Realtime-PCR of Bioterrorism agents

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PubMed

- „Bioterrorism“ = 4.365
  (1996-9/2001 = 205;
  10-12/2001 = 240)
- „qPCR“ = 1.481
- „Bioterrorism“ & „qPCR“ = 132

The „dirty dozen“

Bacteria
- Yersinia pestis
- Bacillus anthracis
- Brucella spp.
- Francisella tularensis
- Burkholderia mallei/
  B. pseudomallei
- Coxiella burnetii

Virus
- Variolavirus
- Venezuelan Equine
  Encephalitisvirus
- Marburgvirus

Toxins
- Botulinumtoxins
- Staphylokokken-
  enterotoxins
- Ricin

CENTERS FOR DISEASE CONTROL AND PREVENTION™

Category	Characteristics	
A	Easy dissemination or contact transmission Cause high mortality rates and have the potential for major public health impact May cause public panic and social disruption Require special action for public health preparedness	Anthrax (*Bacillus anthracis*) Botulism (*Clostridium botulinum* toxin) Plague (*Yersinia pestis*) Smallpox (*Variola major*) Tularemia (*Francisella tularensis*) Viral hemorrhagic fevers
B	Moderately easy to disseminate Result in moderate morbidity rates and low mortality rates Require specific enhancements of CDC's diagnostic capacity and enhanced disease surveillance	Brucellosis (*Brucella* species) Epsilon toxin of *Clostridium perfringens* Food safety threats (*Salmonella* species, *Escherichia coli, Shigella*) Glanders (*Burkholderia mallei*) Melioidosis (*Burkholderia pseudomallei*) Psittacosis (*Chlamydia psittaci*) Q fever (*Coxiella burnetii*) Ricin toxin (from castor beans) Staphylococcal enterotoxin B Typhus fever (*Rickettsia prowazekii*) Viral encephalitis Water safety threats (*Vibrio cholerae, Cryptosporidium parvum*)
C	Emerging pathogens engineered for mass dissemination Availability Ease of production and dissemination Potential for high morbidity and mortality rates and major health impact	Emerging infectious diseases such as Nipah virus and hantavirus

but.....“most wanted“:

PubMed

„PCR and bacteria“ =

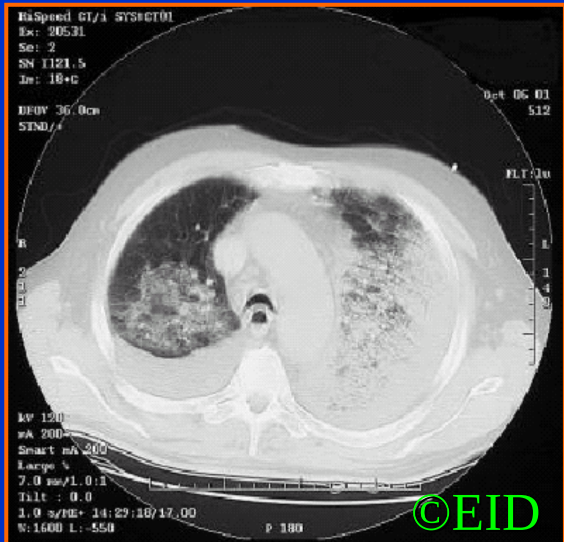
95.976

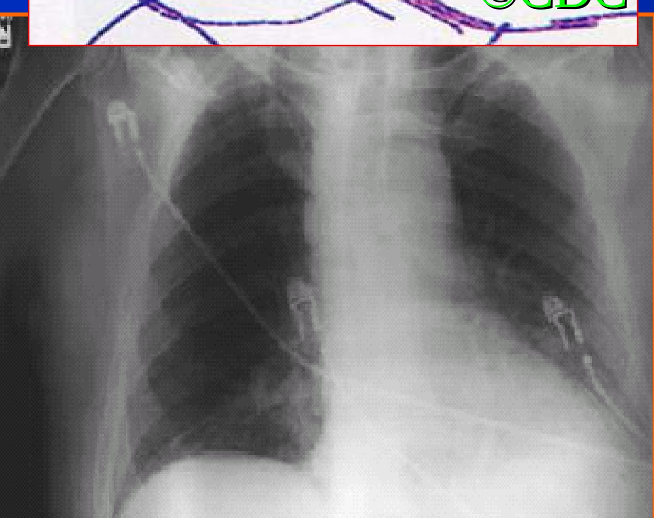
Identification of Bioterrorism agents

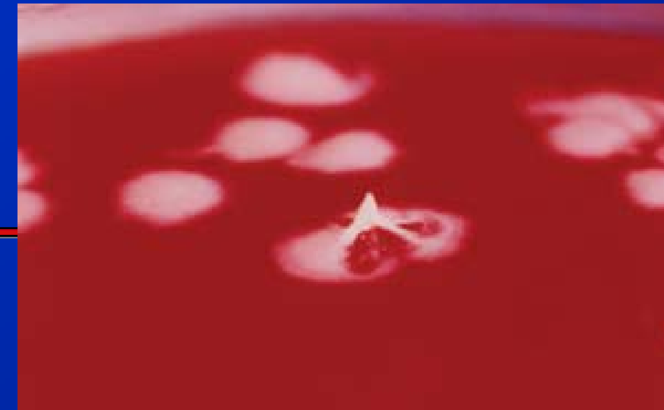
- **UNAMBIGUOUS**
- Specific
- Sensitive
- Fast
- Reproducable
- Safe

