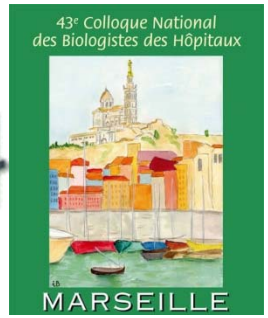
MARSEILLE- CONGRES DES BIOLOGISTES – 05.11.14
«Q fever what's new»

Unité de Recherche sur les Maladies Infectieuses Tropicales Emergentes
URMITE – UMR CNRS 7278, IRD 198, INSERM U1095

Didier Raoult
Marseille - France
didier.raoult@gmail.com
www.mediterranee-infection.com

ACNBH

ODPC N°1495

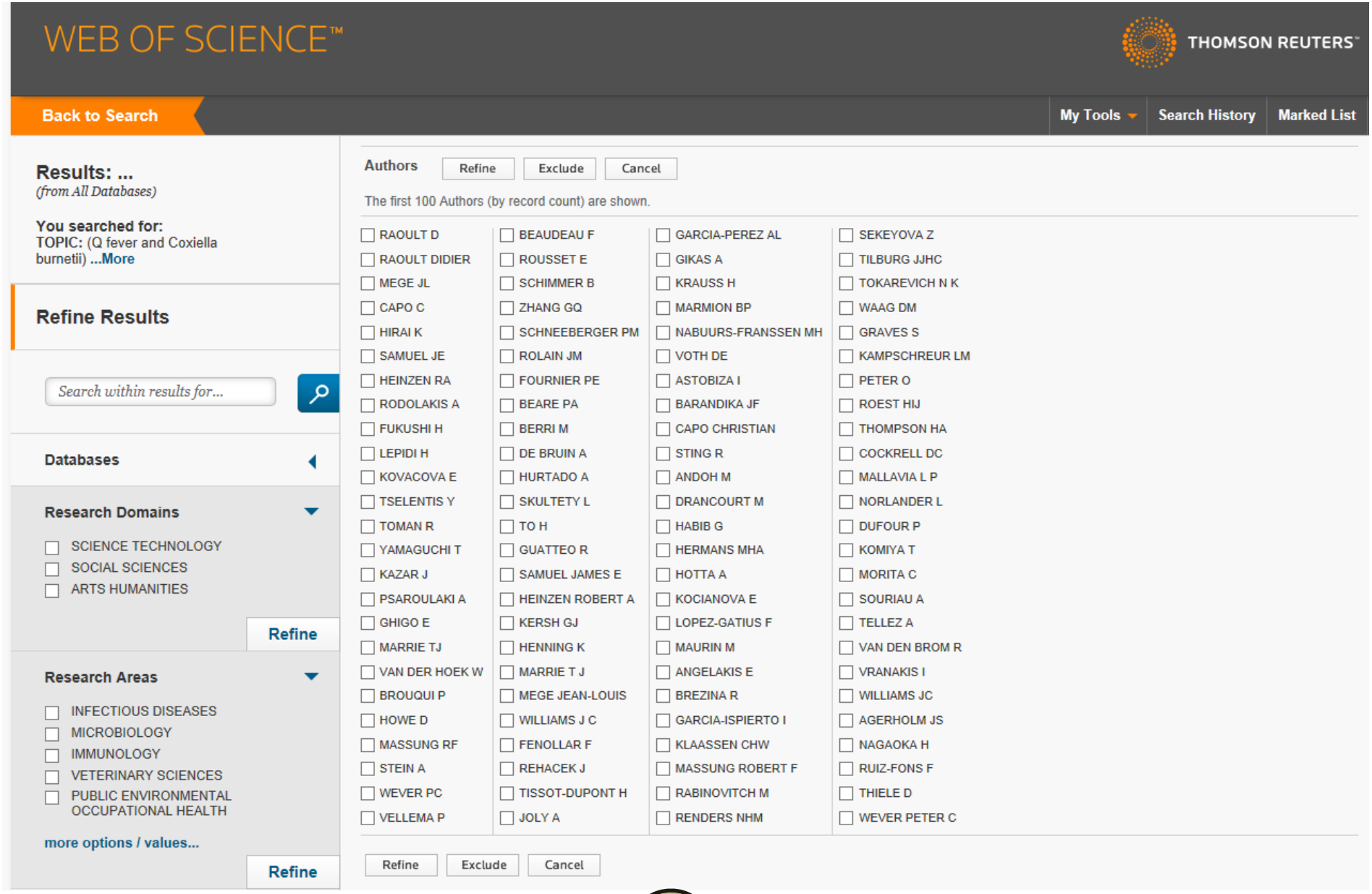


43ème Colloque National des Biologistes des Hôpitaux Marseille, 5-7 novembre 2014

ODPC habilité à dispenser des programmes de DPC

DECLARATION D'INTERET DANS LE CADRE DE MISSIONS DE FORMATION REALISEES POUR L'ACNBH

Pr Didier Raoult
Exerçant au CH la Timone
déclare sur l'honneur **ne pas avoir d'intérêt**, direct ou indirect (financier) avec les entreprises pharmaceutiques, du diagnostic ou d'édition de logiciels susceptible de modifier mon jugement ou mes propos, **concernant le DMDIV et/ou le sujet présenté**.


WEB OF SCIENCE™
THOMSON REUTERS
Back to Search
My Tools
Search History
Marked List
Results: ...
(from All Databases)
You searched for:
TOPIC: (Q fever and Coxiella burnetii) ...More
Refine Results
Search within results for...
Databases
Research Domains
SCIENCE TECHNOLOGY
SOCIAL SCIENCES
ARTS HUMANITIES
Refine
Research Areas
INFECTIOUS DISEASES
MICROBIOLOGY
IMMUNOLOGY
VETERINARY SCIENCES
PUBLIC ENVIRONMENTAL OCCUPATIONAL HEALTH
more options / values...
Refine
Authors
Refine
Exclude
Cancel
The first 100 Authors (by record count) are shown.
RAOULT D
RAOULT DIDIER
MEGE JL
CAPO C
HIRAI K
SAMUEL JE
HEINZEN RA
RODOLAKIS A
FUKUSHI H
LEPIDI H
KOVACOVA E
TSELENTIS Y
TOMAN R
YAMAGUCHI T
KAZAR J
PSAROULAKI A
GHIGO E
MARRIE TJ
VAN DER HOEK W
BROUQUI P
HOWE D
MASSUNG RF
STEIN A
WEVER PC
VELLEMA P
BEAUDEAU F
ROUSSET E
SCHIMMER B
ZHANG GQ
SCHNEEBERGER PM
ROLAIN JM
FOURNIER PE
BEARE PA
BERRI M
DE BRUIN A
HURTADO A
SKULTETY L
TO H
GUATTEO R
SAMUEL JAMES E
HEINZEN ROBERT A
KERSH GJ
HENNING K
MARRIE T J
MEGE JEAN-LOUIS
WILLIAMS J C
FENOLLAR F
REHACEK J
TISSOT-DUPONT H
JOLY A
GARCIA-PEREZ AL
GIKAS A
KRAUSS H
MARMION BP
NABUURS-FRANSSEN MH
VOTH DE
ASTOBIZA I
BARANDIKA JF
CAPO CHRISTIAN
STING R
ANDOH M
DRANCOURT M
HABIB G
HERMANS MHA
HOTTA A
KOCIANOVA E
LOPEZ-GATIUS F
MAURIN M
ANGELAKIS E
BREZINA R
GARCIA-ISPIERTO I
KLAASSEN CHW
MASSUNG ROBERT F
RABINOVITCH M
RENDERS NHM
SEKEYOVA Z
TILBURG JJHC
TOKAREVICH N K
WAAG DM
GRAVES S
KAMPSCHREUR LM
PETER O
ROEST HIJ
THOMPSON HA
COCKRELL DC
MALLAVIA L P
NORLANDER L
DUFOUR P
KOMIYA T
MORITA C
SOURIAU A
TELLEZ A
VAN DEN BROM R
VRANAKIS I
WILLIAMS JC
AGERHOLM JS
NAGAOKA H
RUIZ-FONS F
THIELE D
WEVER PETER C
Refine
Exclude
Cancel

Coxiella burnetii

- I was single

- Then outbreak in Netherland
-Dutch consensus
- E-CDC Recommandation

- Expert Vs consensus !

Chronic Q fever: expert opinion versus literature analysis and consensus. Raoult D.
J Infect. 2012 Aug;65(2):102-8.

Reevaluation of the Risk of Fetal Death and Malformation After Q Fever.
Million M, Roblot F, Carles D, D'Amato F, Protopopescu C, Carrieri MP, Raoult D.
Clin Infect Dis. 2014 Apr 18

Evolution from acute Q fever to endocarditis is associated with underlying valvulopathy and age and can be prevented by prolonged antibiotic treatment.
Million M, Walter G, Thuny F, Habib G, Raoult D.
Clin Infect Dis. 2013 Sep;57(6):836-44.

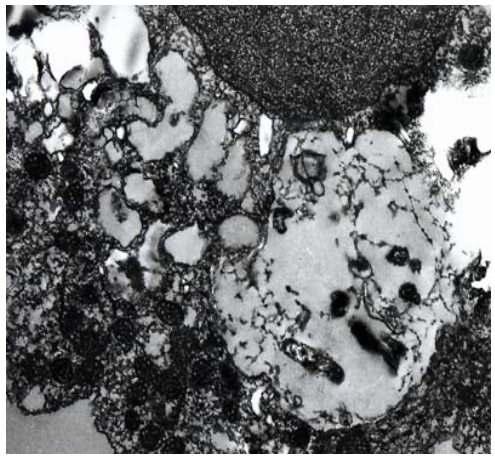
Immunoglobulin G anticardiolipin antibodies and progression to Q fever endocarditis.
Million M, Walter G, Bardin N, Camoin L, Giorgi R, Bongrand P, Gouriet F, Casalta JP, Thuny F, Habib G, Raoult D.
Clin Infect Dis. 2013 Jul;57(1):57-64

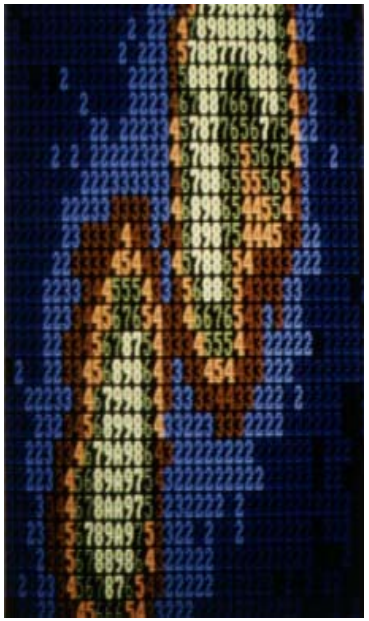
Coxiella burnetii and Q fever

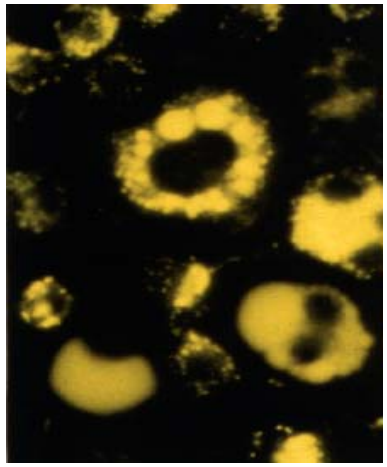
- **A Bacteriology**
- B Epidemiology
- C Clinical presentation
- D Pathophysiology
- E Specificity in Guyana
  - Clinical
  - Epidemiological
  - Bacteriological
  - Immune response
- F Diagnostic
- G Treatment
- H Prophyllaxis
- I Controversy

Coxiella burnetii : Bacteriology

- Obligate intracellular bacterium
- Any animals
- Gene exchanges in Amoeba with Legionella
- Multiplication within the phagolysosome of macrophages
- Survives in acidic vacuole (low pH activates metabolism)
- Growing in axenic medium

