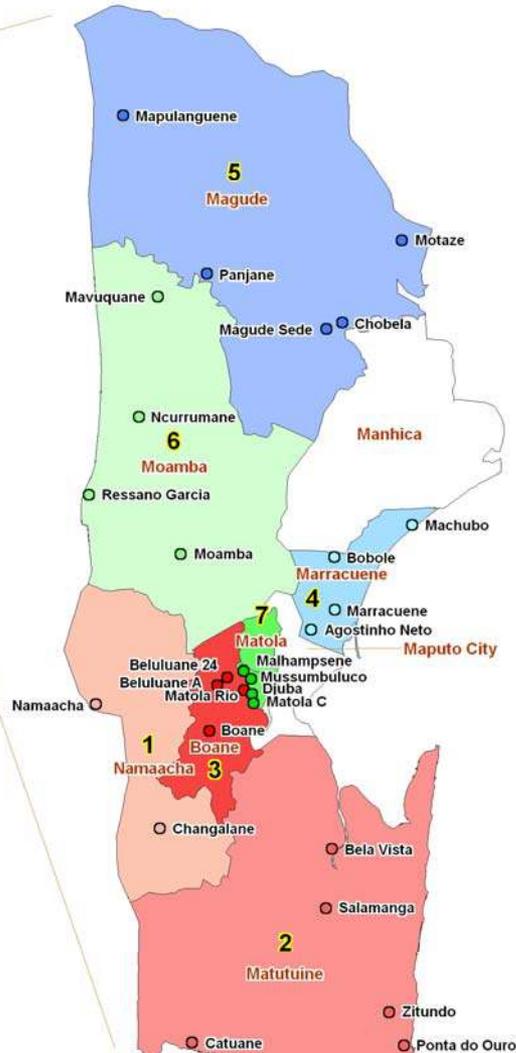


### Sentinel Sites By Date of Implementation of ACT's

#### Maputo Province, Mozambique Lubombo Spatial Development Initiative



0 20 40  
kilometers



#### Legend

Districts

#### Date of Implementation

- April 2004
- July 2004
- June 2005
- March 2006
- May 2006
- November 2004
- October 2005

Apr 2004 → May 2006



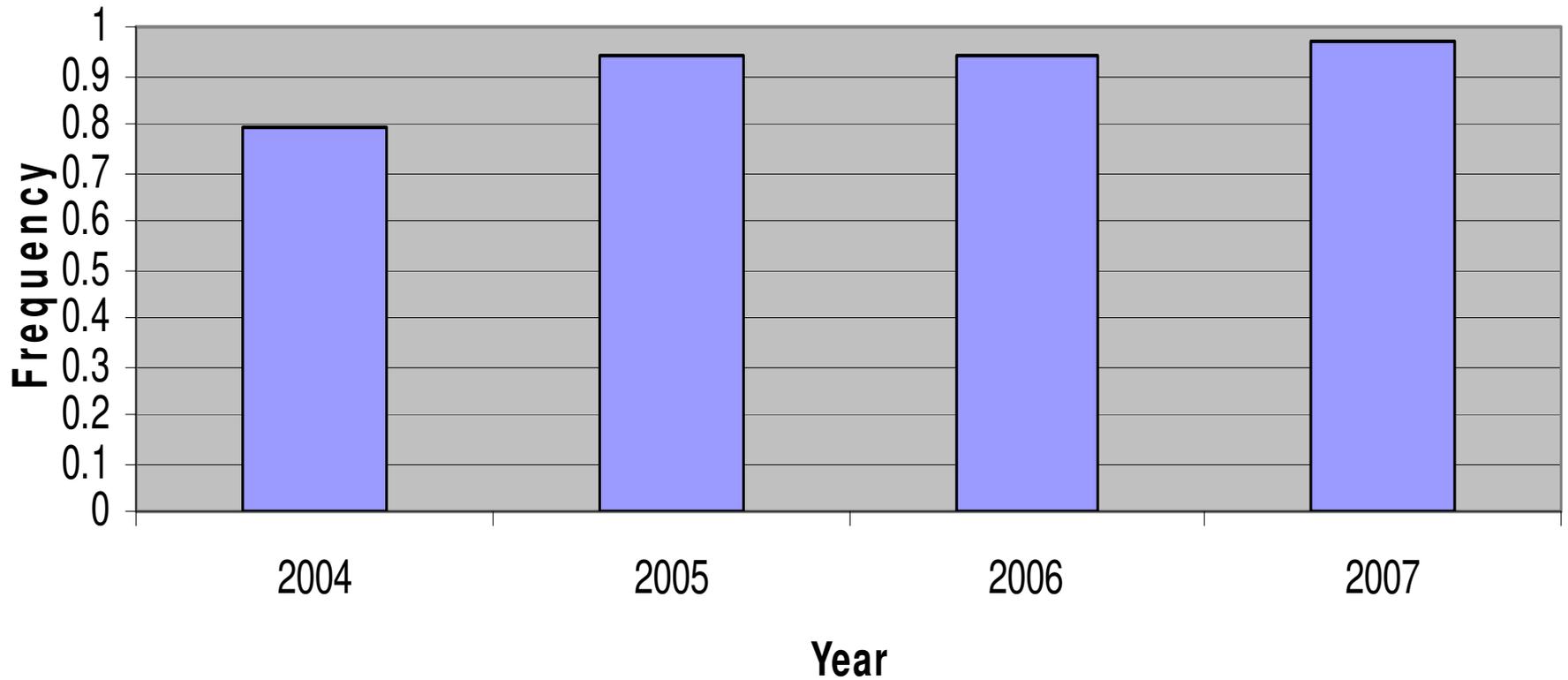
-In 2004 phased implementation of the ACT, artesunate plus SP, commenced

