

**Pneumonia**

Frequent, significant mortality

Crucial to define the etiology

- to choose an optimal antibiotic treatment
- to guide public health measures

Unknown etiology in 50% of cases

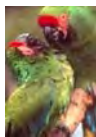
→ intracellular bacteria

### Intracellular bacteria

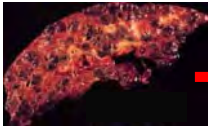


#### Mainly discovered during outbreaks

***Chlamydia psittaci***  
 - outbreak in Uster in 1880  
 - pandemia in 1929

} psittacosis



***Legionella pneumophila***  
 Fraser et al. 1977 New Engl J of Med  
 « Legionnaires' disease: description of an epidemic of pneumonia »


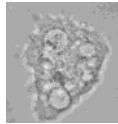
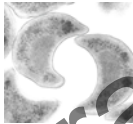
Lung      Guinea pig      Agar

### *Parachlamydia*

#### Discovered during an outbreak

- Isolation of an amoeba containing *Parachlamydia* from the water of an humidifier associated to an outbreak of fever

Birtles et al. Lancet 1997

Humidifier      Amoeba (x100)      *Parachlamydia* (x70000)

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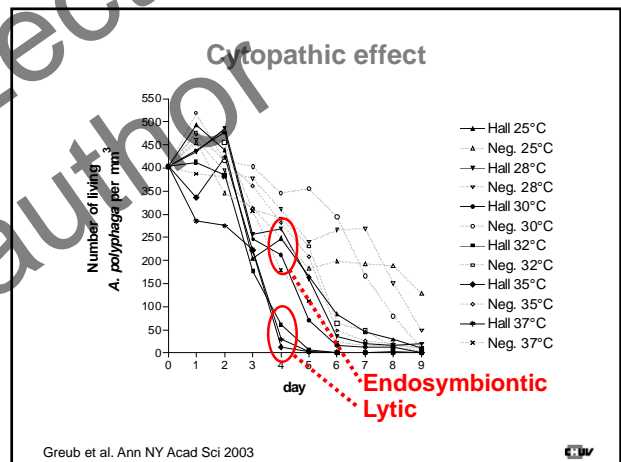
Birtles et al. Lancet 1997

#### Serological hint

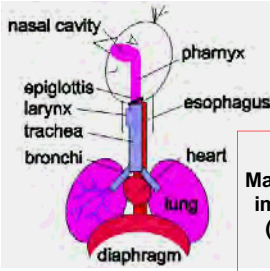
- in patients with community-acquired pneumonia  
Marrie et al. Emerg Infect Dis 2001
- in patients with aspiration pneumonia  
Greub et al. Ann NY Acad Sci, 2003;990:311-319

#### Molecular hint

- amplification of *Parachlamydia* DNA from BAL and sputum  
Corsaro et al. Microbiology 2001,  
Greub et al. Emerg Infect Dis 2003;9:755-756.  
Ossewaarde et al. Microbiol 1999;  
Casson et al. J Clin Microbiol



### Temperature-dependant liberation of the intracellular host

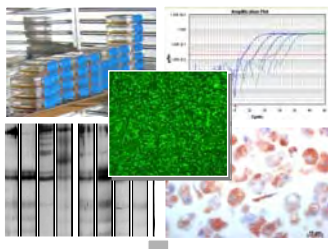


nasal cavity      pharynx  
 epiglottis      esophagus  
 larynx  
 trachea  
 bronchi      heart  
 lung  
 diaphragm

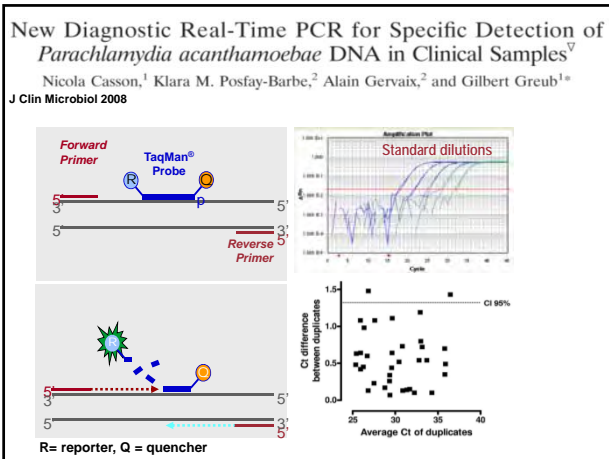
**High inoculum**  
 May overcome the first line of immune defense in the lung (i.e. human macrophages)  
 ↓  
**Aspiration pneumonia**

