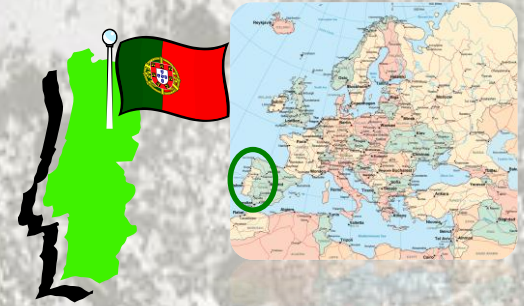


nucleus



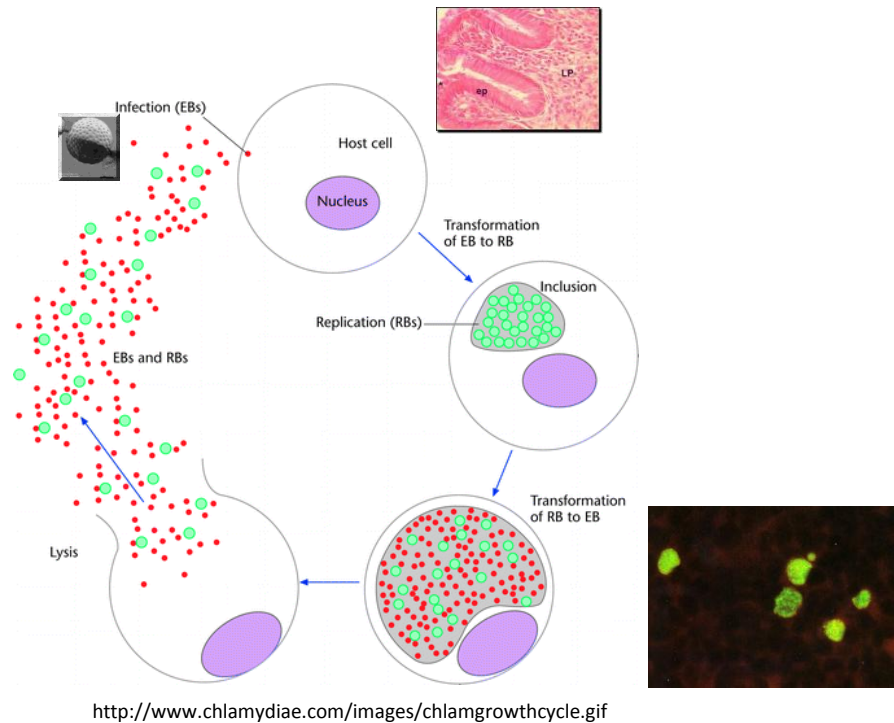
LYMPHOGRANULOMA VENEREUM RECENT OUTBREAK IN EUROPE: THE PORTUGUESE EXPERIENCE

MARIA JOSÉ BORREGO

JURMALA, 26 MAY 2011

www.chlamydiae.com
Michael Ward

Chlamydiae are characterized by their unique life cycle...



and based on the Major Outer Membrane Protein (MOMP)
(major chlamydial antigen)

15 serovars were defined for *Chlamydia trachomatis* (A to L3)

Later on,
Molecular biology studies with the MOMP coding gene, *ompA*,
evidenced several new variants of the 'classical' 15 *ompA*-genotypes ...

C. trachomatis serovars / *ompA*-genotypes are responsible for:

- **Trachoma** (world leading cause of preventable blindness)
(A, B, Ba, C)

- **Sexually Transmitted Infections** (major bacterial etiology)
(D, E, F, G, H, I, J, K)

- **LGV**
(L1, L2, L3)

Considering *C. trachomatis* STI (serovars D-K) ...



Many *C. trachomatis* epidemiological and clinical studies were developed

(defining populations at risk)

(describing cost-effectiveness - early diagnose / *versus* / sequelae development)

Fully automated diagnostic platforms were developed

(based on NAATS)

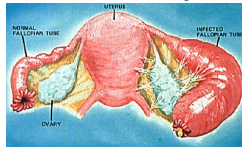
(non-invasive, self-collected biological samples)