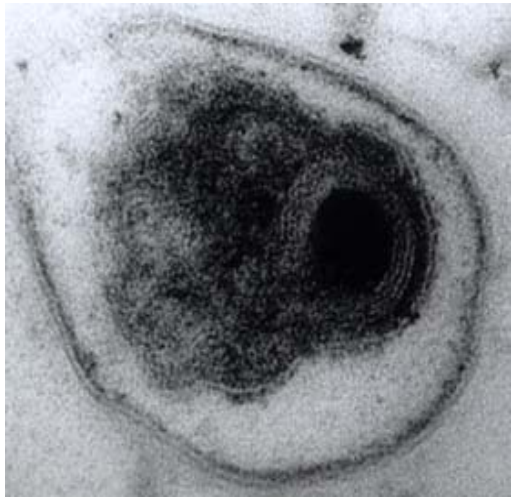


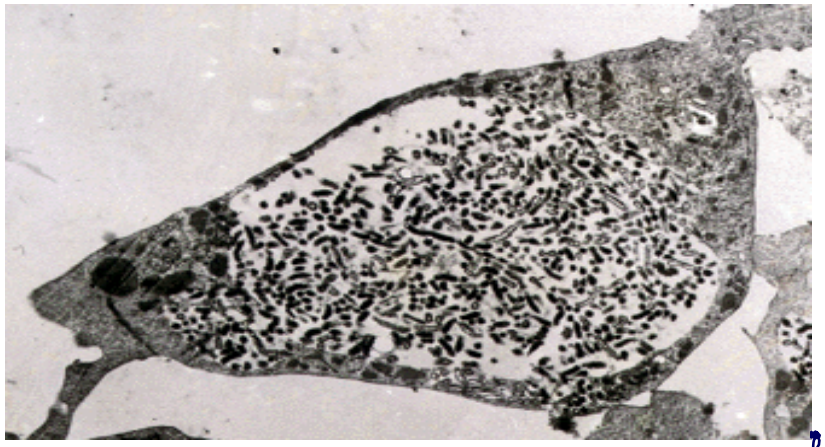




Coxiella burnetii : Bacteriology

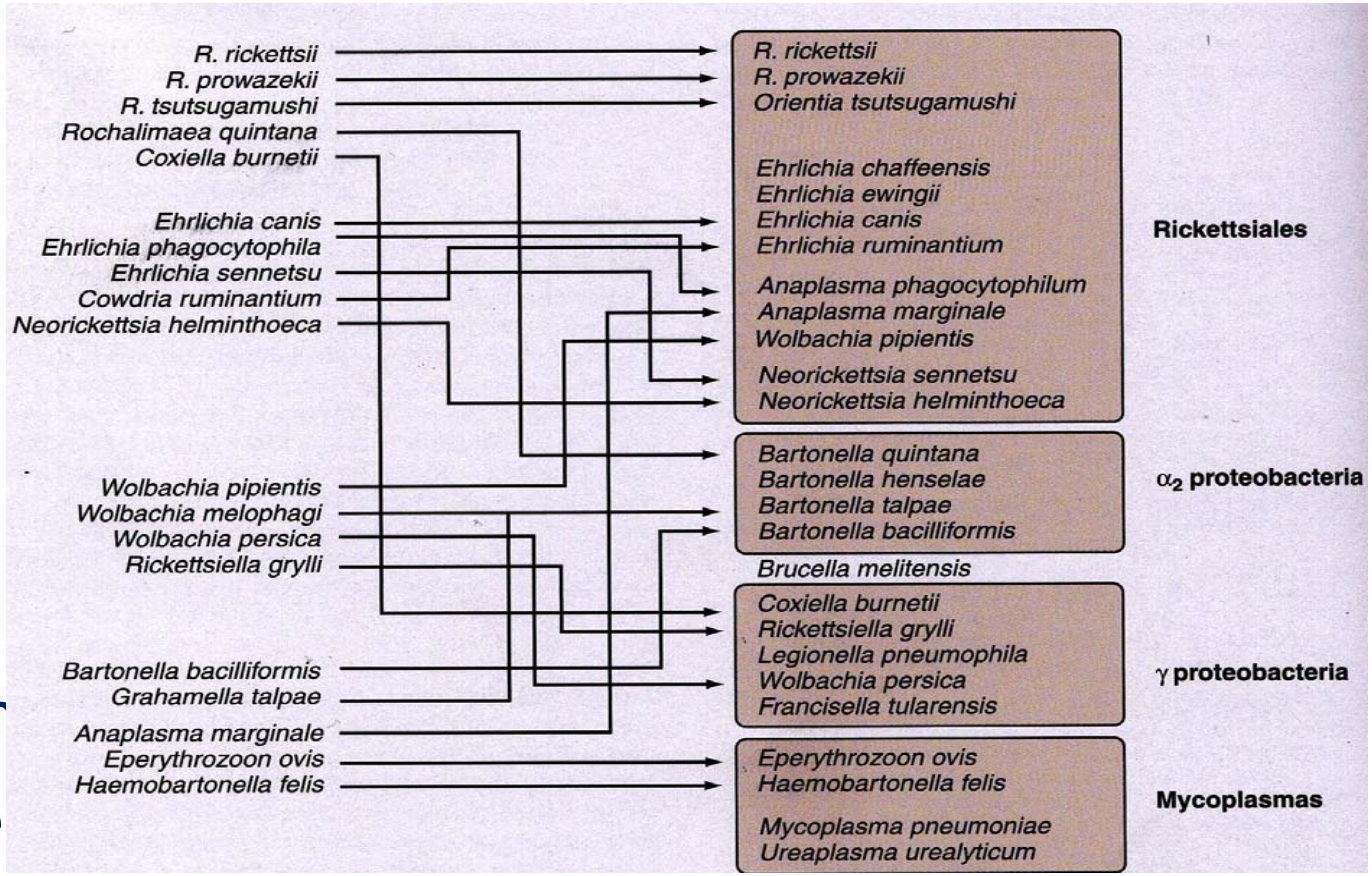
- Spore-like form
- Extremely resistant
- Survival under harsh conditions :
  - 60 min at 60^0 C
  - 10 month at 20^0 C
  - in formalin 0.5 %
  - UV-irradiation





Coxiella burnetii : Bacteriology

- Pleomorphic
- Coccobacillus
- Gram-negative
- 0.2 - 0.7 mm
- Gimenez stainir
- Agent of Q feve



Complete genome sequence of the Q-fever pathogen *Coxiella burnetii*

Rekha Seshadri*, Ian T. Paulsen*†, Jonathan A. Eisen*†, Timothy D. Read*, Karen E. Nelson*, William C. Nelson*, Naomi L. Ward*‡, Hervé Tettelin*, Tanja M. Davidsen*, Maureen J. Beanan*, Robert T. Deboy*, Sean C. Daugherty*, Lauren M. Brinkac*, Ramana Madupu*, Robert J. Dodson*, Hoda M. Khouri*, Kathy H. Lee*, Heather A. Carty*, David Scanlan*, Robert A. Heinzen§, Herbert A. Thompson¶, James E. Samuel‖**, Claire M. Fraser*††, and John F. Heidelberg*‡

*The Institute for Genomic Research, 9712 Medical Center Drive, Rockville, MD 20850; †Johns Hopkins University, Charles and 34th Streets, Baltimore, MD 21218; ¶Centers for Disease Control and Prevention, National Center for Infectious Diseases, Division of Viral and Rickettsial Diseases, Viral and Rickettsial Zoonoses Branch, 1600 Clifton Road, Atlanta, GA 30333; ‖Department of Medical Microbiology and Immunology, Texas A&M University System Health Science Center, College Station, TX 77843-1114; §Department of Molecular Biology, University of Wyoming, Laramie, WY 82071-3944; ††Departments of Pharmacology and Microbiology and Tropical Medicine, George Washington University School of Medicine, 2300 Eye Street N.W., Washington, DC 20037; and ‡Center of Marine Biotechnology, University of Maryland Biotechnology Institute, Baltimore, MD 21202

Communicated by Harley W. Moon, Iowa State University, Ames, IA, February 11, 2003 (received for review November 25, 2002)

Proc Natl Acad Sci U S A. 2003 Apr 29;100(9):5455-60

The 1,995,275-bp genome of *Coxiella burnetii, Nine Mile phase I* RSA493, a highly virulent zoonotic pathogen and category B bioterrorism agent, was sequenced by the random shotgun method. This bacterium is an obligate intracellular acidophile that is highly adapted for life within the eukaryotic phagolysosome. Genome analysis revealed many genes with potential roles in adhesion, invasion, intracellular trafficking, host-cell modulation, and detoxification. A previously uncharacterized 13-member family of ankyrin repeat-containing proteins is implicated in the pathogenesis of this organism. Although the lifestyle and parasitic strategies of *C. burnetii resemble that of Rickettsiae and Chlamydiae,* their genome architectures differ considerably in terms of presence of mobile elements, extent of genome reduction, metabolic capabilities, and transporter profiles. The presence of 83 pseudogenes displays an ongoing process of gene degradation. Unlike other obligate intracellular bacteria, 32 insertion sequences are found dispersed in the chromosome, indicating some plasticity in the *C. burnetii genome. These analyses suggest that the obligate* intracellular lifestyle of *C. burnetii may be a relatively recent* innovation.

Coxiella burnetii : Genome

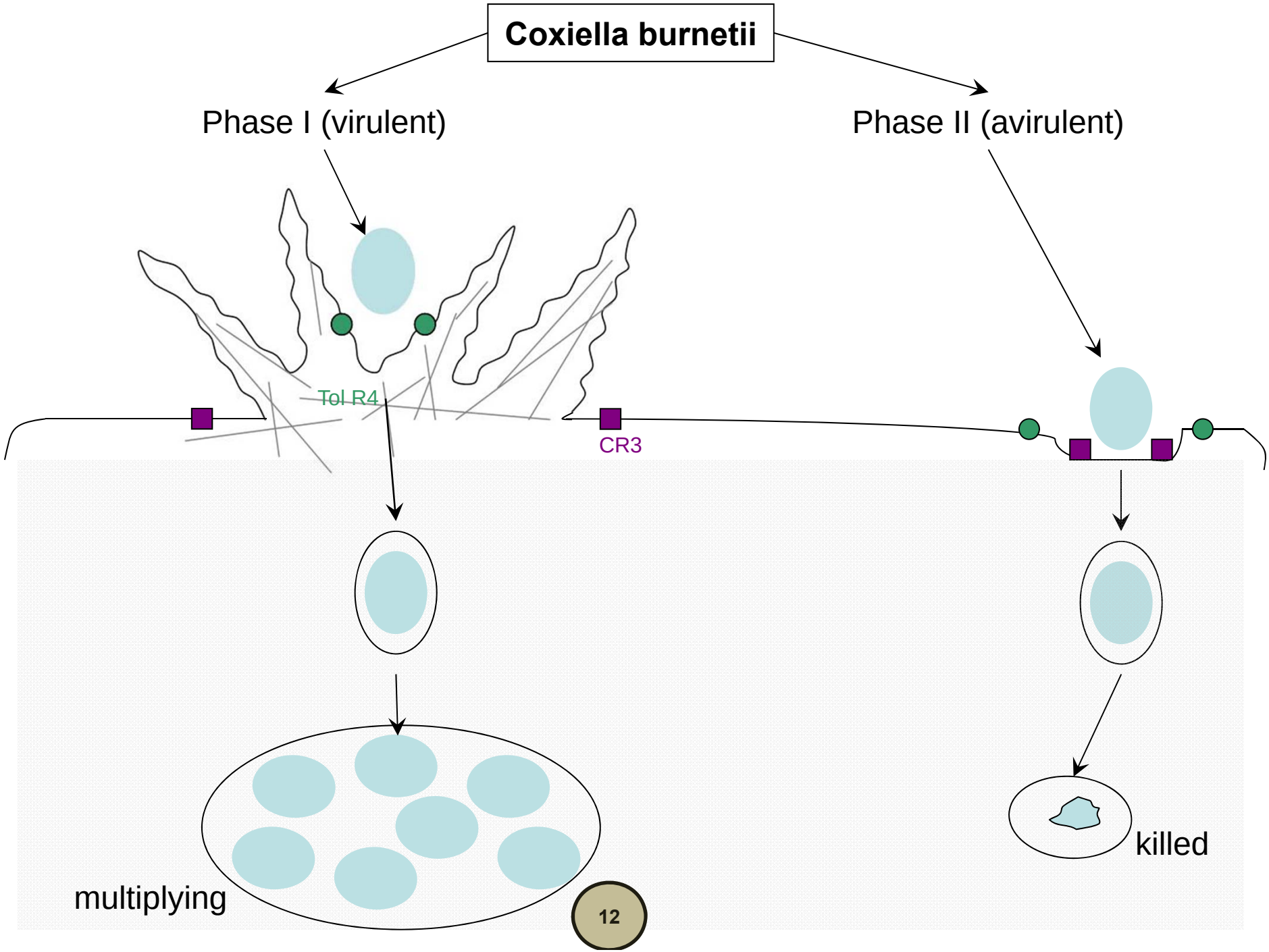
Table 1. General features of the *C. burnetii* genome

	Chromosome	QpH1
Size, bp	1,995,275	37,393
G + C content, %	42.6	39.3
Protein-coding genes		
No. similar to known proteins	1,022	11
No. similar to proteins of unknown function*	179	5
No. of conserved hypotheticals†	200	1
No. of hypotheticals‡	693	23
Total	2,094	40
Average ORF size, bp	849	736
Coding, %	89.1	78.8
Stable RNAs		
rRNA	3	0
tRNA	42	0

Seshadri R. *et al.* Complete genome sequence of the Q-fever pathogen *Coxiella burnetii*. PNAS, 2003;100:5455-60

Bacteriology : *Coxiella burnetii*

Phase I	Phase II
Infectious	Non infectious Deleted (17 KB) mutant of phase I
Resists macrophage killing	Killed by macrophage
Only form present in nature	Absent in nature
Multiply slowly in cells	Multiply rapidly in cells
Enters by αvB3	Enters by αvB3 and complement receptor
Determine late antibody response and high antibody response in chronic disease	Determine early antibody response (acute infection)


Coxiella burnetii
Phase I (virulent)
Phase II (avirulent)
Tol R4
CR3
multiplying
killed