Kochs „Postulate“

- The anthrax bacillus, ***Bacillus anthracis*,** was the first bacterium shown to be the cause of a disease. In 1877, Robert Koch grew the organism in pure culture, demonstrated its ability to form endospores, and produced experimental anthrax by injecting it into animals.



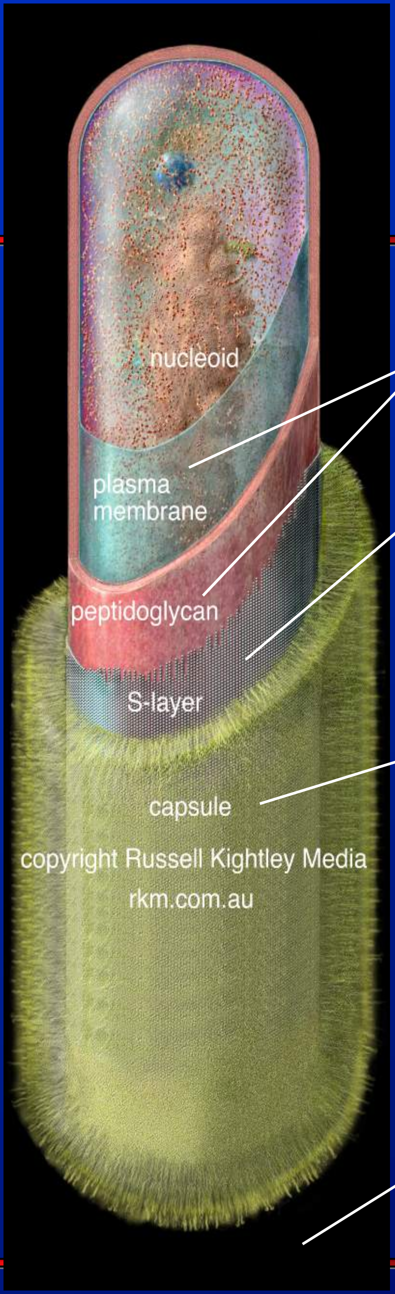




„Anthrax“

***Bacillus anthracis*:**

* Gram-positive
* aerobe rod
* 1 - 1.2µm in width x 3 - 5µm in length
* sporeforming
* Genotypically and phenotypically very similar to *Bacillus cereus* and *thuringiensis*
* Toxin producer