-*in-vivo* studies (2003 - 2005) showed artesunate plus SP to be highly effective with cure rates of 98% at 42 days

-For the period 2004-2007, 2 223 (18%) samples were rapid test positive

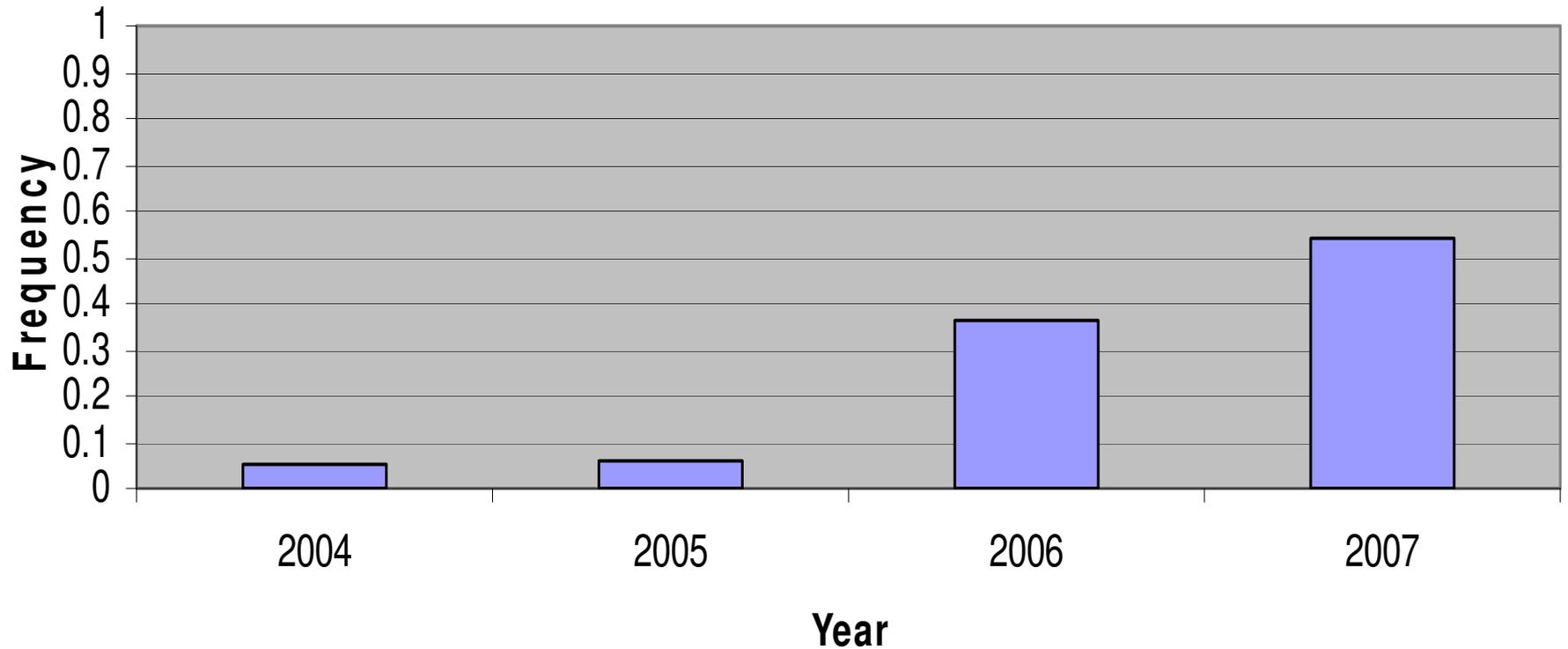
-DNA was extracted from 2 199 samples

***dhfr* triple frequency in Maputo Province, Mozambique  
(2004-2007)**



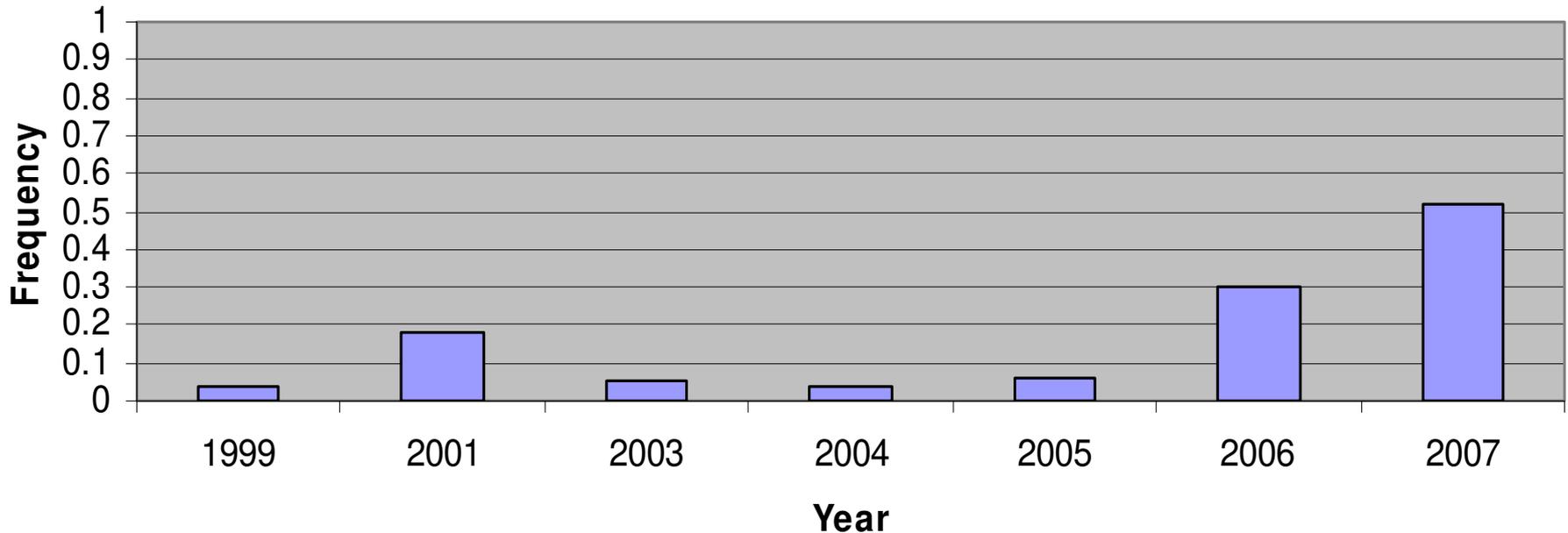
*dhfr* triple frequency remained close to fixation throughout the study period

## *dhps* double frequency in Maputo Province (2004-2007)



- significant increases in *dhps* double frequency ( $p < 0.0001$ )

## Quintuple Mutation Frequency in Maputo Province, Mozambique (1999-2007)



- significant increases in quintuple mutation frequency ( $p < 0.0001$ )
- positive association between quintuple mutation frequency and months since artesunate plus SP deployment (OR: 1.12; 95% CI: 1.09-1.15;  $p < 0.001$ )
- negative association between asexual parasite prevalence and quintuple mutation frequency (OR: 0.98; 95% CI: 0.97-1.00;  $p = 0.008$ )