Coxiella burnetii and Q fever

- A Bacteriology
- **B Epidemiology**
- C Clinical presentation
- D Pathophysiology
- E Specificity in Guyana
    - Clinical
    - Epidemiological
    - Bacteriological
    - Immune response
- F Diagnostic
- G Treatment
- H Prophylaxis
- I Controversy

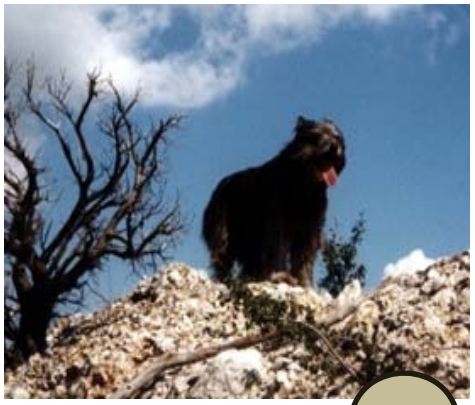
Epidemiology

Q fever: Modes of transmission to man

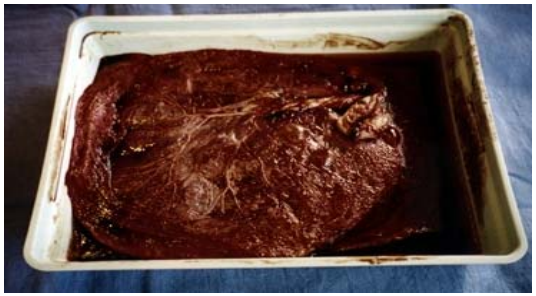
- Inhalation of contaminated aerosols +++
- Oral route (contaminated milk and cheese)
- Percutaneous route (intradermal inoculation)
- Vertical transmission
- Person-to-person transmission (autopsies, deliveries, blood transfusion)
- Sexual transmission

Epidemiology

- Primary reservoirs
  - Sheep
  - Goats
  - Cattle
  - Cats
  - Dogs
  - Pigeons
  - Humans



- Sources of transmission
  - Uterus
  - Placenta
  - Feces
  - Urine
  - Milk
  - Straw, manure
  - Ticks
  - Sperm
  - Blood
  - Intentional



Epidemics described by CNR (1)

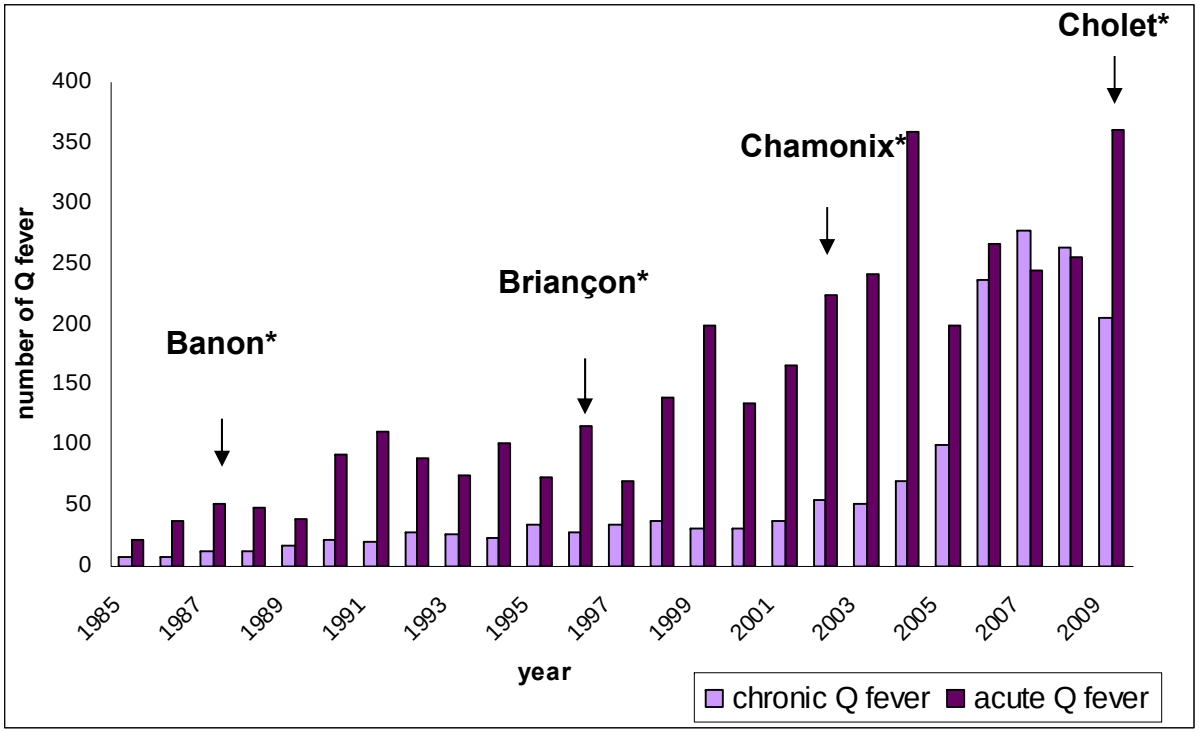


Fig 2. Acute and Chronic Q fever from 1985 to 2009. * places where outbreaks were reported.

Epidemics in Europe from 1987 to 2009

Year Pub	outbreak	n° of cases	ref
Scotland:			
2006	2006	51	25
2008	2006	138	26
UK :			
2004	2002	95	27
2010	2007	30	28

Netherland :

Year Pub	outbreak	n° of cases	ref
2010	2007	168	29
	2008	1000	
	2009	2357	
2010	2008	28	30

Germany:

Year Pub	outbreak	n° of cases	ref
1997	1996	45	b [23]
1996	1994	18	c [23]
2006	2003	299	31
2007	2005	331	32

France :

Year Pub	outbreak	n° of case	ref
1992	1987	40	f [23]
1999	1990-1995	289	m [24]
2002	1996	29	d [23]
1999	1996	5	h [23]
2003	2000	10	j [24]
2003	2000	5	k [24]
2004	2000	17	41
2006	2002	101	14
unpub	2009	50	unp

Year Pub	outbreak	n° of cases	ref
Poland:			
1996	1992-94	25	e [23]
Slovakia :			
1998	1993	113	g [23]
Slovenia:			
2007	2007	36	33
Croatia:			
2005	2004	14	34
Bosnia			
2003	1997	26	l [24]
Kosovo:			
2007		59	35
Bulgaria:			
2009	2004	220	36

Spain :

Year Pub	outbreak	n° of cases	ref
2007	2003	60	39
2006	2004	22	40

Italy :

Year Pub	outbreak	n° of cases	ref
1999	87-88	235	n [24]
1996	93	58	a [23]
2004	2003	133	i [24]
2005		65	33

Fig 8. European outbreaks from 1987 to 2009

Seasonal variation (1)

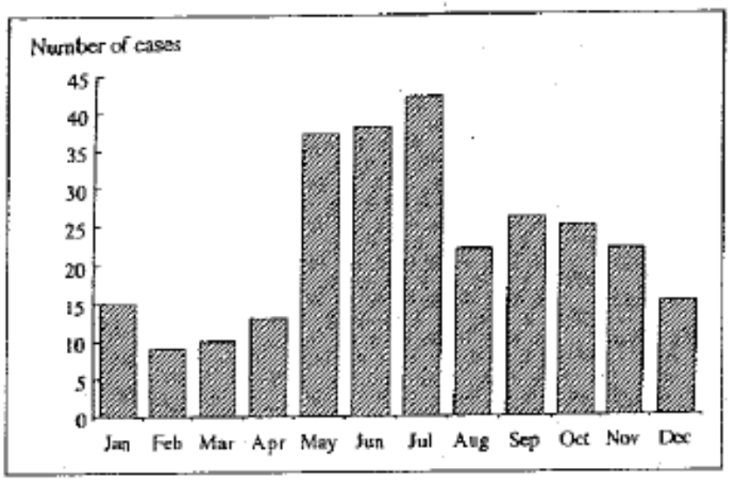


Figure 2. Seasonal distribution of 274 cases of acute Q fever detected at the French National Reference Center between 1982 and 1990.

- 274 cases of acute fever Q of 1982 to 1990 at CNR
- Significant growth of the frequency in May, June and July (42.7% of cases, $p<0.001$)

Epidemiologic features and clinical presentation of acute Q fever in hospitalized patients: 323 French cases.

Tissot Dupont H, Am J Med. 1992 Oct;93(4):427-34.

Epidemiological acute Q fever

Findings in 1070 cases (1985-1998)

Feature	Percent
Males	71
Rural life	36
Occupational exposure	8
Contact with animals	35
Raw cheese consumption	23
Immunodepression	5

Rickettsia follows rickettsiologists

Acute cases diagnosed (may be 2% of infection without active search)

- France $3/10^6$/year
- In our region $19/10^6$/year
- In our departement $40/10^6$/year (2 %real incidence)

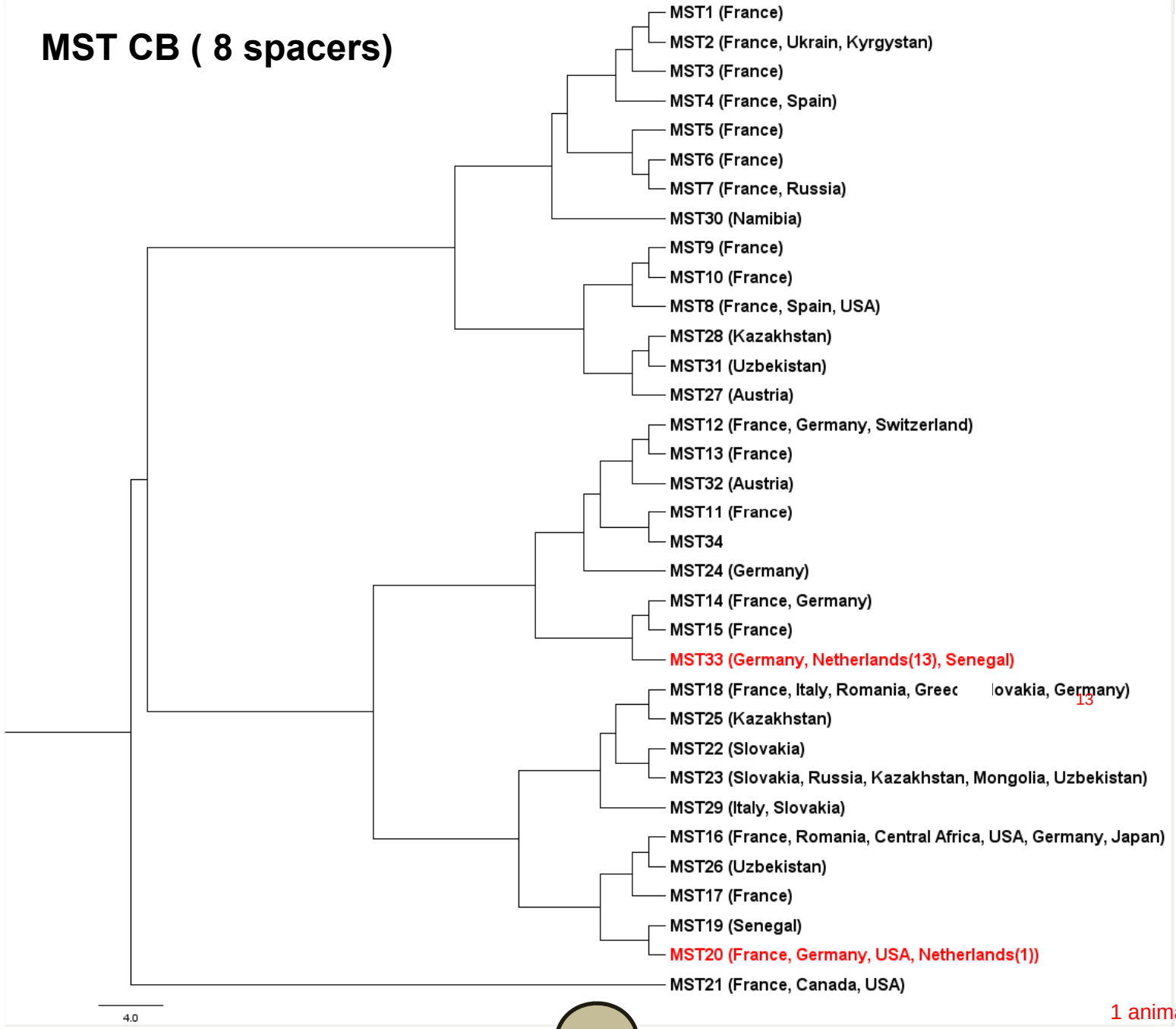
Molecular Epidemiology of *Coxiella burnetii* from Ruminants in Q Fever Outbreak, the Netherlands

Hendrik I.J. Roest, Robin C. Ruuls, Jeroen J.H.C. Tilburg, Marrigje H. Nabuurs-Franssen, Corné H.W. Klaassen, Piet Vellema, René van den Brom, Daan Dercksen, Willem Wouda, Marcel A.H. Spierenburg, Arco N. van der Spek, Rob Buijs, Albert G. de Boer, Peter Th.J. Willemsen, and Fred G. van Zijderveld

Q fever is a zoonosis caused by the bacterium *Coxiella burnetii*. One of the largest reported outbreaks of Q fever in humans occurred in the Netherlands starting in 2007; epidemiologic investigations identified small ruminants as the source. To determine the genetic background of *C. burnetii* in domestic ruminants responsible for the human Q fever outbreak, we genotyped 126 *C. burnetii*–positive samples from ruminants by using a 10-loci multilocus variable-number tandem-repeat analyses panel and compared them with internationally known genotypes. One unique genotype predominated in dairy goat herds and 1 sheep herd in the human Q fever outbreak area in the south of the Netherlands. On the basis of 4 loci, this genotype is similar to a human genotype from the Netherlands. This finding strengthens the probability that this genotype of *C. burnetii* is responsible for the human Q fever epidemic in the Netherlands.