Sacculus

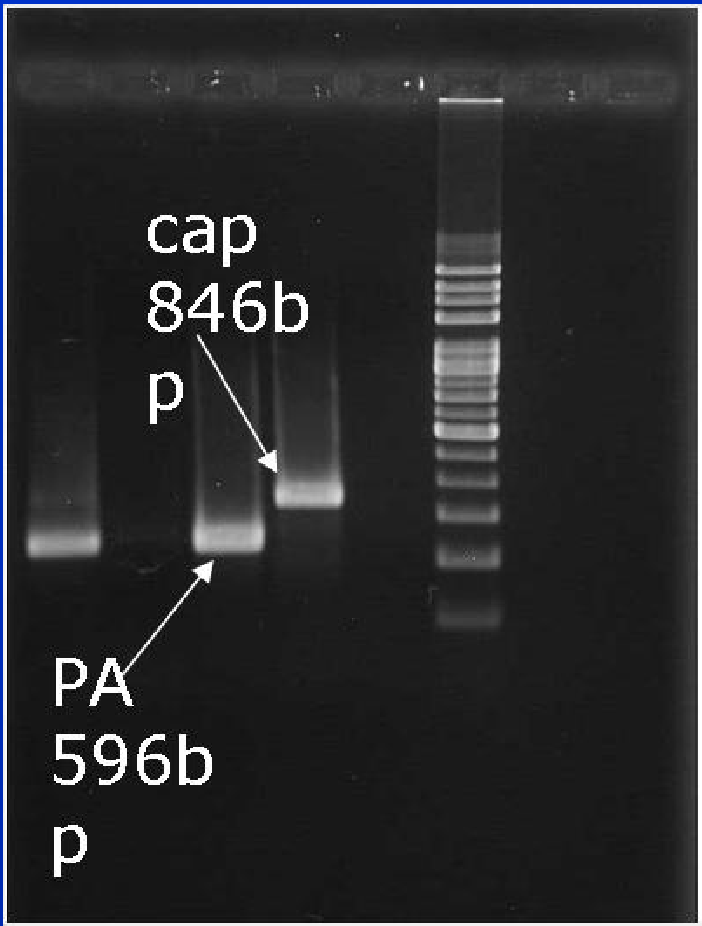
** S-Layer
Sap + EA1

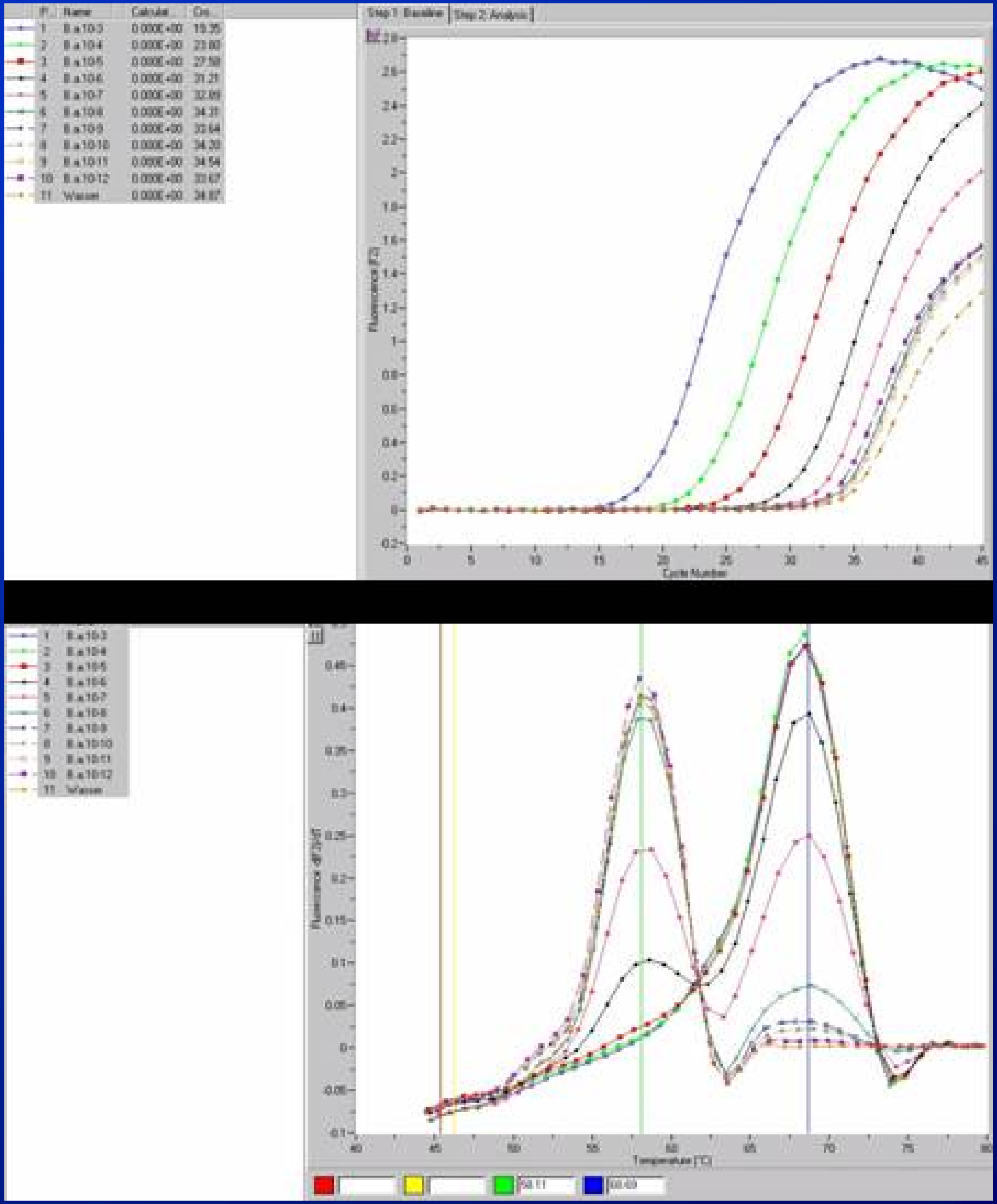
** Capsule: poly-γδ-Glutamin-acid

** Toxins

VIRULENCE-FACTORs (plasmid-coded)

- **Poly-γ-D-Glutamyl-capsule: pX01**
- **Exotoxin-complex: pX02**
  - **Lethalfactor (LF)**
  - **Edemafactor (EF)**
  - **protective Antigen (PA)**


cap
846b
p
PA
596b
p


Name
Step 1: Baseline
Step 2: Analysis
Cycle Number
Wasser
Temperature (°C)