# CONCLUSIONS

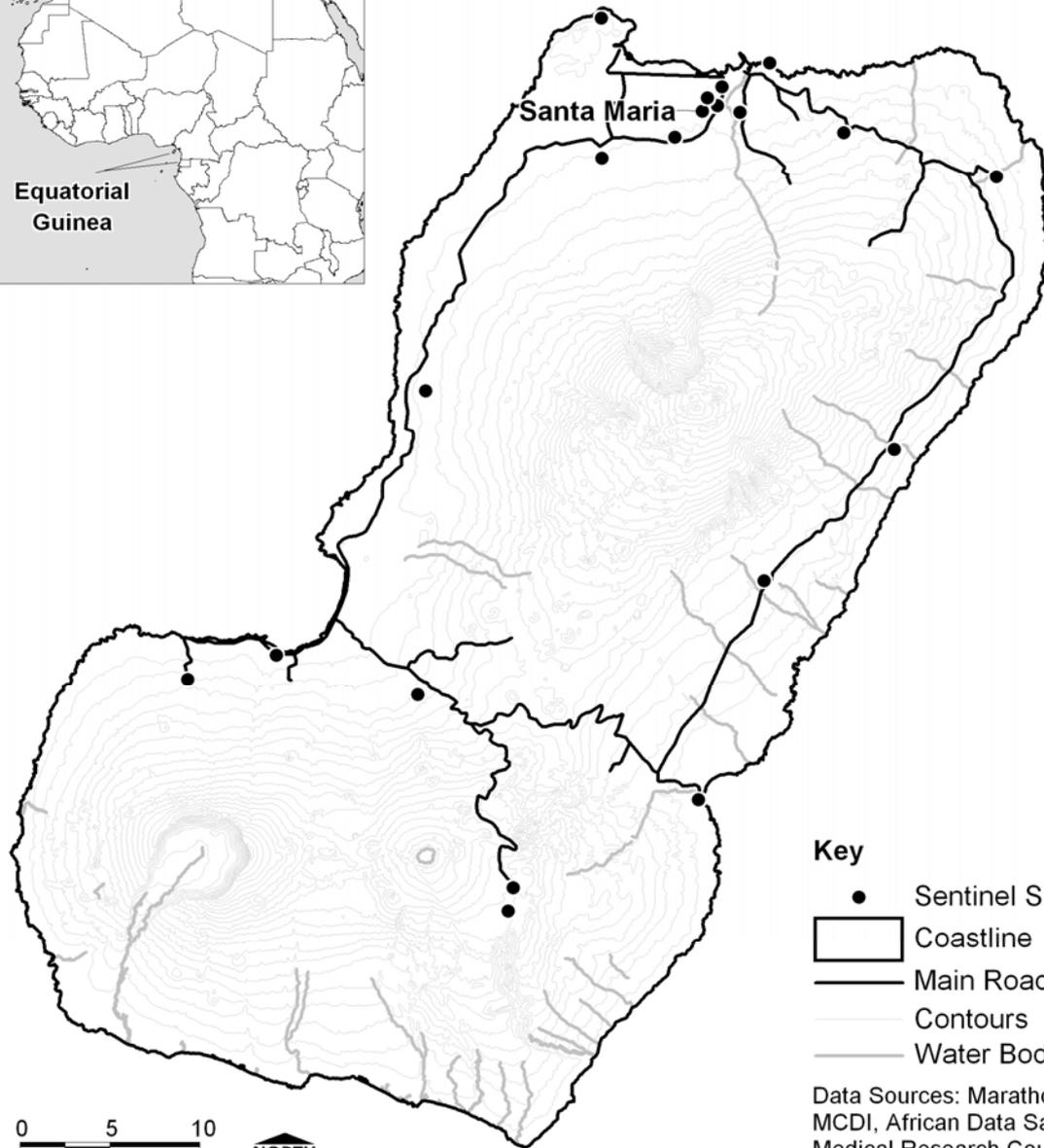
--artesunate plus SP selecting for or at least not limiting spread of SP resistant parasites.

--contribution of pre-existing fixation of *dhfr* triple mutation and SP monotherapy for ITPp unclear

--microsatellite analyses have shown that *dhfr* and *dhps* lineages are shared between Tanzania and South Africa (*Roper et al., Lancet, 2003*)

--this study supports the recent policy decision to replace artesunate plus SP with artemether plus lumefantrine, despite recent high artesunate plus SP cure rates.