Emerg Infect Dis. 2011;17(4):668-675.

MST CB (8 spacers)

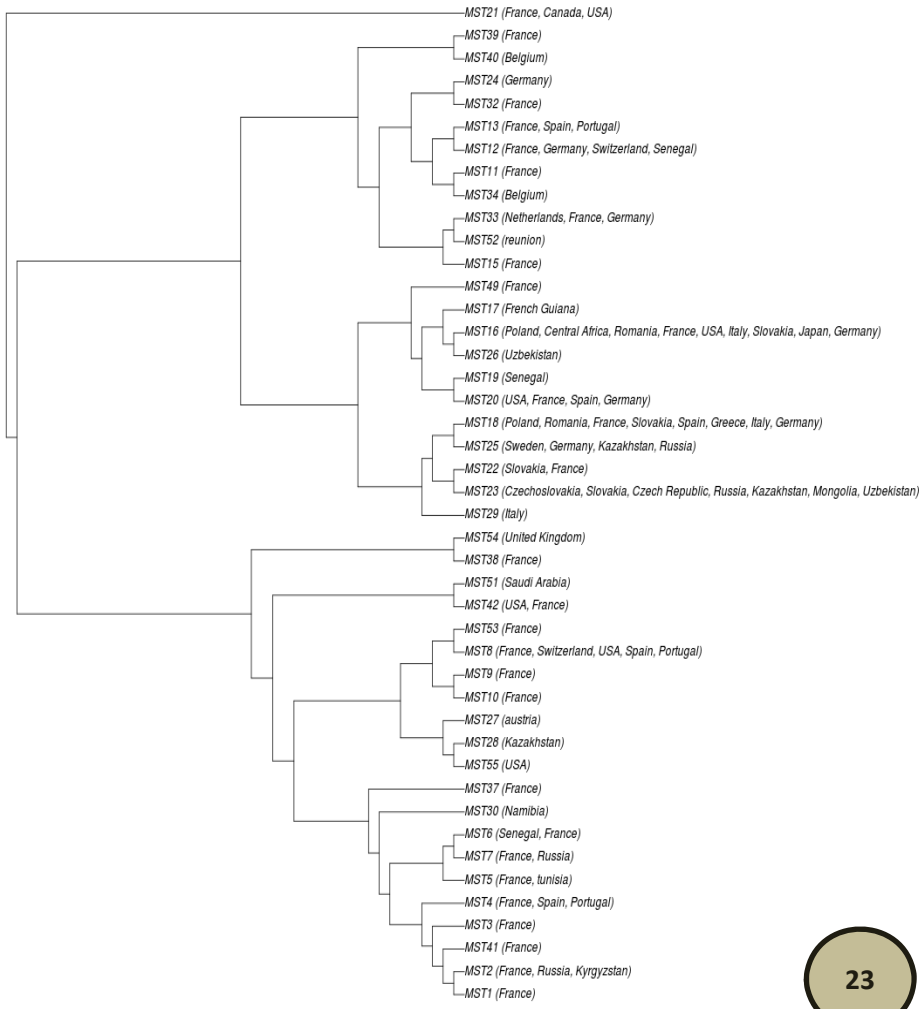


1 animal

RESULTS

• We tested 250 humans and 135 animal samples with MST. this genotyping method reveals a genetic diversity by identifying 46 different *C. burnetii* genotypes

Figure 1. Phylogenetic diversity of 48 genotypes of *C. burnetii* identified by using MST



•Among, the 385 specimens tested, the MST 33 (19%), 8(15%) and 20 (10%), are the most frequent genotype found

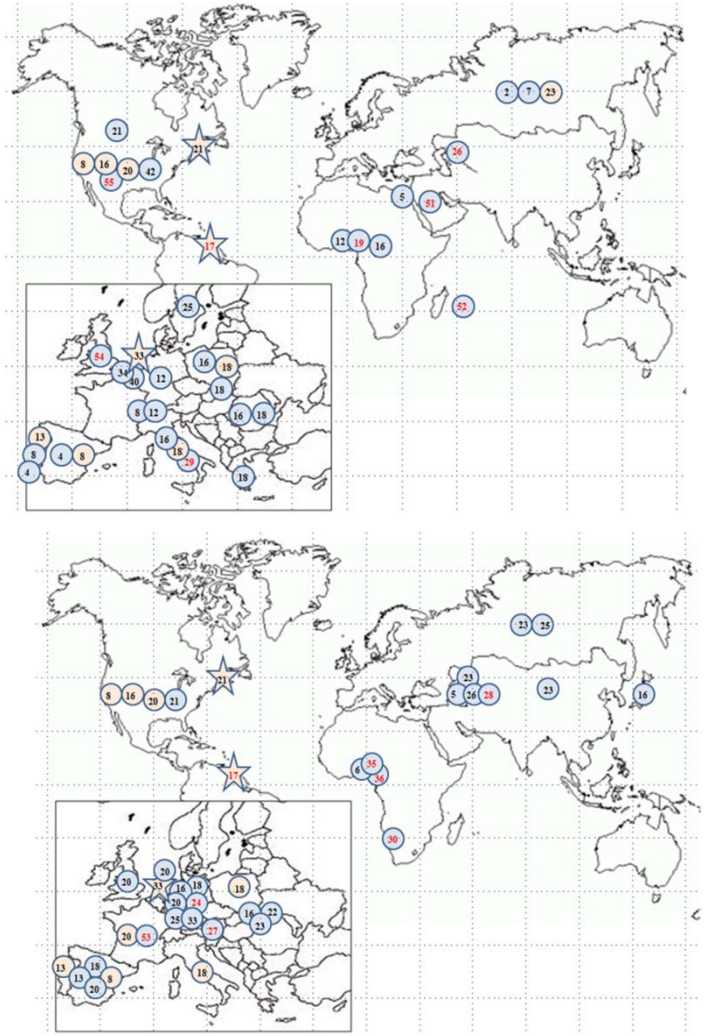
•To date, we identified specific *C. burnetii* genotype in French Guiana (MST17), Senegal (MST 19, 35, 36), Italia (MST29), Reunion Island (MST52), Belgium (MST34 and MST40), United Kingdom (MST54), Austria (MST27), Namibia (MST30), Kazakhstan (MST28), United states (MST55) and Saudi Arabia (MST51).

•Two distinct clones were identified as responsible of Q fever epidemic. *C. burnetii* MST 33 was identified as responsible of the largest outbreak of Q fever in Netherland and MST 17 is an epidemic genotype circulating in French Guiana causing severe acute pneumonia in human.

RESULTS

• 38 different genotypes was found in humans and 20 in animals samples (figure 2) distributed as below.

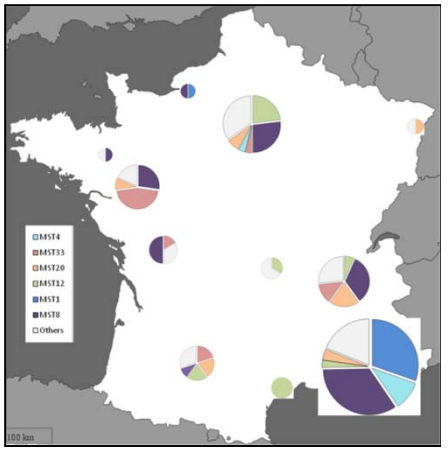
Figure 2. Worldwide distribution of MST-defined clone of human (A) and animals (B) samples



*Specific MST for a country was indicated in red

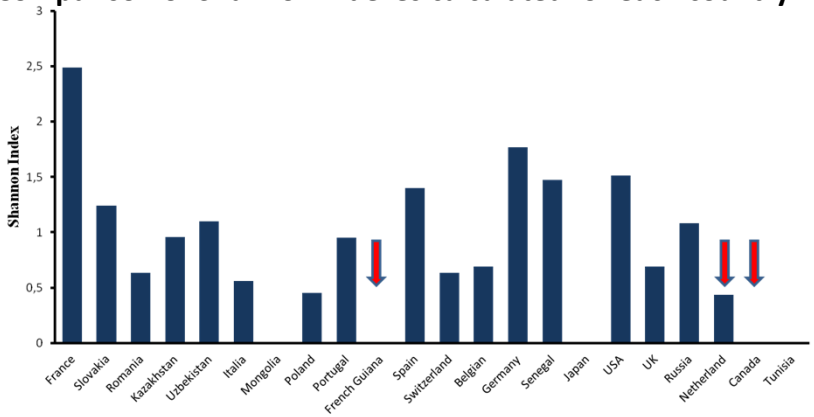
Figure 3 : Distribution of the different MST genotypes in France

• Among the 157 samples tested from France, we found 27 different genotypes circulating. MST 8, 1, 12, 20, 33 and 4 are the most common genotype with a proportion of 31, 16, 9, 7, 7 and 6% respectively. These frequent clones were also found in many countries (up to 9 countries). The majority of these genotypes are distributed throughout the country but still there is the predominance of certain genotype based on regions



• We calculated the Shannon index to quantified the biodiversity of the MST genotype per country. We showed that diversity is very important in France, Germany, Spain and poor in French Guiana, Canada and Netherland suggesting an epidemic situation (Figure 4)

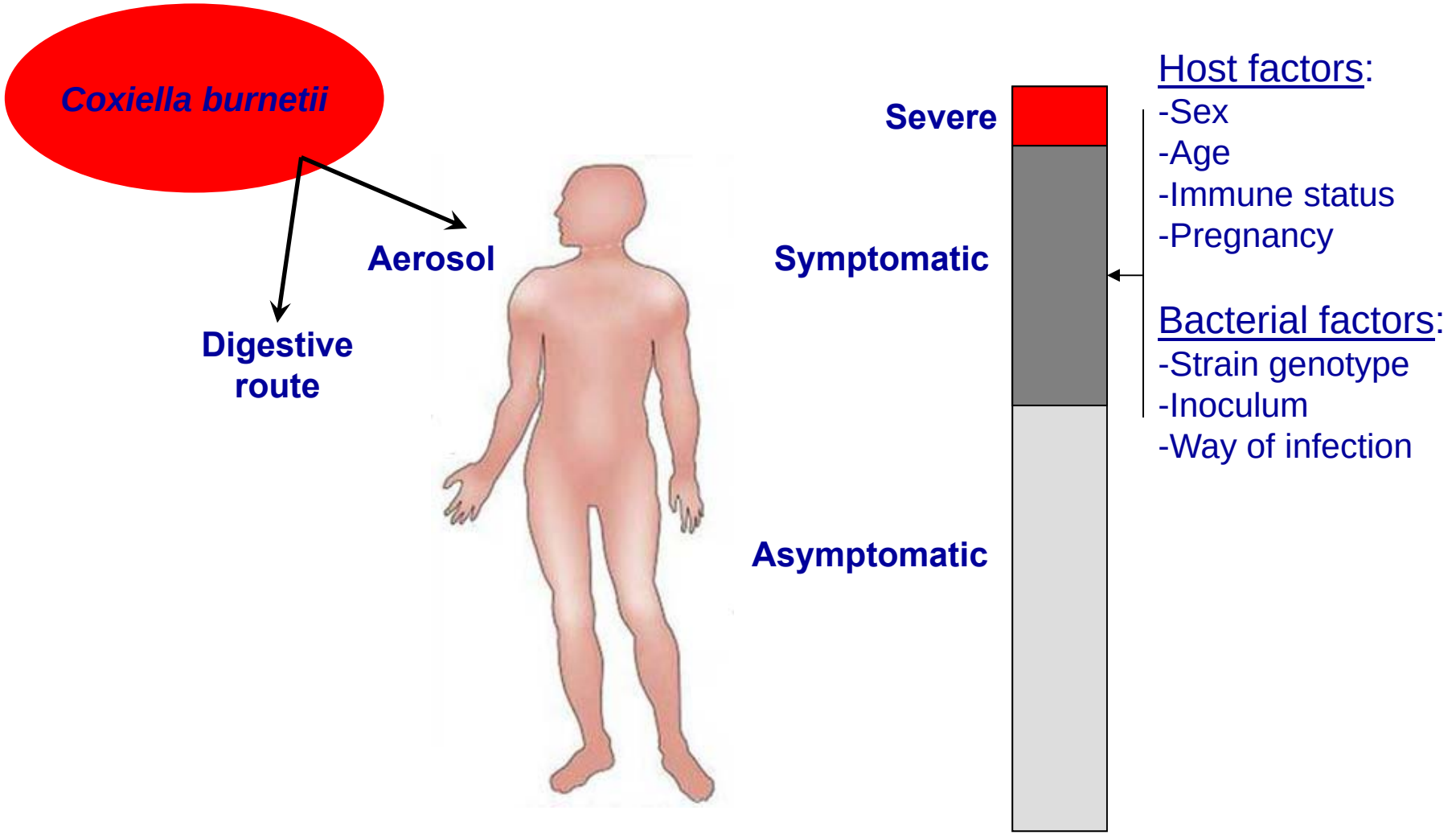
Figure 4 : Comparison of Shannon indexes calculated for each country



Number of samples	159	6	3	7	3	4	2	6	10	13	18	3	2	11	7	5	17	2	15	77	7	2

Coxiella burnetii and Q fever

- A Bacteriology
- B Epidemiology
- **C Clinical presentation**
- D Pathophysiology
- E Specificity in Guyana
  - Clinical
  - Epidemiological
  - Bacteriological
  - Immune response
- F Diagnostic
- G Treatment
- H Prophylaxis
- I Controversy



Acute Q fever

Acute Q fever :
Clinical manifestations

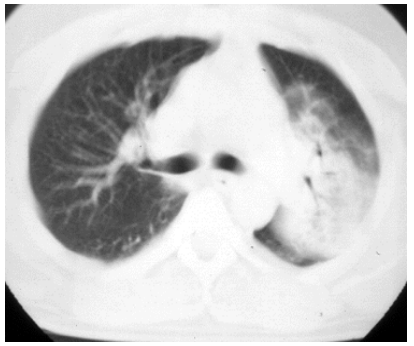
«Then the suspicion arose and gradually grew into a conviction that we were dealing with a type of fever which had not previously been described.
It became necessary to give it a name, and «Q fever» was chosen to denote it until fuller knowledge should allow a better name.»