JOURNAL OF CLINICAL MICROBIOLOGY, Sept. 2006, p. 3352–3360 Vol. 44, No. 9
0095-1137/06/$08.00+0 doi:10.1128/JCM.00561-06

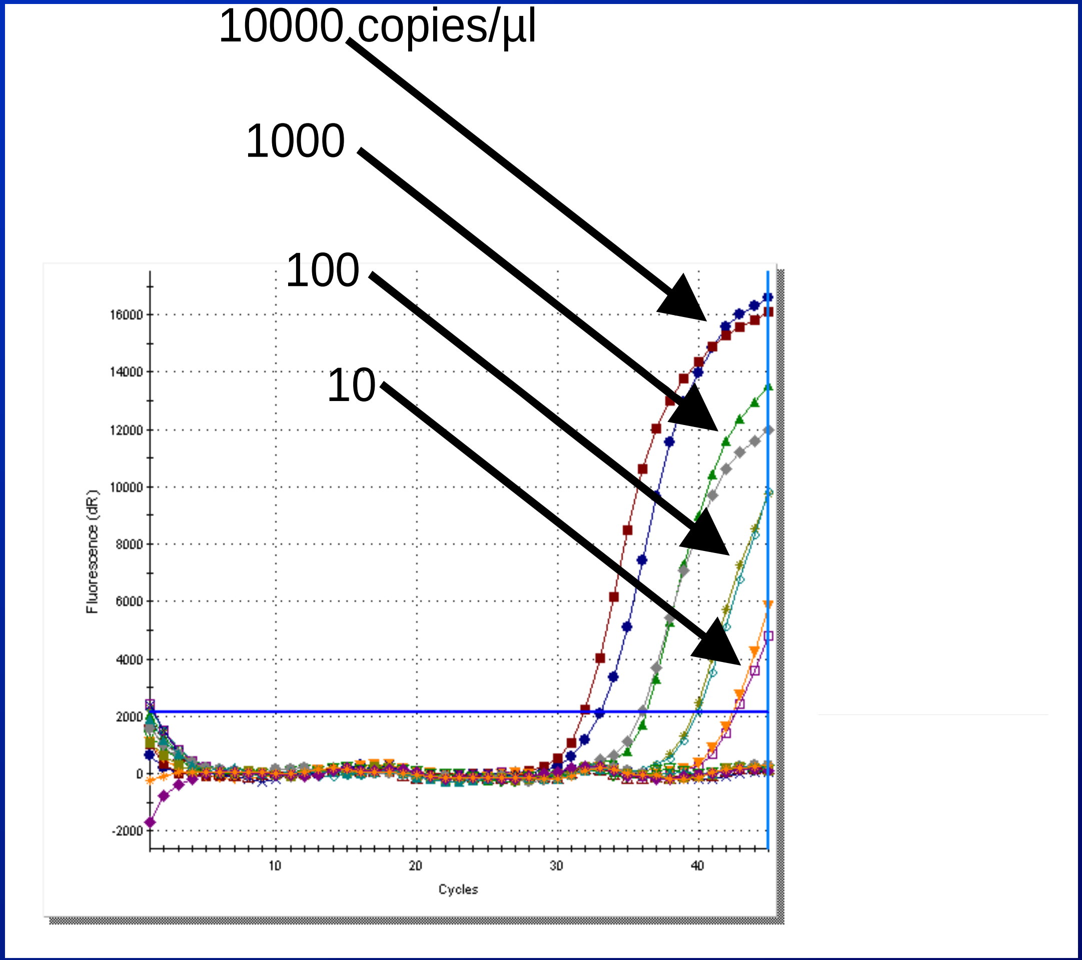
Characterization of *Bacillus cereus* Isolates Associated with Fatal Pneumonias: Strains Are Closely Related to *Bacillus anthracis* and Harbor *B. anthracis* Virulence Genes†

Alex R. Hoffmaster,[1*] Karen K. Hill,[4] Jay E. Gee,[1] Chung K. Marston,[1] Barun K. De,[1] Tanja Popovic,[2] David Sue,[1‡] Patricia P. Wilkins,[1] Swati B. Avashia,[3,4§] Rahsaan Drumgoole,[4] Charles H. Helma,[5] Lawrence O. Ticknor,[6] Richard T. Okinaka,[5] and Paul J. Jackson[7]

Marker	**Number of strains testes versus amplified**		**Reference**
	B. anthracis	**B. cereus**	
Ba831	47/47	4/60	Ramisse et al 1996, JAM, 87, 224ff
rpoB	14/14	5/36	Ellerbrock et al FEMS Microb Lett. 2002, 214, 51ff
BA5510	4/4	2/289	Olsen et al JMM 2007, 71, 265ff
gyrB	1/1	2/23	La Duc et al JMM 2004, 56, 383ff.
saspB	392/392	0/56	Hoffmaster et al EID 2002, 8, 1178ff
plcR	89/89	0/29	Easterday et al JCM 2005, 43, 1995ff
gyrA	43/43	0/49	Hurtle JCM 2004, 42, 179ff
BA5345	**142/142**	**0/236**	**Antwerpen et al, MCP 2008, 22, 313ff**

„Dhp61-PCR“

The detection limit was calculated using Probit-analysis
to be 12.7 copies per µl
(95% confidence interval, 10.2 - 17.5 copies).