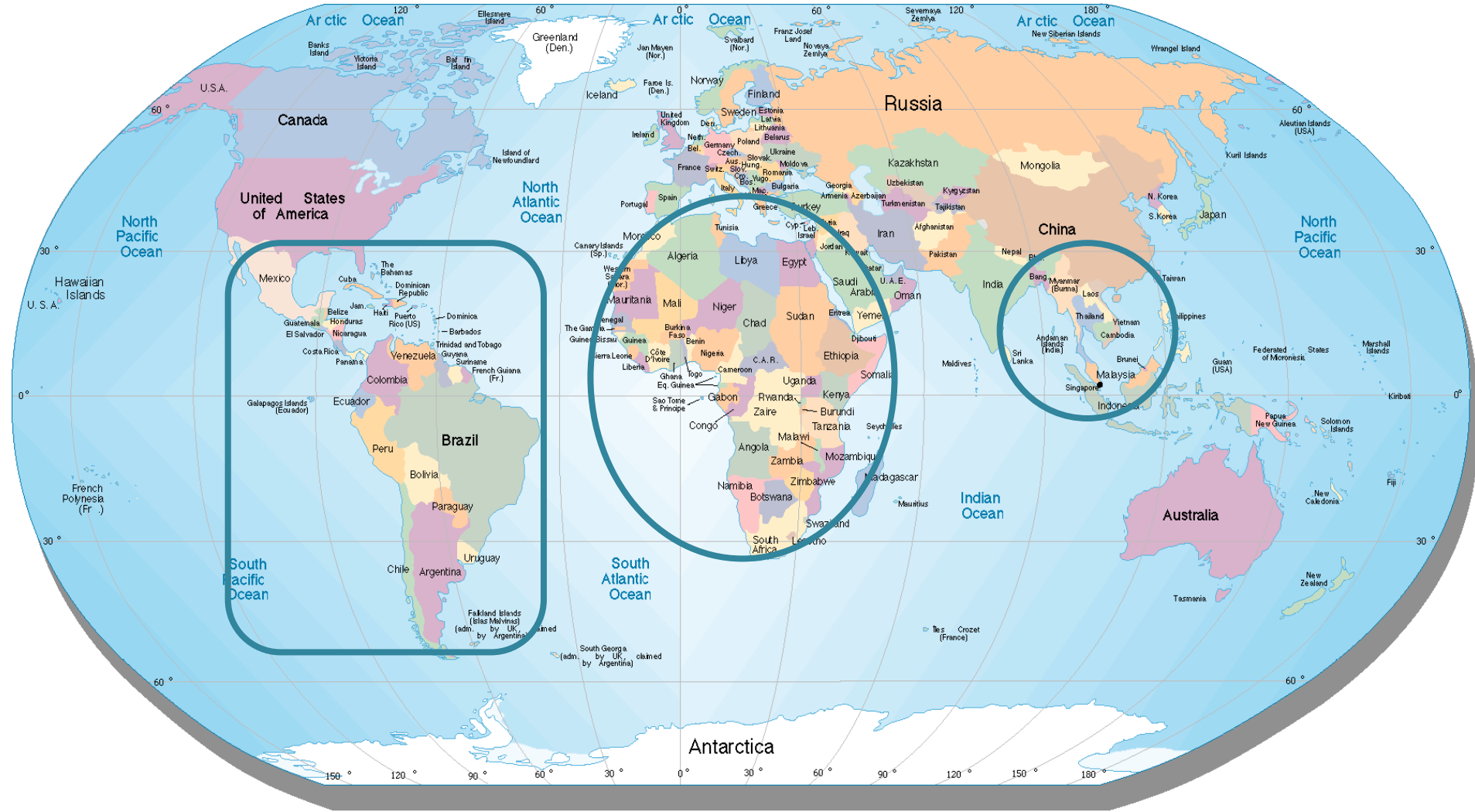
***C. trachomatis* surveillance schemes were implemented**

(target – populations more at risk)