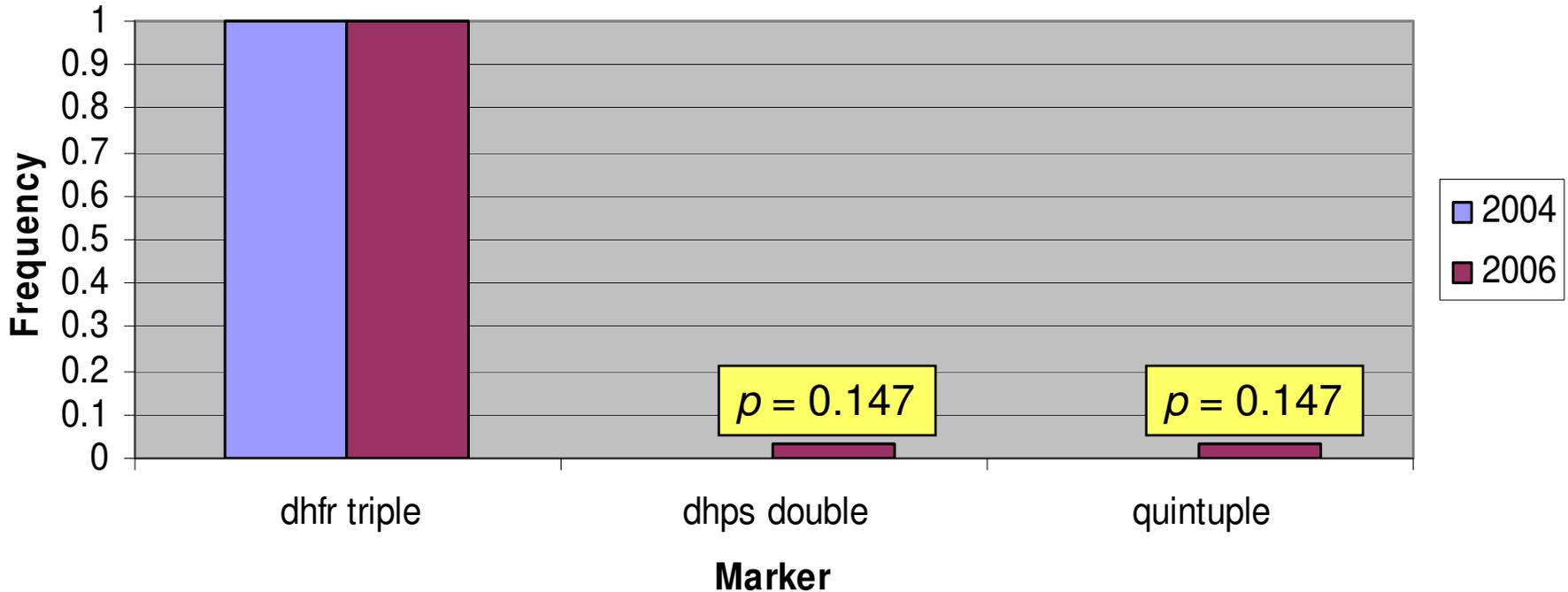


- Key
- Sentinel Sites
  - Coastline
  - Main Roads
  - Contours
  - Water Bodies

Data Sources: Marathon Oil, MCDI, African Data Sampler, Medical Research Council SA

- Malaria control programme implemented in 2004
- Indoor residual spraying
- Artesunate plus SP replacing CQ, as first line treatment in 2005
- SP monotherapy for IPTp also introduced in 2005
- DNA extracted from 153 samples in 2004 and 126 samples in 2006

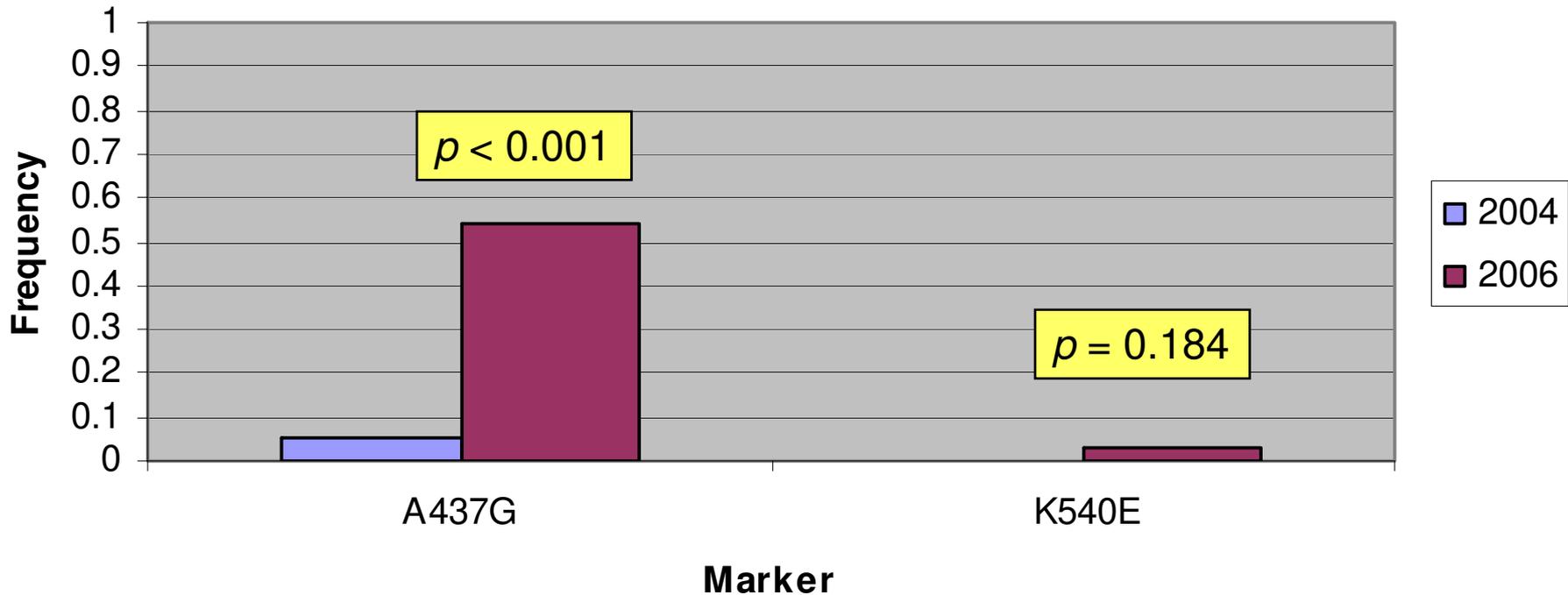
## SP mutational marker frequency on Bioko Island (2004, 2007)