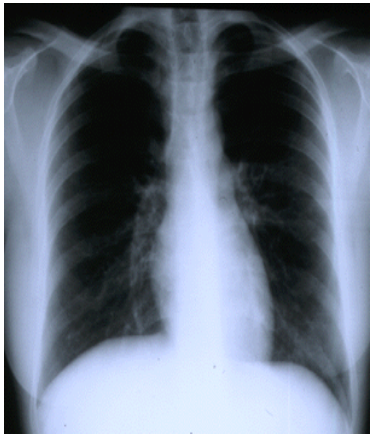
- Onset nearly always abrupt
- Symptoms vary from country to country
- At least 50 % of infections are totally asymptomatic
- Mortality < 0.5 %
- G. Dupuis *et al.*: Outbreak of 415 cases in a Swiss alpine valley of 4652 inhabitants
  - **191 cases (46 %): febrile illness**
  - **224 cases (54 %): asymptomatic**
  - **8 cases (2 %): admission to hospital**
  - **Antibiotics did not shorten duration of fever**

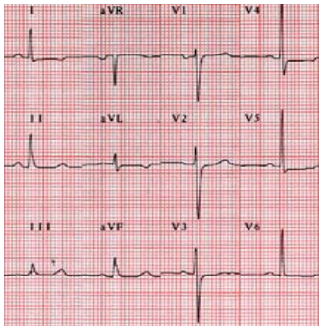
Acute Q fever :

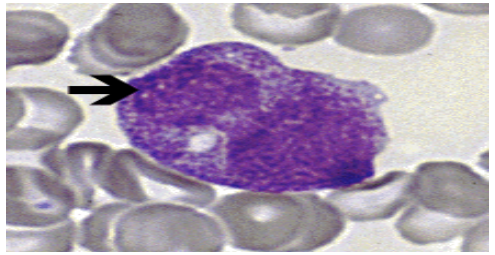
Clinical manifestations

- Asymptomatic
- Self limited febrile illness
- Pneumonia
- Hepatitis
- Meningoencephalitis
- Pericarditis
- Myocarditis
- Exanthema
- Others: bone marrow necrosis, hemophagocytosis, hemolytic anemia, lymphadenopathy, erythema nodosum, diarrhea, splenic rupture, pancreatitis...



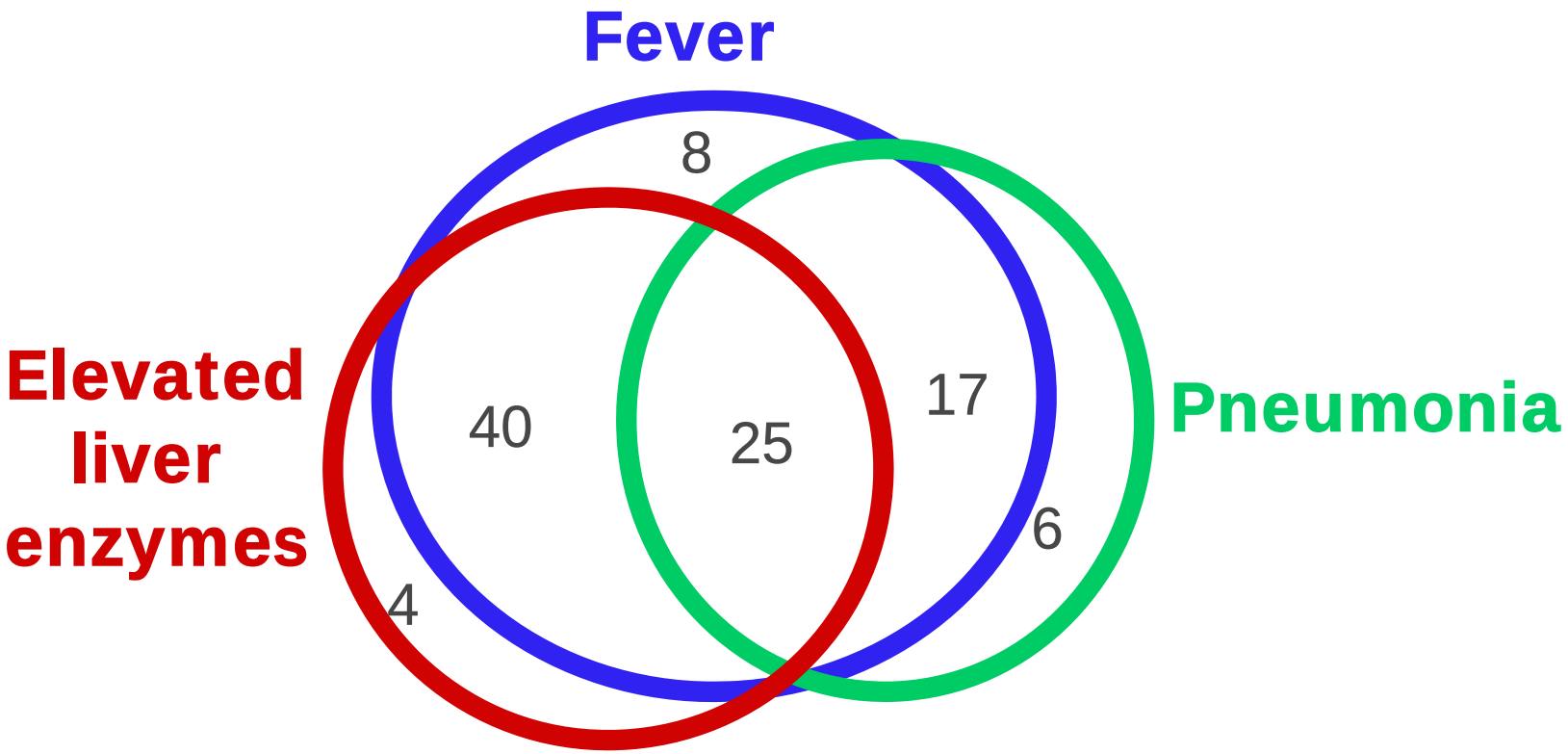






Acute Q fever

Distribution of the three major clinical manifestations of acute Q fever



Tissot-Dupont H. et al. Epidemiologic features and clinical presentation of acute Q fever in hospitalized patients: 323 French cases. Am J Med. 1992;93:427-34.

Acute Q fever in 1,070 patients*

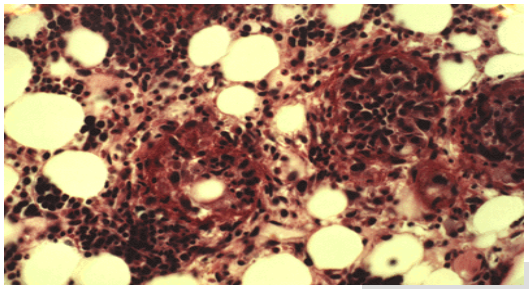
Clinical presentation	%
Isolated fever	14
Hepatitis	40
Pneumonia	17
Pneumonia + Hepatitis	20
CSF sampling	4
Meningitis	0,5
Meningoencephalitis	1
Pericarditis	1
Myocarditis	1
Not determined	3

*Patients classified in 1 category only

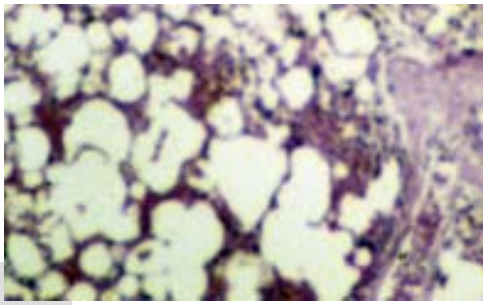
Raoult D., et al. Q fever 1985-1998. Clinical and epidemiologic features of 1,383 infections. Medicine (Baltimore). 2000;79:109-23.)

Acute Q fever :

Variations from country to country



Hepatitis or pneumonia ?



	Hepatitis	Pneumonia	Febrile illness
Basque county	+	+++	+
Andalusia	+++	+	++
France	+++	+	+
Canada	+	+++	+
Australia	+++	+	+

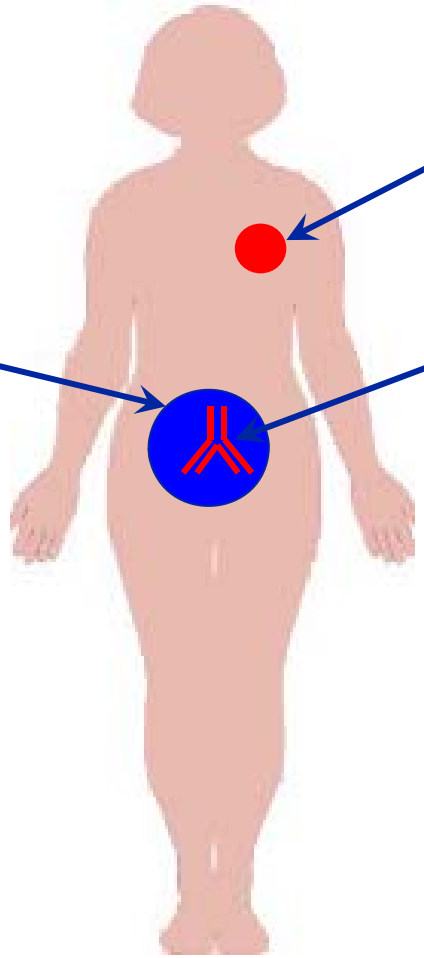
Maurin M, Raoult D. Q fever. Clin Microbiol Rev. 1999 ;12:518-53.