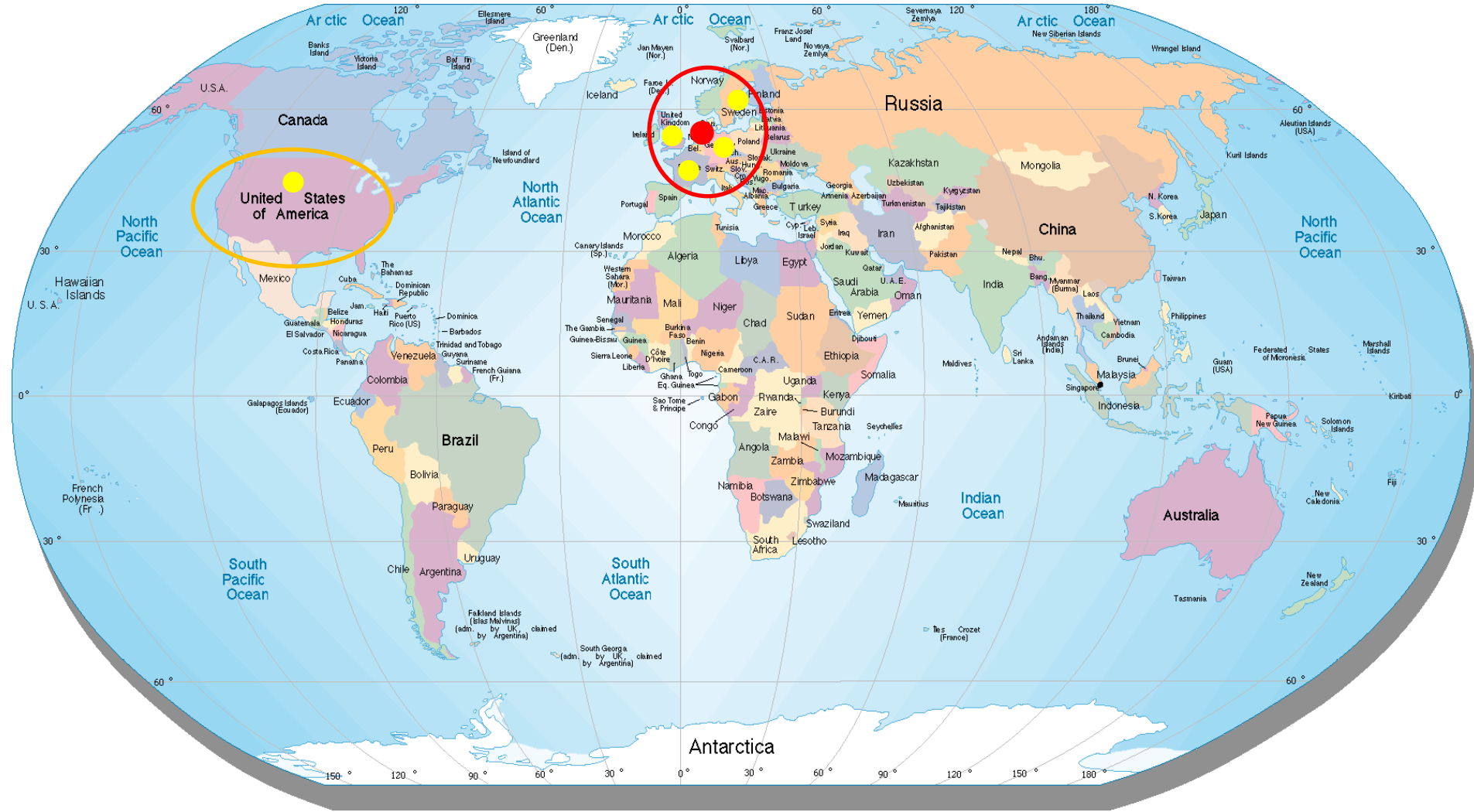
(objective – prevent sequelae // save money)



And what about LGV ?



and then...





In Portugal there is ...

↪ No surveillance system for chlamydial infections

↪ No knowledge on how many labs are detecting *C. trachomatis*

↪ No knowledge on what methods are being used

10 (or less) laboratories perform NAATs for *C. trachomatis* in the whole country



In contrast with some other countries...

No surveillance measures were launched as a consequence of the 2004 LGV outbreak in Europe

However...



All *C. trachomatis* specimens that are sent to the Portuguese NIH



ompA genotyped



Opportunistic detection of Portuguese circulating LGV strains

The Portuguese NIH acts as a research, reference and routine diagnosis laboratory

NIH customers

mostly asymptomatic
sexual orientation and habits unknown
routine laboratory exams

Prescription by
general practice, gynecology,
family planning clinics

Symptomatic or Asymptomatic
Complete clinical data
demographical data
sexual orientation and habits
routine STI laboratory exams

Prescription by
major Portuguese STI clinic
(Lapa Health Centre, Lisbon)

(MSM reporting unprotected anal intercourse have a rectal swab for evaluation of LGV-genotype carriage)