-*dhfr* triple remained at fixation

-both the *dhps* and quintuple mutations increased in frequency, it was not statistically significant.

## Frequency of the A437G and K540E mutations on Bioko Island, Equatorial Guinea (2004, 2006)



- increase in frequency of 437 mutation
- high frequency of infections with both wild and mutant genotypes at both these codons

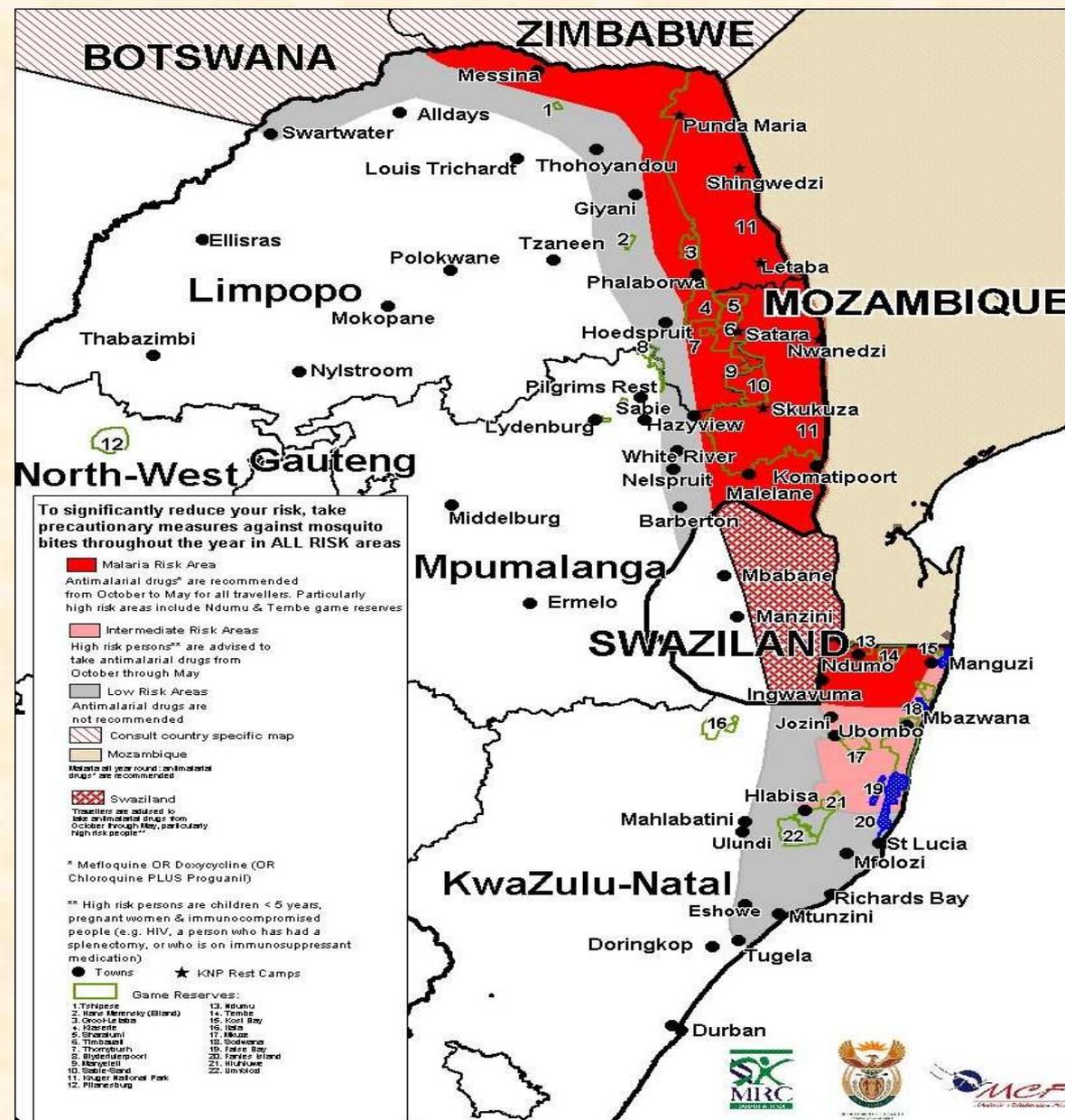
## CONCLUSIONS

--the emergence of SP resistant alleles not halted following artesunate plus SP deployment