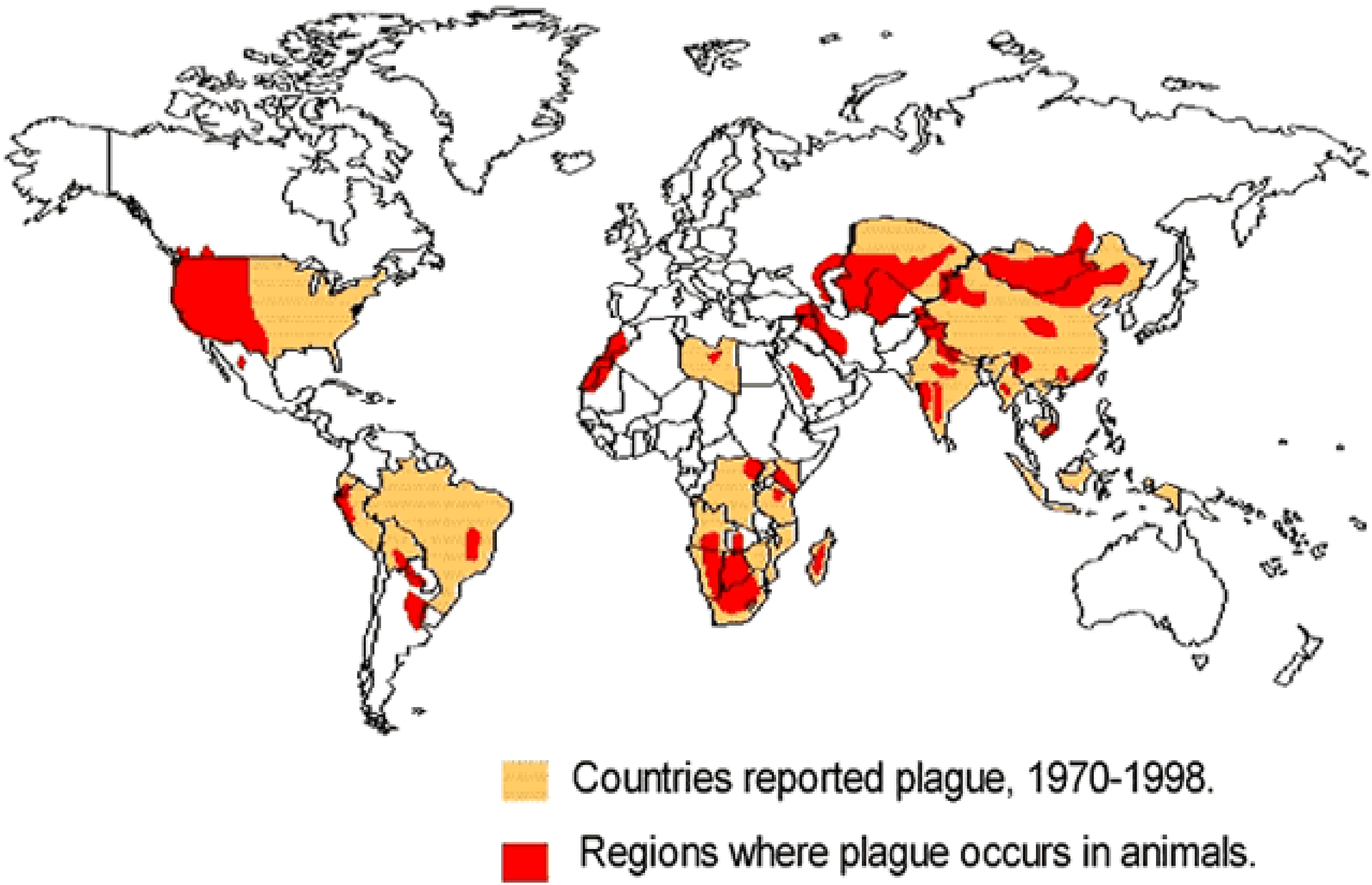
Yersinia pestis

1. Gram-negative, short rod

2. Family: Enterobacteriaceae

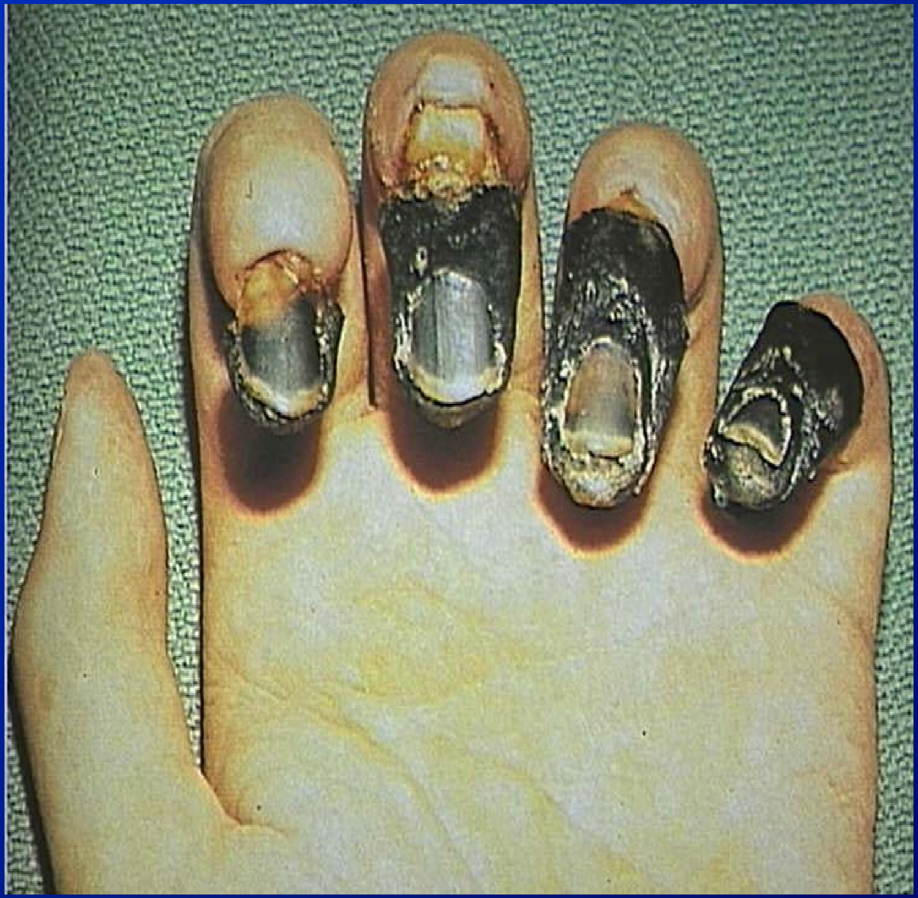
3. 11 *Yersinia*-Species,
   3 human-pathogen
   - *Y. pestis*
   - *Y. pseudotuberculosis*
   - *Y. enterocolitica*

World Distribution of Plague, 1998