2007 - 2010

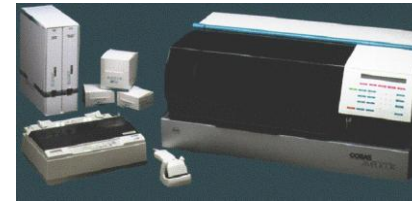
9000 samples were tested for *C. trachomatis*

[Cobas-Amplicor (2007-2009)]

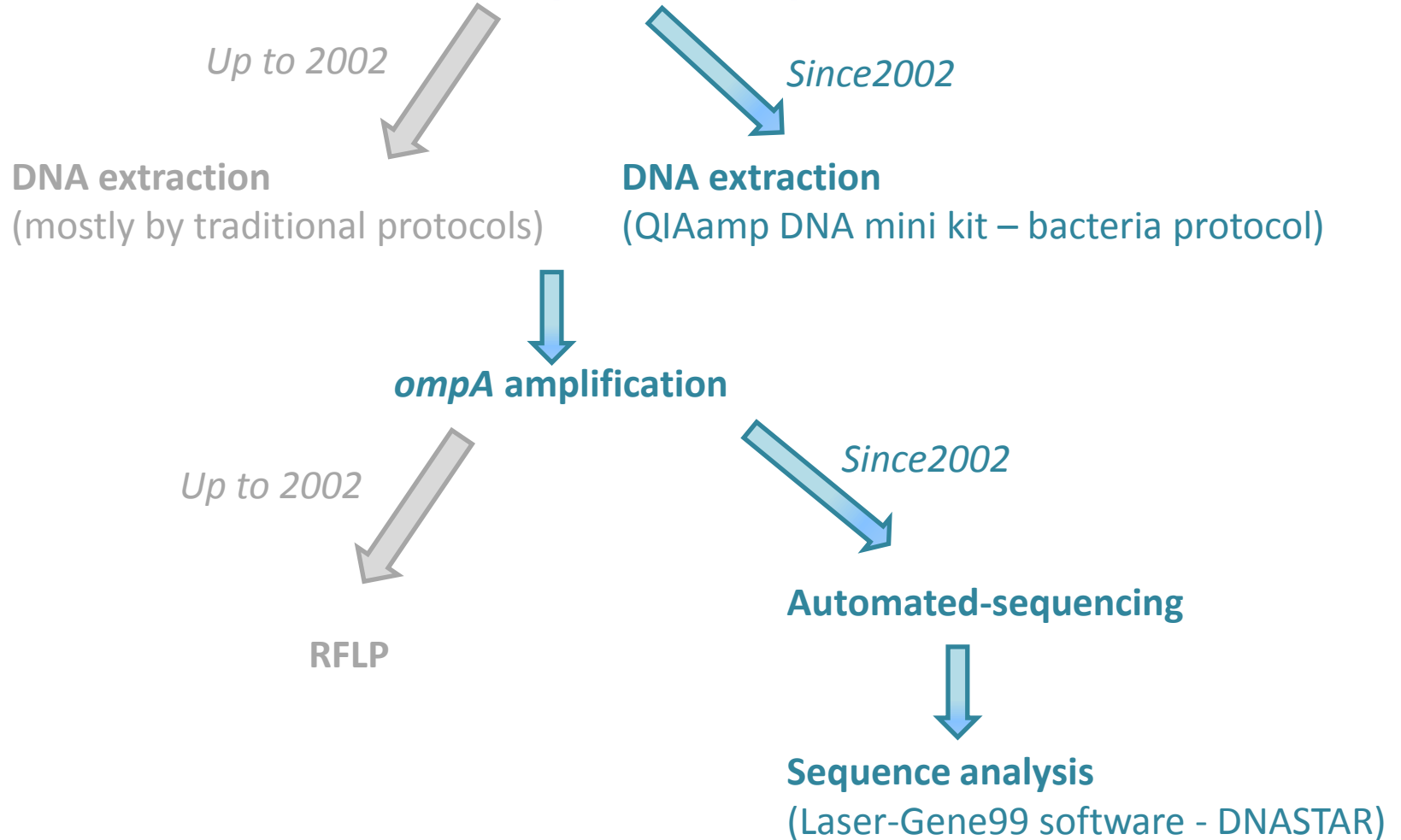
or

[Cobas 4800 (2010)]