Greub et al. Ann NY Acad Sci 2003

### To further assess the role of *Parachlamydia* as an agent of pneumonia



Samples from patients with and without pneumonia



### *Parachlamydia acanthamoebae*

Bronchoalveolar lavage = 223, ~ 50% from controls without infection

Age (années)	Genre	Signes/symptômes	Autre étiologie	Ct qPCR (copies/ml)	Sérologie
53	M	Patient intubé, fièvre, infiltrat pulmonaire leucocytaire	non	qPCR Pac (2520) 97.1% <i>P. acanthamoebae</i>	Pas de sérum
37	F	Toux, dyspnée infiltrat pulmonaire	non	qPCR Pac (931) PCR conventionnel nég.	IgG 1/256
62	M	Fièvre, infiltrat pulmonaire		Negative	Séroconversion IgG 1/32 à 1/128
48	M	Fièvre, infiltrat pulmonaire leucocytaire	non	Pas de LBA	Séroconversion IgM 1/8 à 1/128

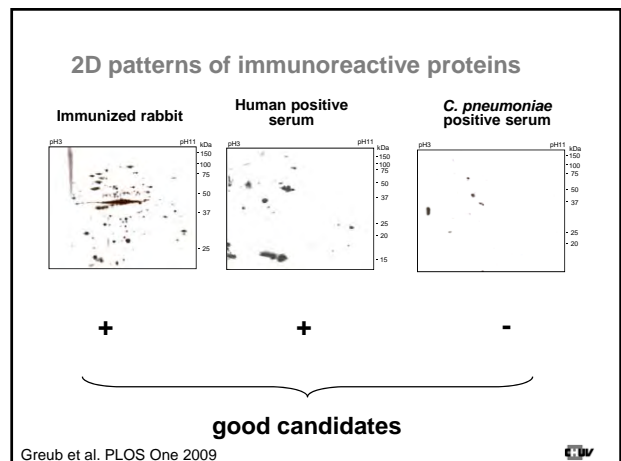
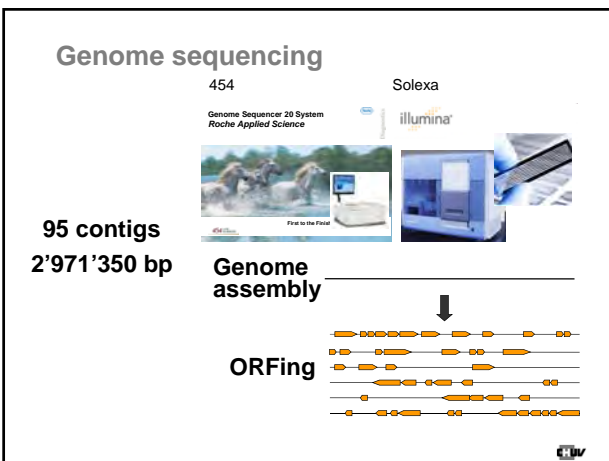
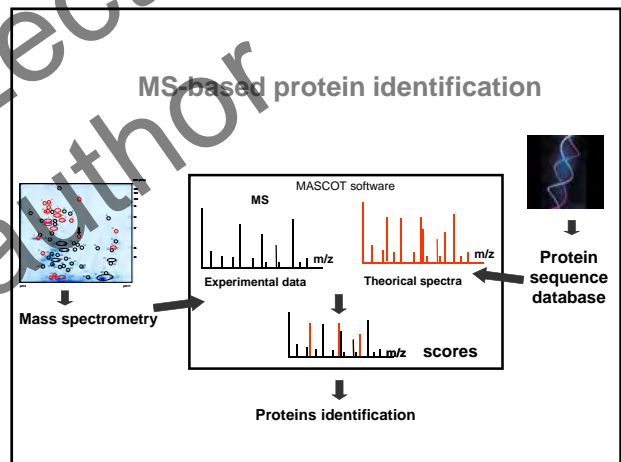
Prevalence of 3.6% among patients with pneumonia