WHO, Geneve, Switzerland

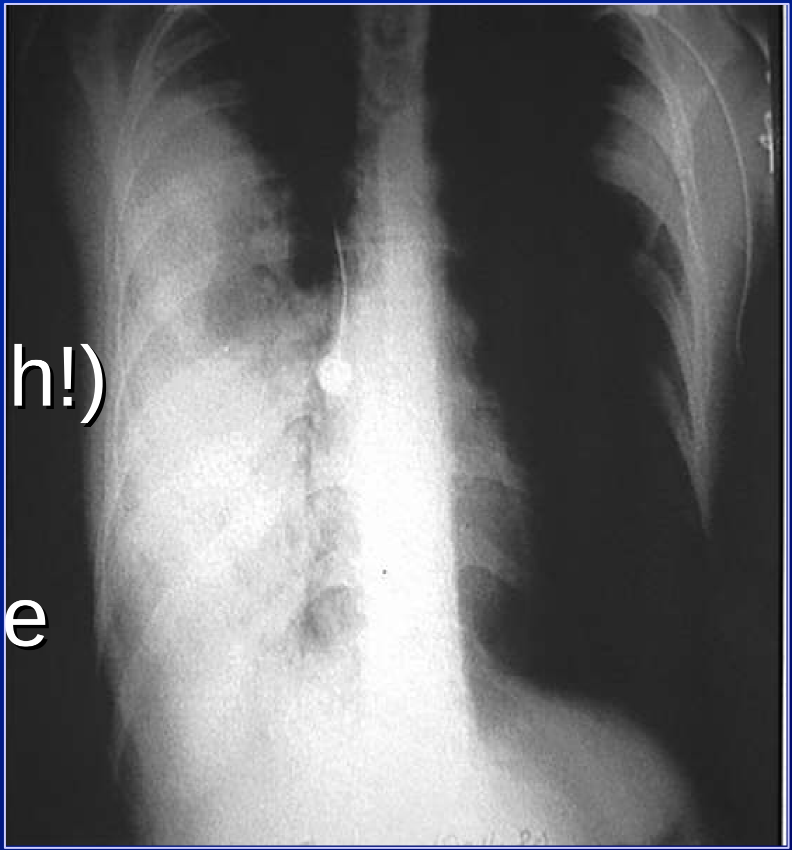
Clinical aspects



CDC, Atlanta, USA

Pulmonary Plague –
„The Pox of Bacteria“

- aerogen Infection (person-to-person)
- less then 100 bacteria
- short Incubationtime (6 h!)
- high letality (>90%)
- no safe vaccine available