9,2% *C. trachomatis* positive results



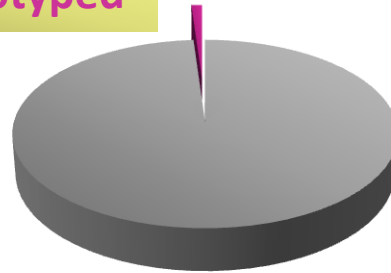
C. trachomatis-positive samples



1991 to 2002

5 L₂ / 463 ompA-genotyped

~ 14000 samples tested
596 *C. trachomatis* (+)



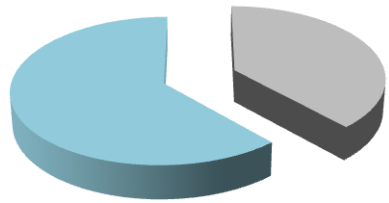
■ non-LGV

■ 'L2'

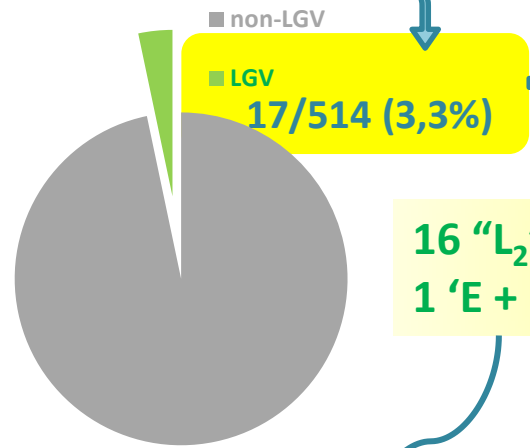
2002-2006 no LGV strain was detected

2007 - 2010

> 9000 samples tested
833 *C. trachomatis* (+)



■ undetermined
■ ompA genotyped
514/833 (61,7%)



■ non-LGV
■ LGV
17/514 (3,3%)

16 "L₂"
1 'E + L₂ undetermined variant'

10 from men

- ◆ 5 MSM / 1 heterosexual / 4 unknown sexual orientation
- ◆ 4 proctitis (2 HIV+)

7 from women

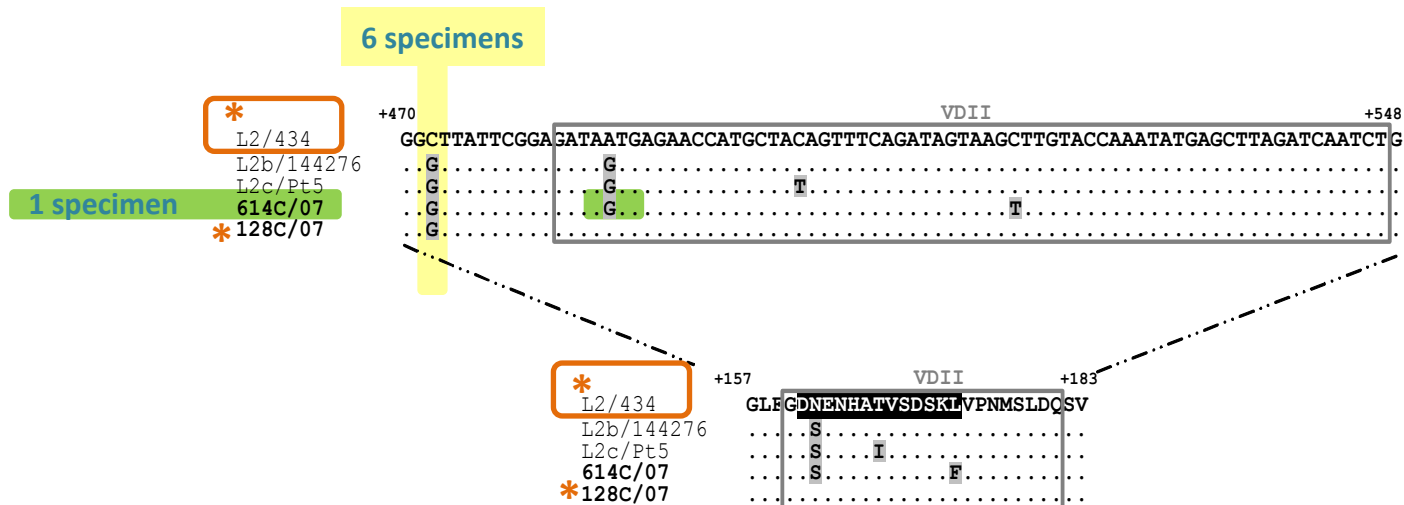
- ▲ all heterosexual
- ▲ 6 asymptomatic and 1 PID