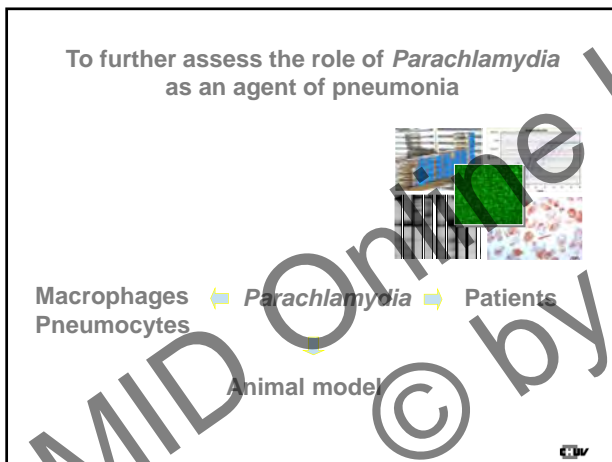
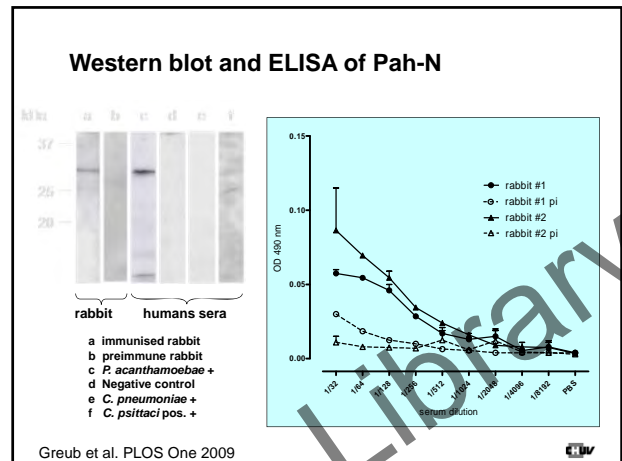
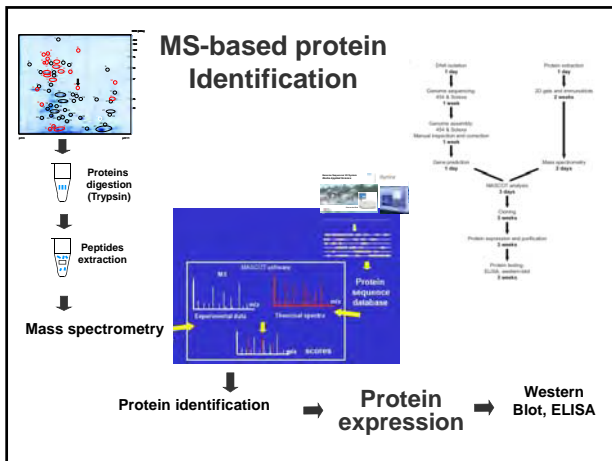
Casson et al., in preparation

### New diagnostic approaches

Combine rapid genome sequencing and proteomics to identify immunogenic proteins that may be used to develop an ELISA

Identify immunogenic proteins





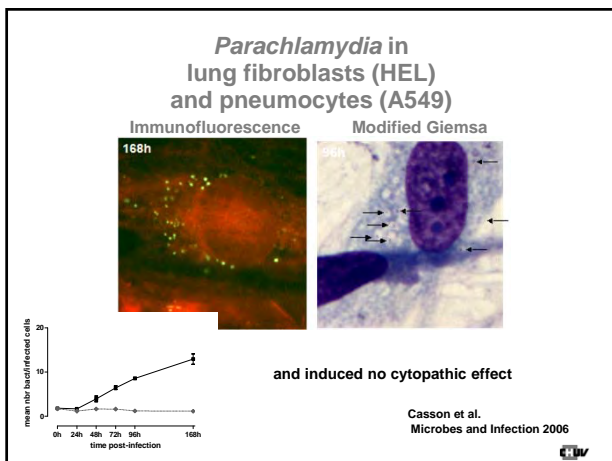
### Replication of *Parachlamydia* within mammalian cells (limited replication)

#### Human/murine macrophages

Greub et al., Infection & Immunity 2003  
Greub et al., Cell microbiol 2005  
Croxatto et al, Microbiology 2010  
Roger et al, Infection & Immunity 2010

#### Human pneumocytes (A549) Human lung fibroblasts (HEL)

Casson et al. Microbes and Infection 2006




### To further assess the role of *Parachlamydia* as an agent of pneumonia

#### Koch postulates

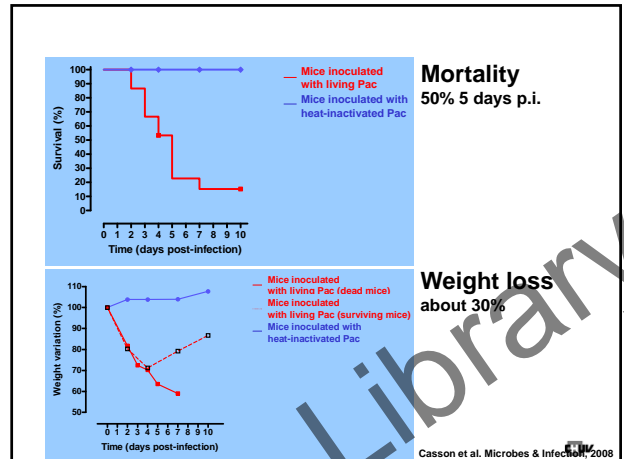
1. Bacteria may be detected in infected host
2. Bacteria may be isolated in pure culture from infected host
3. The same disease may be reproduced in animal
4. The same bacteria may be isolated in pure culture from experimentally-infected animals