--limited therapeutic life span of artesunate plus SP

--results support decision to change policy to artesunate plus amodiaquine.

--significant decrease in K76T mutation frequency ( $p < 0.0008$ )



-Low transmission intensity

-1998 SP replaced chloroquine as first line treatment

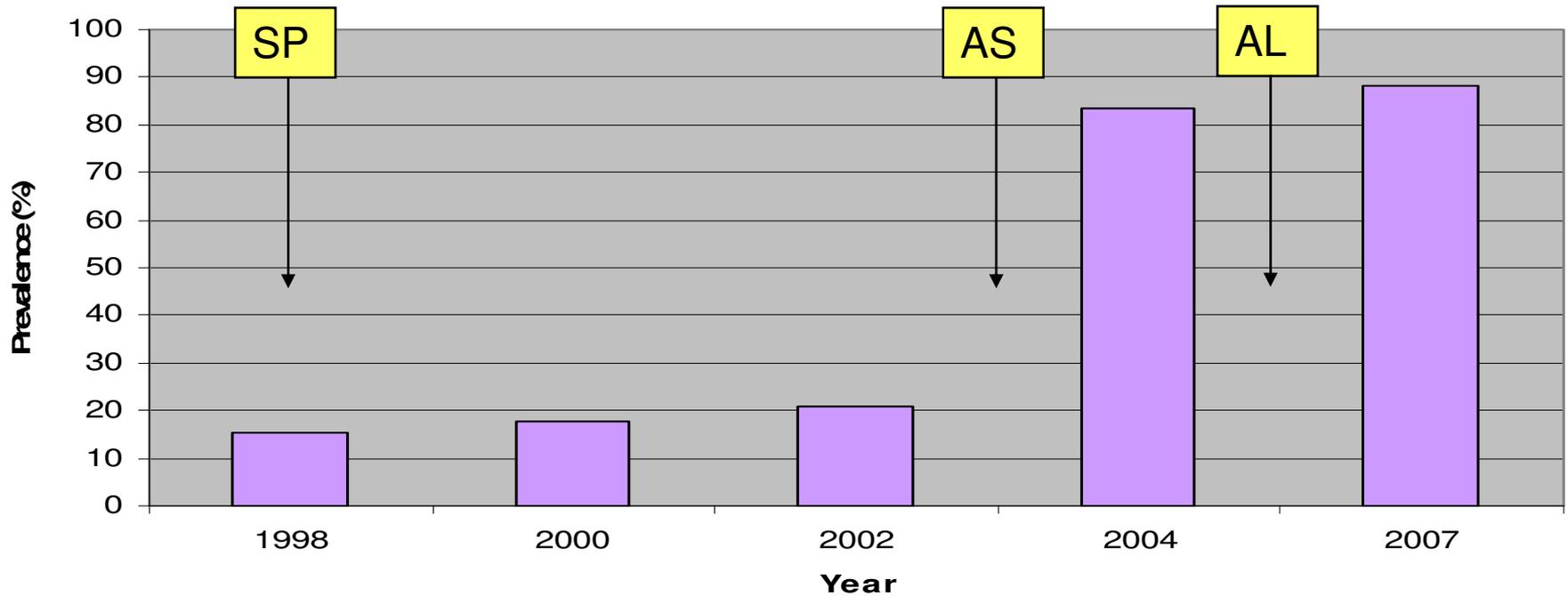
-2003 artesunate plus SP replaced SP

-2006 artemether plus lumefantrine became treatment policy.