Chronic fatigue

- 5-10 % of patients
- 6-36 months
- One report PCR positive from bone marrow
- Cytokine abnormalities (?)
- Treatment ?
- Variations from country to country (cultural?)
- UK and Australia > France, Spain, USA and Canada

3 months – 3 years
After primoinfection
(symptomatic or not)

High antibodies



Valvulopathy

→ endocarditis

Pregnancy

→ Still birth
Fœtal diseases

Aortic Aneurisms
And
Vascular prosthesis

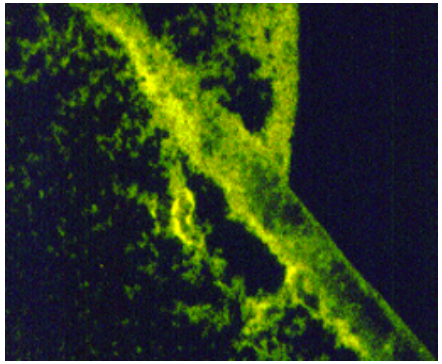
→ vascular infection

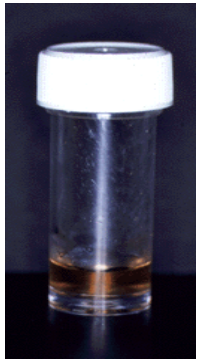
Coxiella burnetii and Q fever

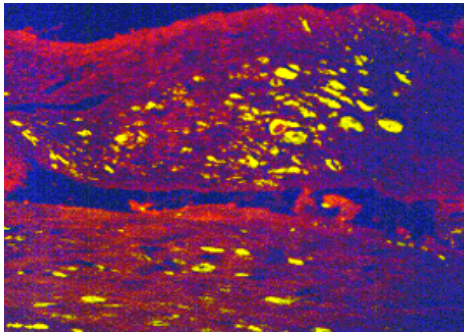
- A Bacteriology
- B Epidemiology
- C Clinical presentation
- D Pathophysiology
- E Specificity in Guyana
  - Clinical
  - Epidemiological
  - Bacteriological
  - Immune response
- **F Diagnostic**
- G Treatment
- H Prophylaxis
- I Controversy

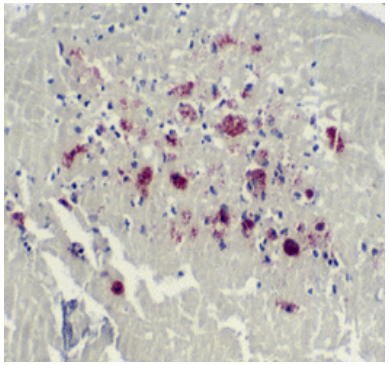
Diagnostic

- Serology
- Isolation (Shell vial technique): blood and heart valves
- Immunofluorescence or Immunohistochemistry: heart valves
- PCR









Diagnostic

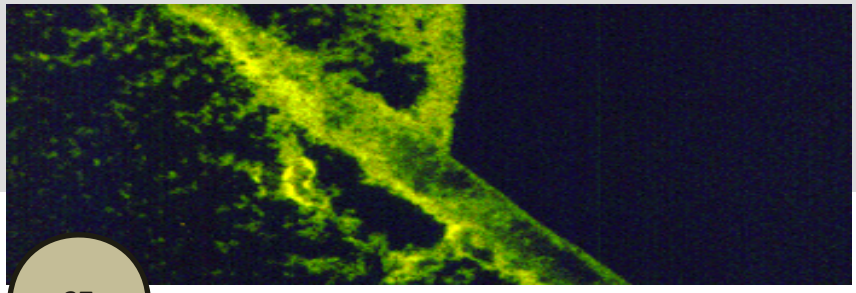
Cutoff proposal for Q fever diagnosis using the microimmunofluorescence and interpretation of serological results obtained with a single serum sample

Phase II antibodies		Phase I antibodies		Interpretation
IgG	IgM	IgG	IgA	
≤ 100				Active Q fever improbable
≥ 200	≥ 50			Acute Q fever (100 % predictive)
		≥ 1600	≥ 100	Chronic Q fever (94 % predictive)

Diagnostic

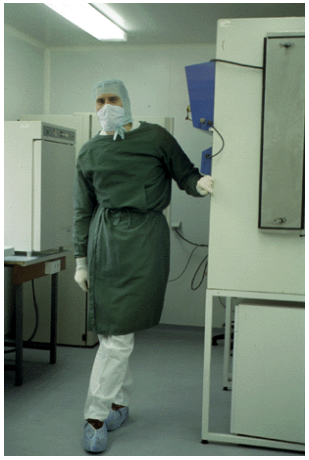
Characteristics of cutoff titers for MIF

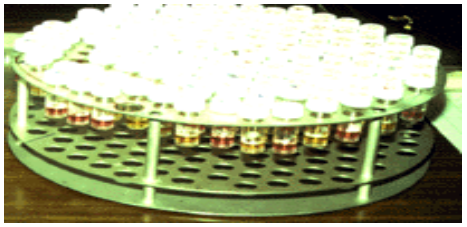
	Cutoff titers	Sens. (%)	Spec. (%)	PPV (%)	NPV (%)
Acute Q fever	IgG II ≥ 1:200 and IgM II ≥ 1:50	58.4	100	100	94
Chronic Q fever	IgG I ≥ 1:1600	80.4	99.8	100	99.6



Diagnostic : Isolation

- Hazardous
  - P3-laboratories
  - Laminar-flow hoods
- 3 systems
  - Laboratory animals (guinea-pig)
  - Embryonated eggs
  - Continuous cell lines (HEL; Vero)
- < 100 isolates worldwide available





Dutch consensus guideline (38)
Proven : Any of the following ! - Positive PCR for Coxiella burnetii in serum, plasma, or tissue in the absence of acute Q fever - IFA phase I titer ≥1,024 with definite endocarditis according to the revised Duke criteria (21) - Indication of vascular infection on PET/CT, CT, MRI, or ultrasound testing
Probable : IFA phase I IgG titer ≥1,024 and any of the following clinical manifestations : - Valvulopathy not meeting the criteria of endocardial involvement of the major modified Duke criteria (39) - Aneurysm, vascular prosthesis or prosthetic valve without signs of infection on PET/CT,CT,MRI, or ultrasound testing - Signs of possible chronic Q fever infection of noncardiac or vascular origin on PET/CT, CT or ultrasound testing -Pregnancy - Clinical symptoms of chronic infection (i.e., fever, night sweats, weight loss, hepatosplenomegaly) -Histopathologic proven granulomatous inflammation - Immune disorder
Possible : IFA phase I IgG titer ≥1,024 without clinical manifestations as described above

Then a little bit competition

- New definition then
- Q fever and pregnancy
- Prophylaxis of endocarditis
- New culture medium

Then a little but of discovery

Multiplexed whole bacterial antigen microarray, a new format for the automation of serodiagnosis: the culture-negative endocarditis paradigm. Gouriet F, Samson L, Delaage M, Mainardi JL, Meconi S, Drancourt M, Raoult D. Clin Microbiol Infect. 2008 Dec;14(12):1112-8

- Lymphoma caused by *C.burnetii*
- French Guyana
  - New strain
  - New reservoir
  - New mechanism of pathogenicity
- New treatments?

Q PCR

- Discrepancies among laboratories
  - Some team report positive PCR in asymptomatic patients or years after infection
  - Our team find positive PCR only during the early phase of acute infection and during chronic infection when antibodies anti phase I IgG are between 1/800 and 1/6400
- Indications
  - Acute cases negative for IgM (pharyngeal swabs):
  - Suspicion of chronic infection with Ig anti phase I: IgG $\geq$ 800
  - Use IS IIII (7 to 20 copies) as a target
  - Increased used of threat samples

Molecular detection of Coxiella burnetii in the sera of patients with Q fever endocarditis or vascular infection.Fenollar F, Fournier PE, Raoult D. J Clin Microbiol. 2004 Nov;42(11):4919-24.

Fournier PE, Raoult D. Comparison of PCR and serology assays for early diagnosis of acute Q fever.J Clin Microbiol. 2003 Nov;41(11):5094-8

Rolain JM, Raoult D. Molecular detection of *Coxiella burnetii* in blood and sera during Q fever. QJM. 2005 ;98:615-7.

Being careful with PCR to avoid erroneous discoveries. Raoult D. Scand J Infect Dis. 2011 May;43(5):323-4.

COXIELLA BURNETII INFECTION AMONG BLOOD DONORS DURING THE 2009 Q-FEVER OUTBREAK IN THE NETHERLANDS.

Hogema BM, Slot E, Molier M, Schneeberger PM, Hermans MH, van Hannen EJ, van der Hoek W, Cuijpers HT, Zaaijer HL.

Transfusion. 2012 Jan;52(1):144-50.

CONCLUSION:

In the area with highest incidence during a large Q-fever outbreak, 3 of 1004 blood donations contained C. burnetii DNA (0.3%; 95% confidence interval, 0.1%-1.0%). A total of 66 of 543 (12.2%) donors tested positive for anti-Coxiella IgG. Ten seroconversions were detected, resulting in an incidence of 5.7% per year, which is more than 10-fold higher than the local number of reported clinical cases (0.47% per year).

0,3 % of PCR positive in asymptomatic cases!

Persistence of DNA in a cured patient and positive culture in cases with low antibody levels bring into question diagnosis of Q fever endocarditis.

Edouard S, Million M, Lepidi H, Rolain JM, Fournier PE, La Scola B, Grisoli D, Raoult D.

J Clin Microbiol. 2013 Sep;51(9):3012-7.

We evaluated the performance of tools for diagnosing Q fever cardiovascular infection. We retrospectively analyzed 162 cardiovascular samples from 125 patients who were tested serologically by immunofluorescence, quantitative PCR (qPCR), 16S rRNA gene amplification, culture, and immunohistochemistry, and we assessed the viability of Coxiella burnetii by measuring the transcription of the 16S rRNA gene. The qPCR technique was significantly more sensitive than 16S rRNA gene amplification ($P < 0.0001$), cell culture ($P = 0.0002$), and immunohistochemistry ($P < 0.0001$). The sensitivity of these techniques was reduced when applied to patients who had been previously treated. The severity of infection appears to be correlated with phase I IgG levels. We report for the first time 4 cases of endocarditis with positive qPCR and/or culture assay result from patients with a low phase I IgG (IgG I) titer (<800), and we have identified the longest (16 years) persistence of DNA described in a heart valve from a patient cured after being previously treated for endocarditis. The active transcription of the 16S rRNA gene was found in 19/59 tested samples, with a positive predictive value of 100% for a positive culture. In conclusion, the diagnosis of Q fever cardiovascular infection should not be excluded in patients with low titers of phase I IgG when they present with valvulopathy. We recommend testing cardiovascular samples using 3 or 4 different biopsy sections by qPCR evaluation for patients with IgG I titers of ≥200.

Dutch consensus guideline (38)	
Proven : Any of the following ! - Positive PCR for Coxiella burnetii in serum, plasma, or tissue in the absence of acute Q fever - IFA phase I titer ≥1,024 with definite endocarditis according to the revised Duke criteria (21) - Indication of vascular infection on PET/CT, CT, MRI, or ultrasound testing	**Proven** : Does not make sense you need to have proof of infection AND (not or) microbiological evidence
Probable : IFA phase I IgG titer ≥1,024 and any of the following clinical manifestations : - Valvulopathy not meeting the criteria of endocardial involvement of the major modified Duke criteria (39) - Aneurysm, vascular prosthesis or prosthetic valve without signs of infection on PET/CT,CT,MRI, or ultrasound testing - Signs of possible chronic Q fever infection of noncardiac or vascular origin on PET/CT, CT or ultrasound testing -Pregnancy - Clinical symptoms of chronic infection (i.e., fever, night sweats, weight loss, hepatosplenomegaly) -Histopathologic proven granulomatous inflammation - Immune disorder	**Probable** : (some positive have no endocarditis or identified infection) -This is valuable only if other factor actively tested (Rheumatoïd factor mycotic aneurism and negative) - Does not make sense for hepatitis – No chronic hepatitis described -I don't know what pregnancy is doing here. -The problem of pregnancy is acute Q fever in the 3 first months -I don't know what granulomateous infection is linked to chronic Q fever - The relation between immune disorder and *C. burnetii* is strange
Possible : IFA phase I IgG titer ≥1,024 without clinical manifestations as described above	Everything is possible!

Then a little bit competition

- New definition then
- Q fever and pregnancy
- Prophylaxis of endocarditis
- New culture medium

Then a little but of discovery

Multiplexed whole bacterial antigen microarray, a new format for the automation of serodiagnosis: the culture-negative endocarditis paradigm. Gouriet F, Samson L, Delaage M, Mainardi JL, Meconi S, Drancourt M, Raoult D. Clin Microbiol Infect. 2008 Dec;14(12):1112-8

- Lymphoma caused by *C.burnetii*
- French Guyana
  - New strain
  - New reservoir
  - New mechanism of pathogenicity
- New treatments?

NEW DEFINITION OF CLINICAL FORMS
(avoiding chronic)

1) Asymptomatic infection
2) Acute Q fever
3) Acute Q fever during pregnancy
4) *C.burnetii* endocarditis
5) *C.burnetii* vascular infection
6) *C.burnetii* osteoarticular infection
7) Fatigue following acute Q fever
8) Others

Chronic Q fever: expert opinion versus literature analysis and consensus.
Raoult D.
J Infect. 2012 Aug;65(2):102-8.

Table 2 Definition of Q fever endocarditis (adapted from Li et al.).

A. Definite criterion

Positive culture, PCR, or immunochemistry of a cardiac valve.

B. Major criteria

Microbiology: positive culture or PCR of the blood or an emboli or serology with IgGI antibodies ≥6400

Evidence of endocardial involvement:

Echocardiogram positive for IE: oscillating intra-cardiac mass on valve or supporting structures, in the path of regurgitant jets, or on implanted material in the absence of an alternative anatomic explanation; or abscess; or new partial dehiscence of prosthetic valve; or new valvular regurgitation (worsening or changing of pre-existing murmur not sufficient).

Pet-scan showing a specific valve fixation and mycotic aneurism.

C. Minor criteria

Predisposing heart condition (know or found on echography)

Fever, temperature > 38 °C

Vascular phenomena, major arterial emboli, septic pulmonary infarcts, mycotic aneurysm (see at Pet-scan), intracranial hemorrhage, conjunctival hemorrhages, and Janeway's lesions.

Immunologic phenomena: glomerulonephritis, Osler's nodes, Roths spots, or rheumatoid factor.