2007 LGV isolates (#7) revealed *ompA* sequences *different** from both L₂-434, and L_{2b}-144276

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Lymphogranuloma Venereum in Portugal Unusual Events and New Variants During 2007

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2008 and 2010 LGV specimens would be similar to L_{2b}-144276

2009 would be similar to the L₂ prototype* strain

Another Portuguese study (7/07 – 7/08)
Detected another 2 L_{2b} specimens
among 8 HIV+ men with proctitis
(Castro R et al, IJSTD&AIDS, 2010)

* but... which is the 'correct' prototype / reference to compare with ???

For L_2 until 2008, similarity with the L_2 -434 GenBank No. M14738 (Stephens et al, J Bacteriol, 1986)

	+470
L2/434	GGC
L2b/144276	..G
L2c/Pt5	..G
614C/07	..G
128C/07	..G

OPEN ACCESS freely available online



Adaptive Evolution of the *Chlamydia trachomatis* Dominant Antigen Reveals Distinct Evolutionary Scenarios for B- and T-cell Epitopes: Worldwide Survey

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Abstract

Background: *Chlamydia trachomatis* is one of the most disseminated human pathogens, for which no vaccine is available yet. Understanding the impact of the host pressure on pathogen antigens is crucial, due to its role as only licensed for highly restricted geographic areas. We aimed to evaluate the evolutionary picture of the chlamydial key antigen (MOMP), which is one of the leading multi-subunit vaccine candidates, in a worldwide basis.

Methodology/Principal Findings: Using genetic, molecular evolution methods and mathematical modelling, we analyzed all MOMP sequences reported worldwide, composed by 5020 strains from 33 geographic regions of five continents. Overall, 72.6% of variants were detected. The evolutionary pattern of MOMP amino acid positions was found to differ from the remaining chromosome, reflecting the demanding constraints of this gene, adhesin and dominant antigen. Amino acid changes were 4.6-fold more frequent in host-interacting domains (P<10⁻¹⁰), specifically within B-cell epitopes (P<10⁻⁵), where 25% of them are at position P<12^o. According to the typical pathogen-host arms race, the rampant B-cell epitopes variation likely represents neutralization escape mutants, as some mutations were previously shown to alter neutralization of chlamydial infectivity in cells. In contrast, T-cell clusters of diverse HLA specifications are under diversifying selection, suggesting a strategy that may lead to immune subversion. Moreover, several distinct mutations are at position 614, generating differential results that may influence acquisition, and may also affect neutralization-induced inhibiting effect from favourable neutral changes. Interestingly, the most prevalent C. trachomatis genotypes, L and F, showed a mutation rate 2.3-fold lower than that of the remainder (P<10⁻⁵), suggesting more fixed antigenic profiles.

Conclusions/Significance: Globally, the adaptive evolution of the C. trachomatis dominant antigen is likely driven by its complex, pathogenesis-related function and reflects distinct evolutionary antigenic scenarios that may benefit the pathogen, and thus should be taken into account in the development of a MOMP-based vaccine.

Citation: Nunes A, Nogueira PJ, Borrego MJ, Gomes JP (2010) Adaptive Evolution of the *Chlamydia trachomatis* Dominant Antigen Reveals Distinct Evolutionary Scenarios for B- and T-cell Epitopes: Worldwide Survey. PLoS ONE 5(10): e13171. doi:10.1371/journal.pone.0131711

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Introduction

Chlamydia trachomatis is an obligate intracellular pathogen that causes ocular genital infections in humans. Trachoma (clonally derived genotype A/C and B/a) is the world's leading cause of preventable blindness with special impact on resource-poor nations, which has been recently placed on the WHO's priority list for intervention [1]. Also, the asymptomatic carriage of most genital chlamydial infections (genotypes D, K, Da, Jb, Jc and L1/L2) makes this pathogen the major cause of heretofore sexually transmitted infections worldwide [2]. Thus, C. trachomatis constitutes a major public health problem, and the development of effective preventive strategies, such as a vaccine, are urgently needed, but vaccine attempts failed to provide broad coverage and cost-effective limited protection [3].

One of the leading multi-subunit vaccine candidates is the C. trachomatis major outer membrane protein (MOMP), coded by ompA, whose variations underlie strain classification into sero-

from 2008, we start using L_2 -434 GenBank No. NC010287 (Thomson et al, Genome Res, 2008)

And so...

