

Amplification of DNA Polymerase I gene of *T. pallidum* from whole blood of persons with syphilis

DSTDP/NCHSTP
DASTLR/NCID
Maricopa County STD Clinic

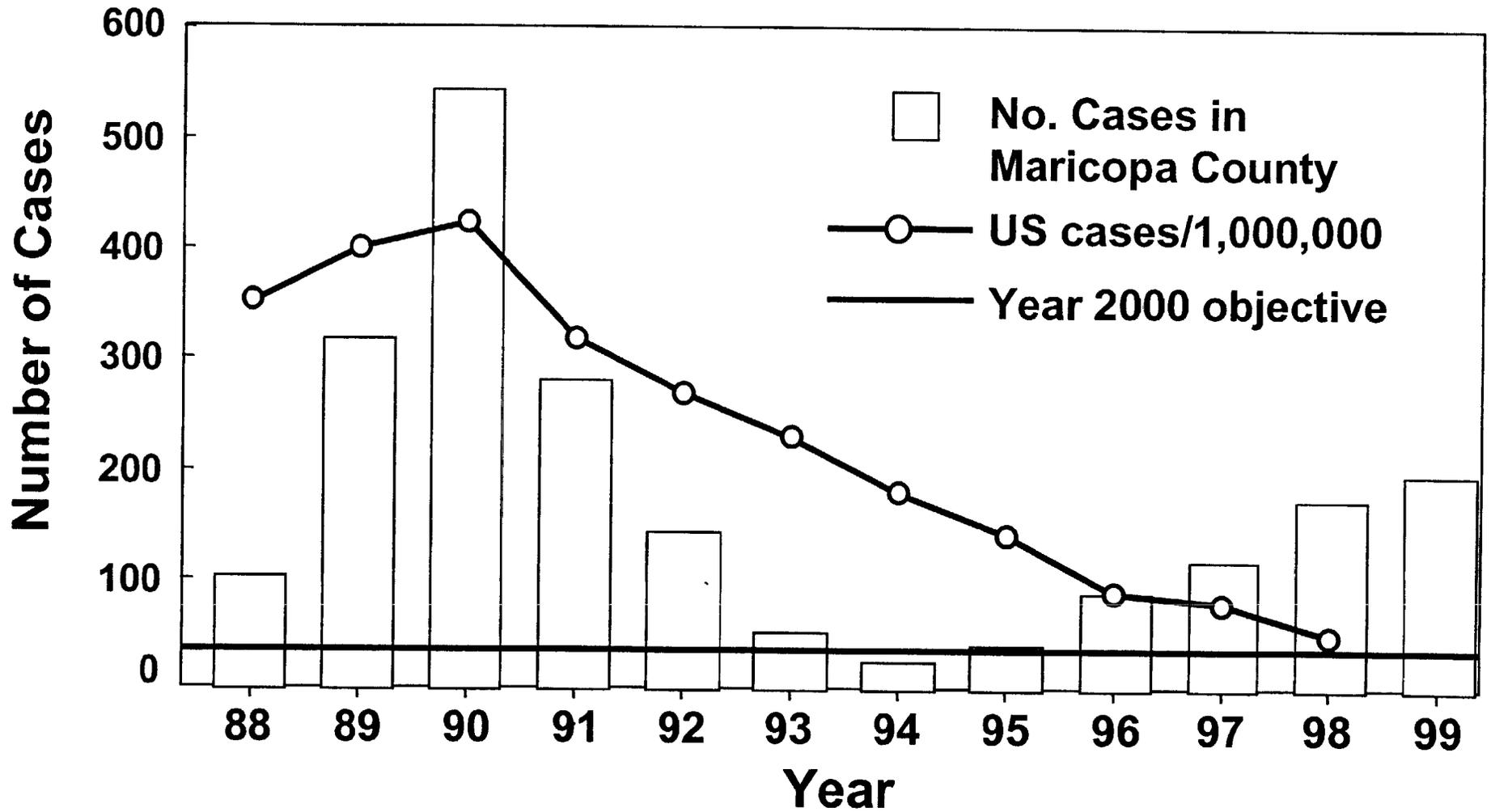


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Data obtained during a study of

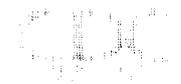
Molecular subtyping of *Treponema pallidum* during an
outbreak investigation of syphilis in
Maricopa County, Arizona
1997-1999

Syphilis Cases in Maricopa County and US, 1988-1999



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Methods

Study population

- Persons attending Maricopa County STD clinic
- Signs or symptoms of syphilis, or
- Sex partner with syphilis

Methods - Case Definitions

Incubating - no signs or symptoms; significant sexual exposure to infectious syphilis; nonreactive RPR and MHA-TP

Primary - genital ulcer with positive dark field

Secondary - rash and/or lymphadenopathy with reactive RPR and MHA-TP

Latent - no signs or symptoms with reactive serology

Methods

Data Collection

- Review of medical records at STD clinic
- RPR and MHA-TP reactivity abstracted

Specimen Collection

- 5-10 ml blood collected in EDTA tubes
- stored at 4°C
- Shipped to CDC for analysis

Laboratory Methods

- Samples screened by PCR to amplify DNA polymerase I gene (*polA*)
- Analyzed by agarose gel electrophoresis
- The validity of *polA* PCR was reconfirmed with two additional targets (*arp*, *tpr*)

Use of *PolA* as Diagnostic PCR for *T. Pallidum*

- House keeping gene - highly conserved
- Primers selected based on 2 unique features
 - Four additional inserts in sequence
 - High in cystein content

Additional Targets used for Molecular Typing

- Acidic repeat protein (arp) gene
 - multiple repeats
 - can be used to distinguish among clinical strains
- Tpr gene
 - multiple gene family
 - can be used to distinguish among clinical strains

RESULTS

PCR attempted on 32 blood specimens



13 (41%) *pol A* positive



7 (22%) positive by at least one additional target (*arp*, *tpr*)

PCR Results in Whole Blood, by Syphilis Disease Stage

Stage	No.	<i>po1A</i> +	<u>≥</u> 2 targets +
incubating	8	4	2
primary	7	1	1
secondary	1	1	1
latent	12	7	3
non-syphilis ulcer	4	0	0

Conclusions

- *T. pallidum* DNA amplified from whole blood samples from persons known to have untreated syphilis or exposure to syphilis
- The viability of *T. pallidum* that yielded amplified DNA is unknown
- Data suggest that potentially infectious spirochetes were present in the blood during incubating, primary, secondary and latent stages

Possible Reasons for Differences between CDC and ARC Study

- Differences in populations: Patients with untreated early syphilis seen at STD clinics vs patients with late latent or treated syphilis
- Differences in blood component: Treponemes may not be present in platelets but are present in other components

PCR Positivity in Sera of Patients in Different Stages of Syphilis

Microbiologica 1999;22