Serological evidence: IgGI antibodies ≥800 <6400

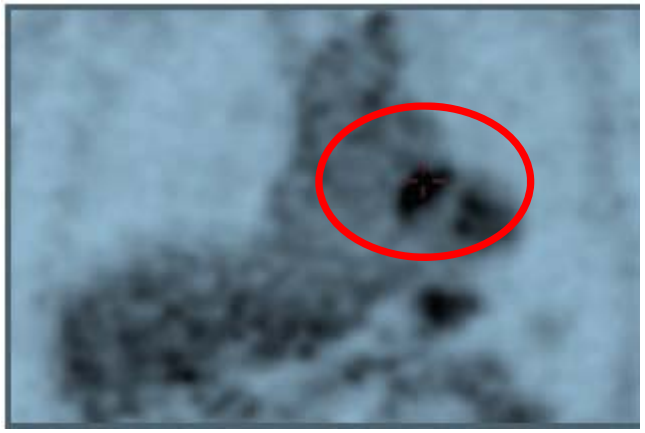
Diagnosis definite

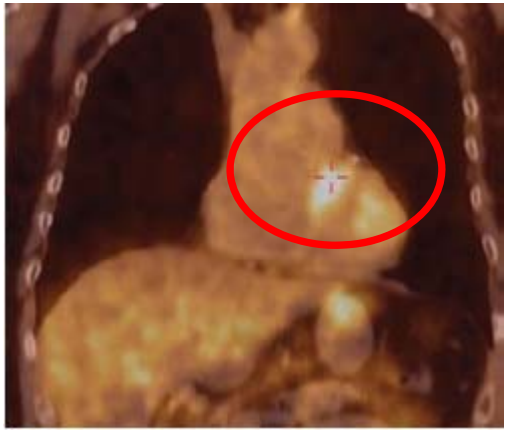
1) 1A criterion
2) 2B criteria
3) 1B criterion, and 3C criteria (including 1 microbiology evidence, and cardiac predisposition)

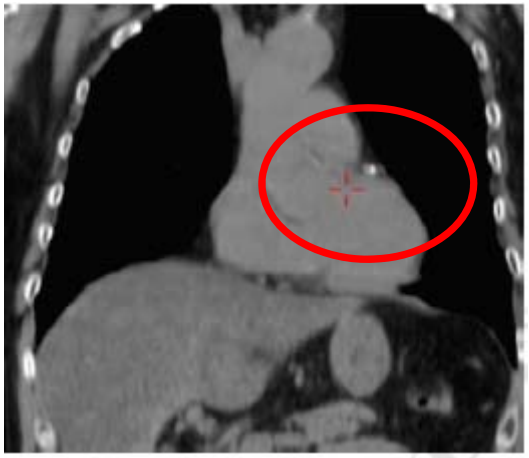
Diagnosis possible

1) 1B criterion, 2C criteria (including 1 microbiology evidence, and cardiac predisposition)
2) 3C criteria (including positive serology, and cardiac predisposition)

A





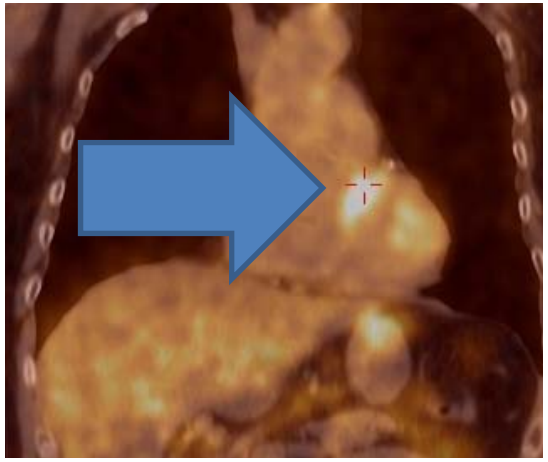


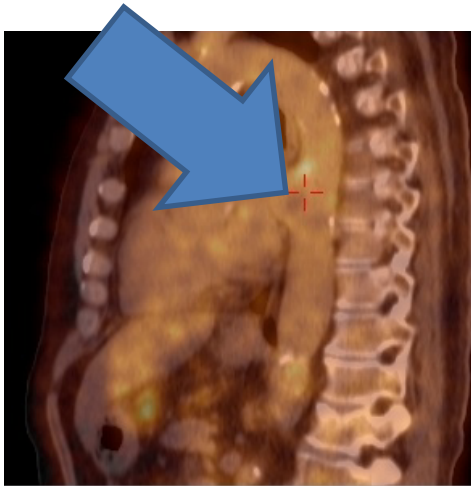
Fixation of aortic valve in a patient with Q fever endocarditis without evidence of endocarditis on echocardiography

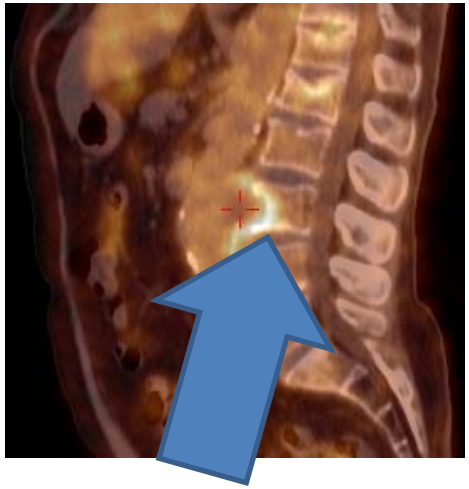
Scintigraphy CT-Scan

F-18 FDG PET scan and Q fever endocarditis

B







Aortic valve

Acuted elevated antibodies

Thoracic aortic aneurysm

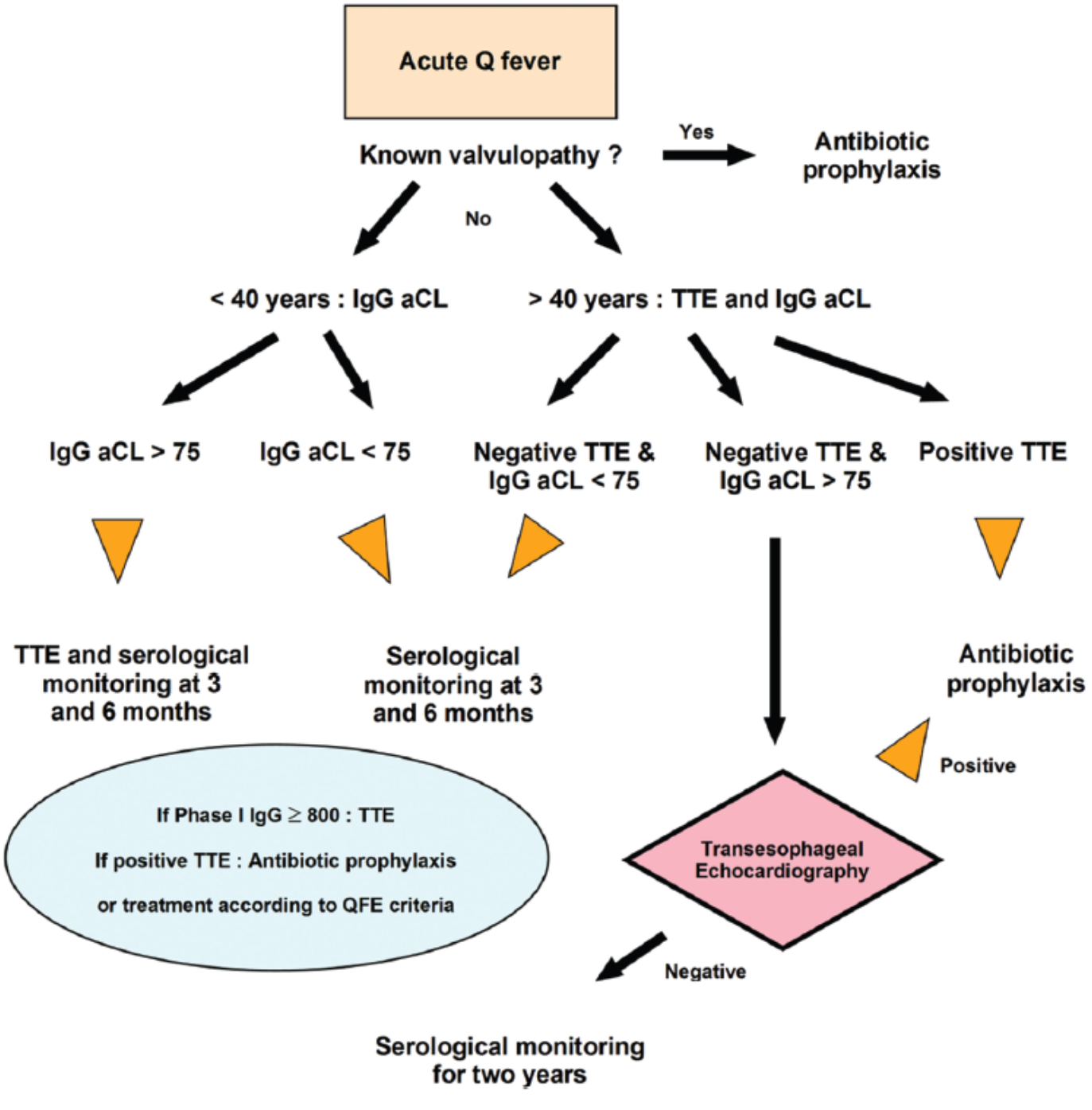
Lombar aortic aneurysm with spondylodiscitis

Immunoglobulin G anticardiolipin antibodies and progression to Q fever endocarditis.

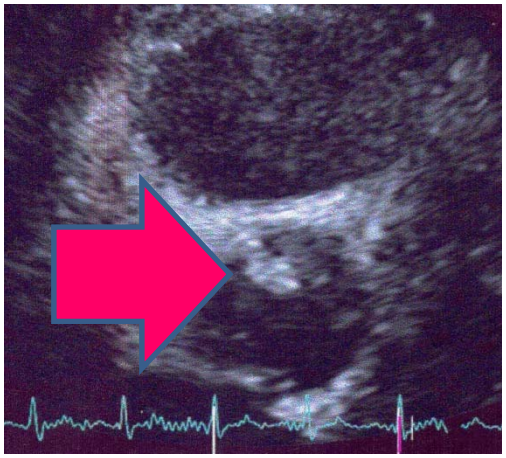
Million M, Walter G, Bardin N, Camoin L, Giorgi R, Bongrand P, Gouriet F, Casalta JP, Thuny F, Habib G, Raoult D.

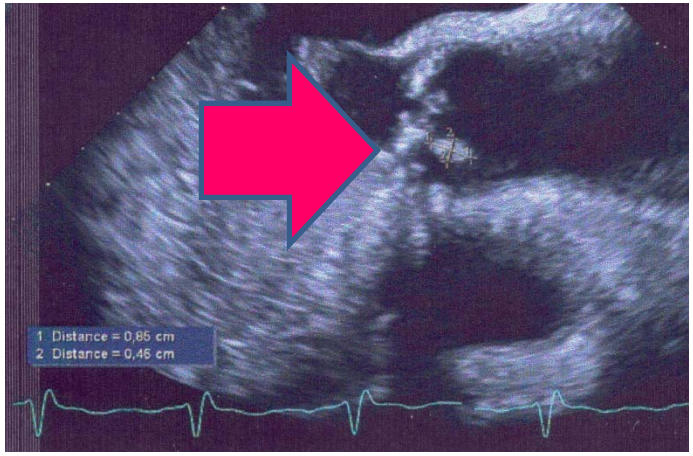
Clin Infect Dis. 2013 Jul;57(1):57-64.

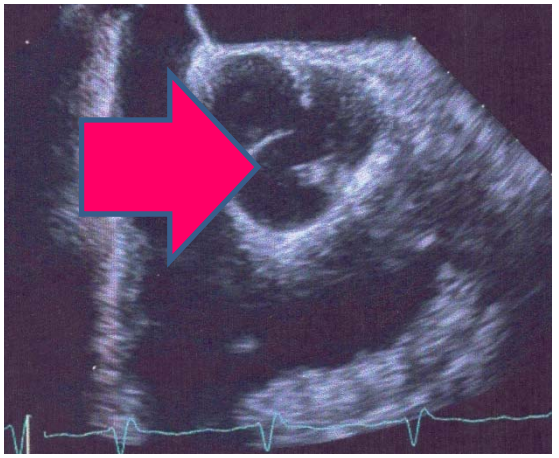
BACKGROUND:
Immunoglobulin G (IgG) anticardiolipin (aCL) antibodies are associated with valvulopathy and endocarditis in patients with lupus and other diseases. During acute Q fever, high IgG aCL prevalence has been reported, but the clinical significance remains unknown.
METHODS:
To test if increased IgG aCL at acute Q fever diagnosis is associated with an increased risk of progression to endocarditis, all patients diagnosed in the French National Referral Center for Q fever from January 2007 to December 2011 were included and followed regularly until January 2013 in a 5-year prospective cohort study. Q fever endocarditis was defined according to recently updated criteria.
RESULTS:
Seventy-two patients were followed for a median time of 31 months (interquartile range, 18-47 months). Of these, 13 patients with valvulopathy without antibiotic prophylaxis progressed to endocarditis. IgG aCL levels were highly prevalent (57%) and significantly higher in the presence of a valvulopathy (P = .005). Using Cox regression analysis, highly increased levels of IgG aCL (adjusted hazard ratio [AHR], 12.95; 95% confidence interval, 2.85-58.95; P = .001) and high levels of phase II immunoglobulin M (IgM; AHR, 6.59; 95% CI, 1.37-31.62; P = .018) were the only independent predictors of progression to endocarditis.
CONCLUSIONS:
Rapid progression from acute Q fever to endocarditis is associated with high levels of IgG aCL and high levels of phase II IgM, findings that should be critical in the prevention of endocarditis.
KEYWORDS:
Coxiella burnetii; Q fever; anticardiolipin; antiphospholipid; endocarditis


Acute Q fever
Known valvulopathy ?
Yes
Antibiotic prophylaxis
No
< 40 years : IgG aCL
> 40 years : TTE and IgG aCL
IgG aCL > 75
IgG aCL < 75
Negative TTE & IgG aCL < 75
Negative TTE & IgG aCL > 75
Positive TTE
TTE and serological monitoring at 3 and 6 months
Serological monitoring at 3 and 6 months
Antibiotic prophylaxis
Positive
If Phase I IgG ≥ 800 : TTE
If positive TTE : Antibiotic prophylaxis
or treatment according to QFE criteria
Transesophageal Echocardiography
Negative
Serological monitoring for two years

Acute Q fever endocarditis







A 45-year-old male patient without any medical history and living in Poitou-charentes were *Coxiella burnetii* MST8 is endemic presented a 8-day fever. Transthoracic echocardiography revealed an aortic vegetation of 85mm. Levels of IgG anti-cardiolipin antibodies were highly increased (742 GPLU, that is 34 fold the normal treshold value (N<22)). Serology diagnosed a *Coxiella burnetii* primary infection. The vegetation disappeared rapidly while patient was treated.