### Animal model

- Intratracheal injection of  $2.5 \times 10^8$  *Parachlamydia acanthamoebae*

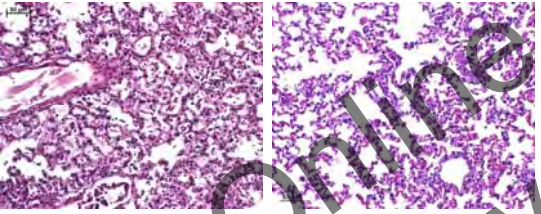


Casson et al. Microbes & Infection 2008



### Histology: severe pneumonia

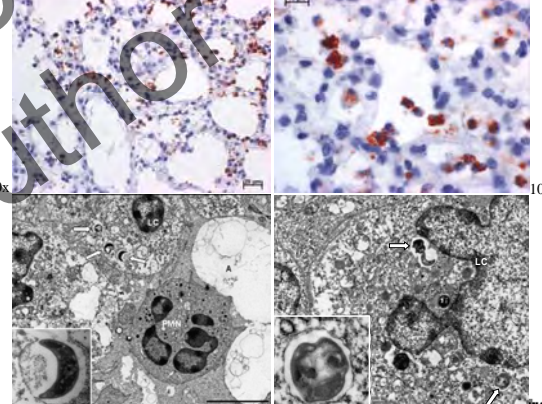
2 days post-infection      Uninfected control



Purulent pneumonia in 100% of infected mice (days 2-4)  
Interstitial pneumonia (days 7-10)

Casson et al. Microbes & Infection 2008

### Presence of *Parachlamydia* in the lesions

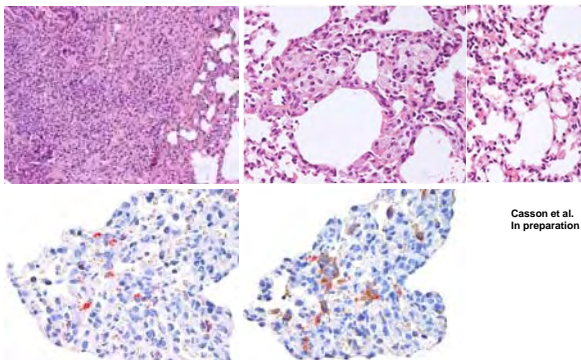


40x      100x

Casson et al. Microbes & Infection 2008

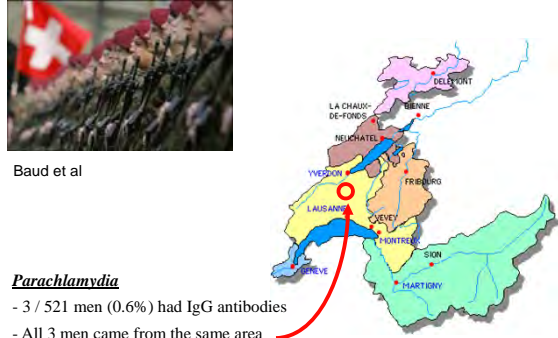
### Intra-nasal model

J2      J10      Neg. control



Anti-*Parachlamydia*      *Parachlamydia*/macrophages

Casson et al. In preparation




Baud et al

***Parachlamydia***

- 3 / 521 men (0.6%) had IgG antibodies
- All 3 men came from the same area (Echallens)

### Amniotic fluid

- 29-year-old woman, 2nd pregnancy
- cough and flu-like symptoms of 3 weeks' duration before amniocentesis
- pregnancy ended prematurely at 35 weeks (2'060 g newborn)
- amniotic fluid remained sterile
- negative vaginal, placental, urinary cultures
- zoonotic contact ?  
... in a rural area known for cattle breeding



### Diagnosis

Amoebal co-culture  
PCR  
Serology

### Treatment

Macrolides  
Doxycycline

(not quinolones: mutation in QRDR)

Maurin et al 2003 / Casson et al 2006

### Conclusions

*Parachlamydia acanthamoebae* should be considered as a new agent of pneumonia

Further works are needed to better understand the biology of this obligate intracellular bacteria and to precise its pathogenic potential

### Acknowledgments