March 3, 2009 | vol. 106 | no. 9 | 2969–3640

PNAS

Proceedings of the National Academy of Sciences of the United States of America www.pnas.org

Yersinia pestis, the cause of plague, is a recently emerged clone of *Yersinia pseudotuberculosis*

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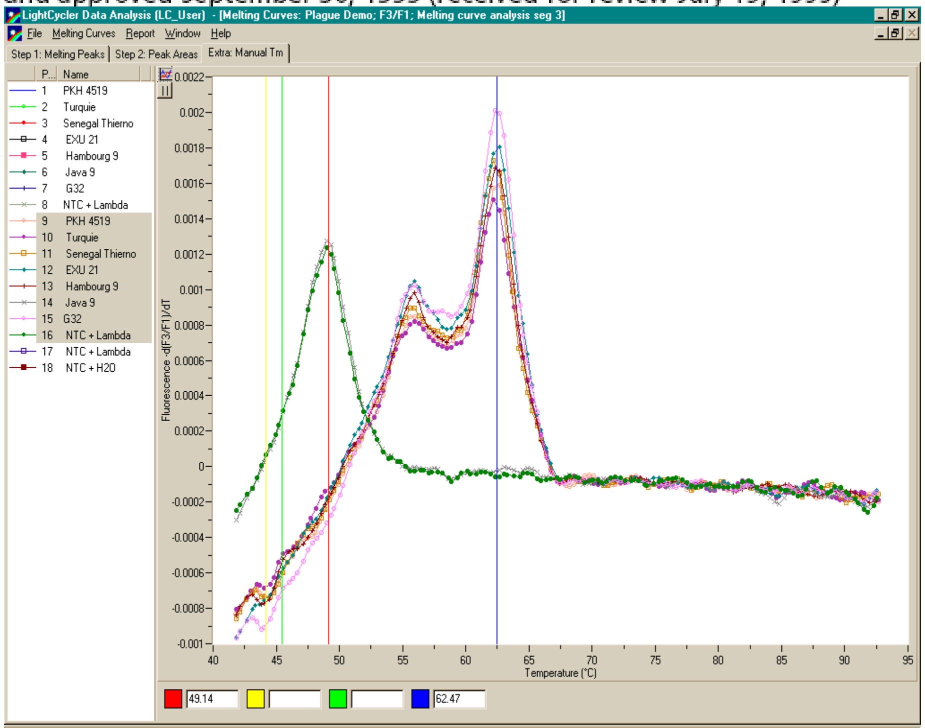
Edited by Richard M. Krause, National Institutes of Health, Bethesda, MD, and approved September 30, 1999 (received for review July 19, 1999)

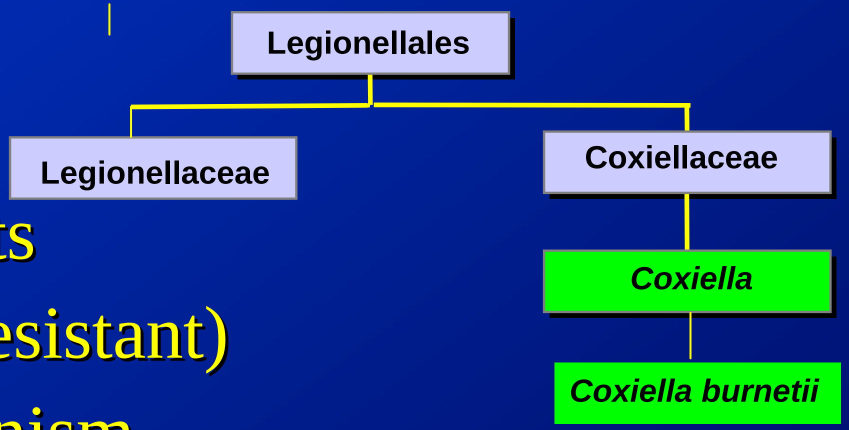


Multiplex LC-PCR assays for the specific detection of *Yersinia pestis*

	Targets		Internal amplification control
Multi A	**16S rRNA gene**	***pla* (pPla)**	**Bacteriophage Lambda DNA**
Multi B	***ymt* (pMT1)**	***caf1* (pMT1)**	**Bacteriophage Lambda DNA**