-present results from *in-vivo* trials conducted in 1998, 2000, 2002, 2004 and 2007

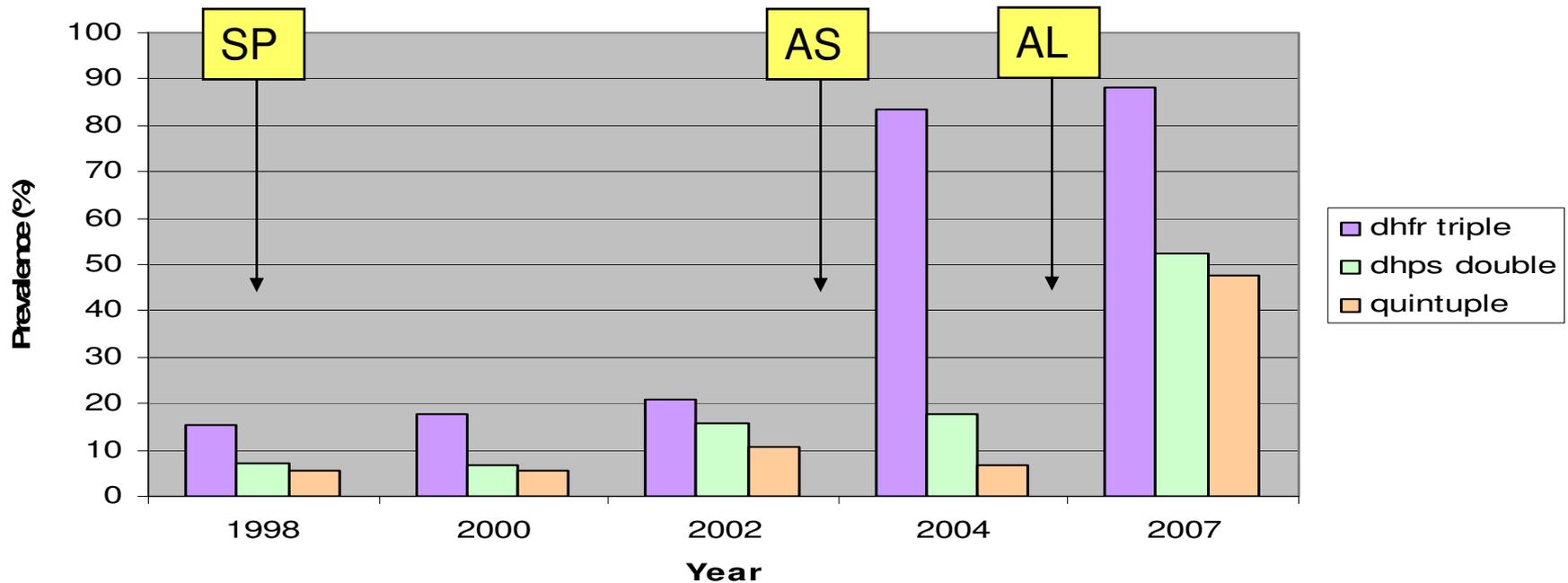
-Samples from 464 patients over 10 years were analysed

**Prevalence of the *dhfr* triple mutation in Mpumalanga, South Africa (1998-2007).**



- marked increase in *dhfr* triple by 2004 ( $p < 0.001$ )
- continued to increase almost reaching fixation by 2007

### Prevalence of mutations associated with SP resistance in Mpumalanga, South Africa (1998-2007)



-by 2007 the increase in both the *dhps* double and quintuple mutations had increased significantly ( $p < 0.0001$  for both)

-artesunate plus SP contributing to or at least not slowing the spread of SP resistant parasites

-majority of malaria cases in Mpumalanga are imported (mostly from Mozambique), so resistance may again reflect regional influence.

## CONCLUDING REMARKS

--Results presented here show that artesunate plus SP deployment has not slowed the spread of SP resistant parasites, and may have contributed to the observed mutation frequency increase.

-- other contributing factors include

- SP drug pressure (Artesunate + SP, SP IPTp )

- short elimination half-life of artemisinin vs. long elimination half-life of SP

- impact of drug pressure exerted in neighbouring countries

--highlights importance of regular drug resistance surveillance especially when ACTs are deployed against a background of resistance to the partner drug.

## ACKNOWLEDGEMENTS

- FUNDERS: Global Fund to fight AIDS, TB and malaria, South African Department of Health, Business Trust of South Africa, Mozambican Ministry of Health, Marathon Oil, Equatorial Guinea Ministry of Health, Medical Research Council of South Africa
- All the staff involved in samples collection during the surveys
- Val Kelly for assistance with the lab analyses
- Natashia Morris for GIS support and producing all the maps used in this presentation
- Database staff for all their assistance with database management