L2/434

L2b/144276

614C/07

128C/07

CS182/07

CS183/07

CS185/07

CS909/07

134C/07

CS19/08

CS784/08

CS798/08

CS1062/08

257C/09

302C/09

490C/10

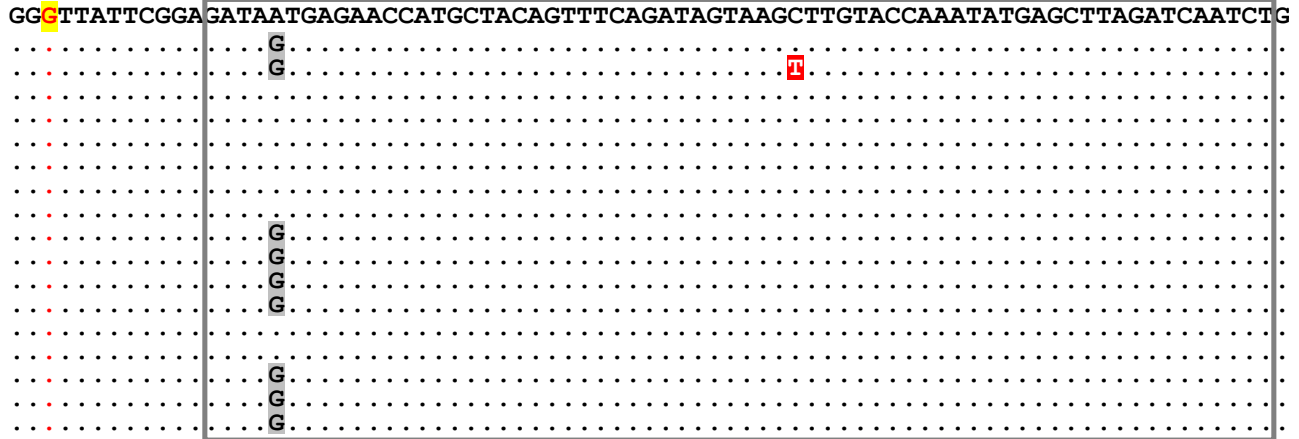
535C/10

CS1309/10

+470

VDII

+548



Antigenic domain

+157

VDII

+183

L2/434

L2b/144276

614C/07

128C/07

CS182/07

CS183/07

CS185/07

CS909/07

134C/07

CS19/08

CS784/08

CS798/08

CS1062/08

257C/09

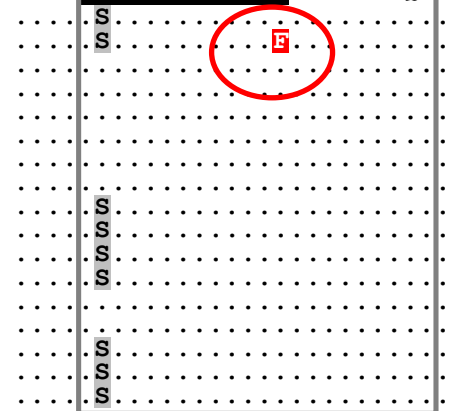
302C/09

490C/10

535C/10

CS1309/10

GLEFDNENHATVSDSKLVPNMSLDQSV



Leucine → Phenylalanine

Last thoughts

No evidence for greater predisposition for genetic variants in anorectal isolates?
(in L serovars? In other serovars?)

Relation with other STI?

No relation with gender ?

No relation with clinical features ?

(50% of men were symptomatic – proctitis, rectal ulcer)

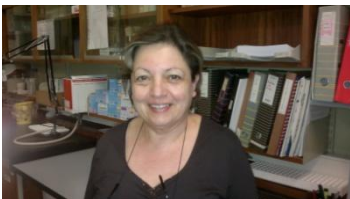
(1 / 7 women was symptomatic – abdominal pain, vaginal discharge)

Relations with ongoing outbreaks?

(no relation to any social venues in Portugal or abroad?)

The Portuguese NIH bacterial-STI team

Arminda Ferreira



João Paulo Gomes



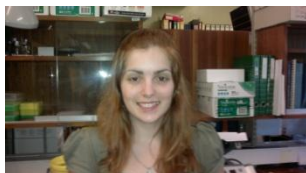
Vítor Borges



Carlos Florindo



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Alexandra Nunes



Maria José Borrego