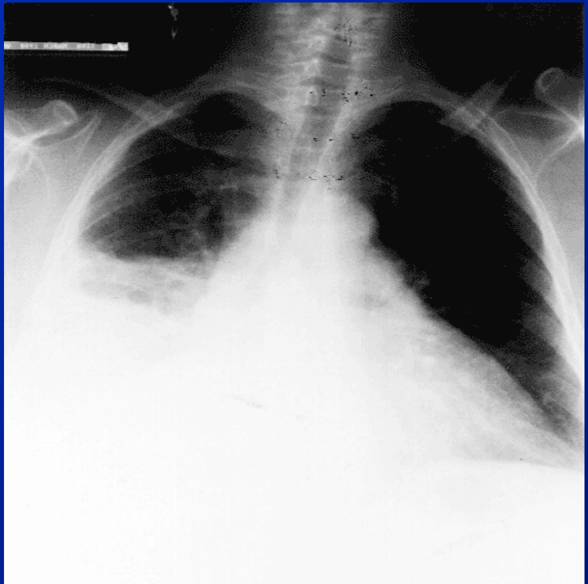
Properties *Coxiella burnetii*

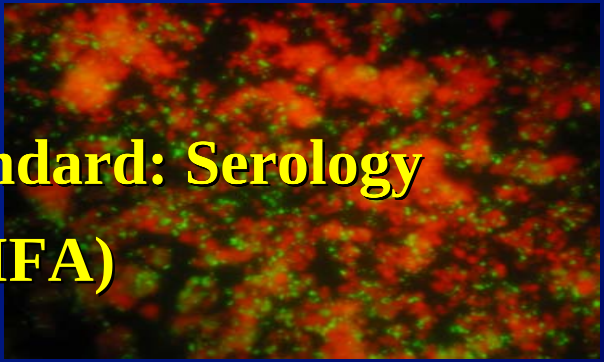
- Pathogen of the Zoonosis Q fever
- gram-negative
- obligate intracellular
- small- u. large cell variants
- „Spore-like“ –particles (resistant)
- extrem low ID: 1-10 organism
- Family of Legionellales
- CDC-Classification 2 (Aerosol, extreme low ID <10 Bacteria)



Coxiella burnetii – Q-Fever

- periodical Outbreaks worldwide incl. Germany (e.g.: Dillenburg, Aschaffen-burg 2008, NL 2008 - >600 cases)
- 98%: Acute (Pneumonia),
  2% : Chronic (Endocarditis)





Diagnostic Gold-Standard: Serology

(ELISA, IFA)

Q Fever in Germany

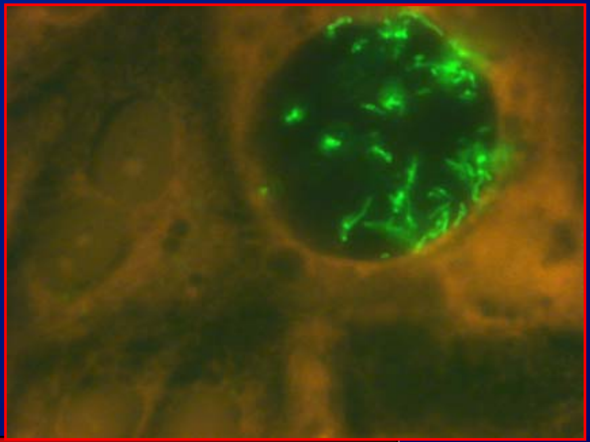
Year	Location	Cases	Transmission
1993	Sontra	84	birth products, sheep
1997	Gießen	68	lambing
2003	Soest	299	lambing
2005	Jena	322	sheep flock

Q Fever - PCR indications?

- acute Q-fever: serum samples, sputum, BAL,...(low DNA amount)
- chronic Q-fever: blood, placenta/amnion fluid, heart valve (low to moderate DNA)
- „Bioterrorism“ samples (moderate to high DNA)
- veterinary medicine (Milk, feces, birth products – moderate to high DNA)

PCR requirements

- Sensitivity (high?!)
- Specificity (high!!)
- Controls (pos., neg., internal), multiple targets?
- Stability of reagents/robustness (RT, 4°/-20°C)
- „Stability“ of results, reproducible?
- Hands-on-time/ „easy-to-use“
- request „frequency“
- Costs
- availability guarantee



„I have a dream…….“

+ 100% Specificity

+ High Sensitivity (<10 cp/µl)

+ Competitive Internal Control

+ „real“ Quantification with pre-diluted standards (4x)
single use or stabil after solution

+ Melting-Curve-Analysis

+ „Ready-to-use“ reagents

+ (multi-target Assay)

+ availability guarantee