Chronic Q fever: expert opinion versus literature analysis and consensus.
Raoult D.
J Infect. 2012 Aug;65(2):102-8.

Table 3 Criteria for diagnosis of Q fever vascular infection.

A. Definite

Positive culture, PCR or immunochemistry of an arterial samples (prosthesis or aneurism) or a periarterial abscess or a spondylodiscitis linked to aorta.

B. Major criteria

Microbiology: Positive culture, PCR of the blood or emboli, or serology with IgGI antibodies $\geq$6400

Evidence of vascular involvement:

CT-scan: aneurism or vascular prosthesis + periarterial abscess, fistula, or spondylodiscitis.

Pet-san specific fixation on an aneurism or vascular prosthesis.

C. Minor criteria

Serological IgGI $\geq$800 $<$6400

Fever, temperature $\geq$38 °C

Emboli

Underlying vascular predisposition (aneurism or vascular prothesis)

Diagnosis definite

1) A criterion
2) 2B criteria
3) 1B criterion and 2C criteria (including microbiology and vascular predisposition)

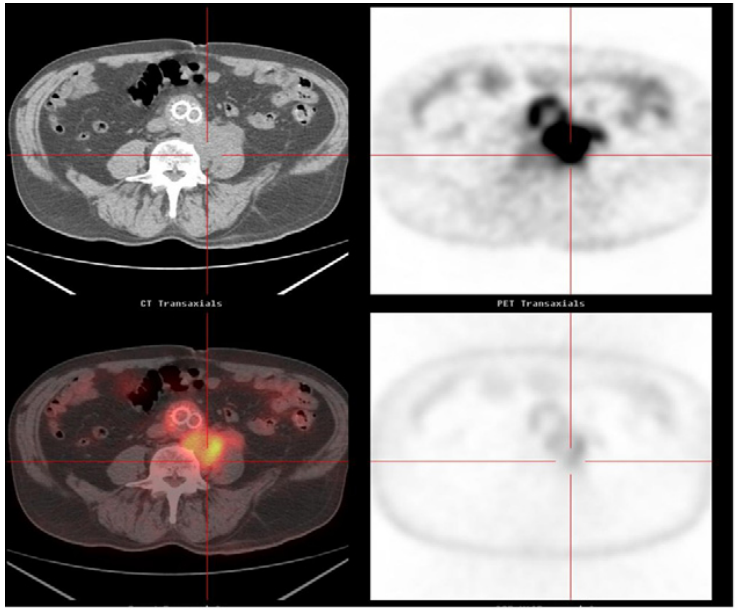
Diagnosis possible

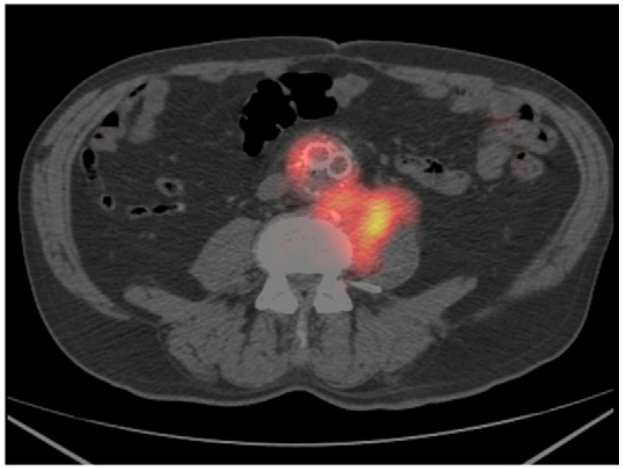
Vascular predisposition, serological evidence and fever or emboli.

Relevance of the positron emission tomography in the diagnosis of vascular graft infection with Coxiella burnetii.

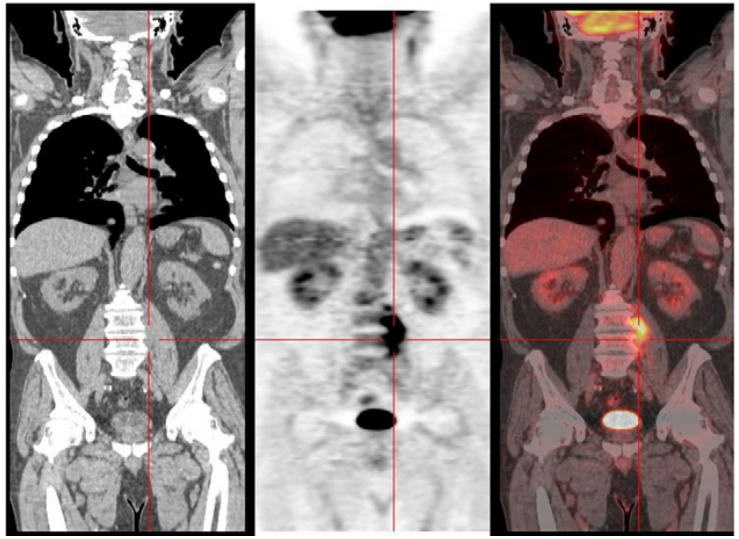
Merhej V, Cammilleri S, Piquet P, Casalta JP, Raoult D.

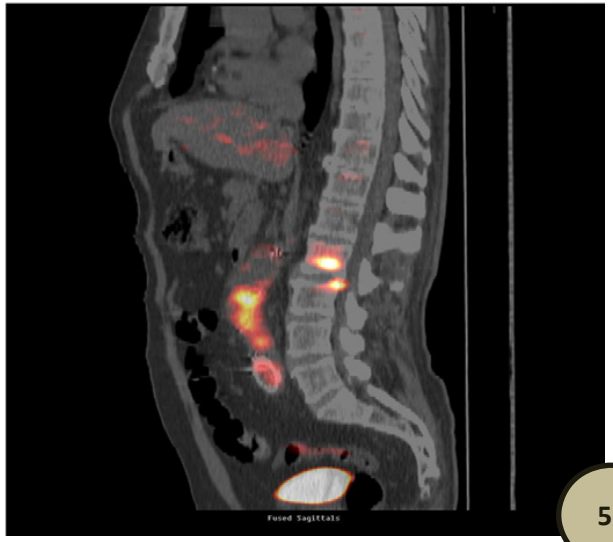
Comp Immunol Microbiol Infect Dis. 2012 Jan;35(1):45-9.





A FDG-PET CT scan demonstrates an increased radiotracer accumulation in the aortic abdomen and in the lower lumbar spine leading to a diagnosis of undercurrent active infection





A case of Q fever prosthetic joint infection and description of an assay for detection of Coxiella burnetii.

Tande AJ, Cunningham SA, Raoult D, Sim FH, Berbari EF, Patel R.

J Clin Microbiol. 2013 Jan;51(1):66-9.

Abstract

We present the first published case of Coxiella burnetii prosthetic joint infection. Diagnosis was established with PCR and culture of periprosthetic tissue and synovial fluid (and serology). A novel PCR assay is described herein. Q fever should be considered in patients with prosthetic joint infection without an identified pathogen.

Emergence of Q fever arthritis in France.
Angelakis E, Edouard S, Lafranchi MA, Pham T, Lafforgue P, Raoult D.
J Clin Microbiol. 2014 Apr;52(4):1064-7.

Osteoarticular infection is an uncommon presentation of Q fever. Positron emission tomography (PET) scanning is a valuable tool for the diagnosis of Coxiella burnetii graft prosthesis infection and endocarditis. Our objective was to test a series of culture-negative osteoarticular samples using molecular assays for Coxiella burnetii. We tested for C. burnetii by molecular assays targeting the IS1111 and the IS30A spacer regions, using culture-negative osteoarticular samples obtained in our laboratory between January 2011 and December 2012. We examine a total of 1,410 osteoarticular samples, and we observed two cases of arthritis and subacromial bursitis caused by C. burnetii. The infections were localized using PET scanning, and the diagnosis was confirmed through serology. For one, a C. burnetii strain with a multispacer sequence type 8 genotype was isolated from synovial fluid culture. Q fever articular infections could be undiagnosed because of the long evolution of articular attack, and patients with high antibody titers against C. burnetii should be tested using PET scanning to localize the site of infection.

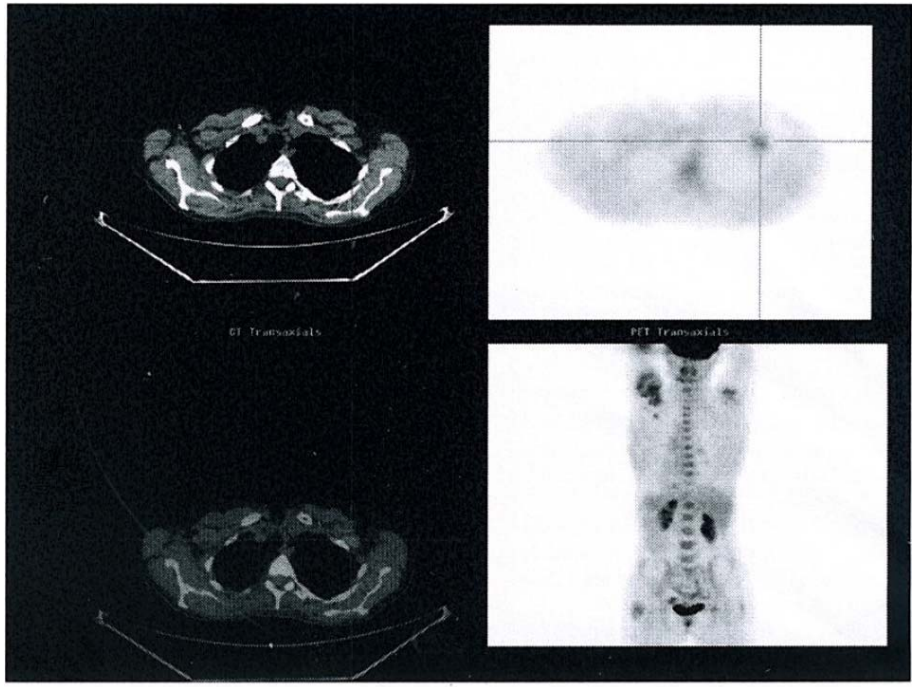


FIG 2 Analysis of *C. burnetii* subacromial bursitis using PET scanning.

Then a little bit competition

- New definition then

- Q fever and pregnancy
- Prophylaxis of endocarditis
- New culture medium

Then a little but of discovery

Multiplexed whole bacterial antigen microarray, a new format for the automation of serodiagnosis: the culture-negative endocarditis paradigm. Gouriet F, Samson L, Delaage M, Mainardi JL, Meconi S, Drancourt M, Raoult D. Clin Microbiol Infect. 2008 Dec;14(12):1112-8

- Lymphoma caused by *C.burnetii*
- French Guyana
  - New strain
  - New reservoir
  - New mechanism of pathogenicity
- New treatments?

Reevaluation of the Risk of Fetal Death and Malformation After Q Fever.

Million M, Roblot F, Carles D, D'Amato F, Protopopescu C, Carrieri MP, Raoult D.

Clin Infect Dis. 2014 Apr 18

Abstract

A meta-analysis of 136 Q fever pregnancies, including 4 new cases and 7 population-based serological studies, revealed significant increases in fetal death and malformation after Q fever during pregnancy. This poor obstetric outcome is prevented by antibiotic treatment.

KEYWORDS:

Coxiella burnetii; Q fever; fetal death; fetal malformation; pregnancy

Reevaluation of the Risk of Fetal Death and Malformation After Q Fever

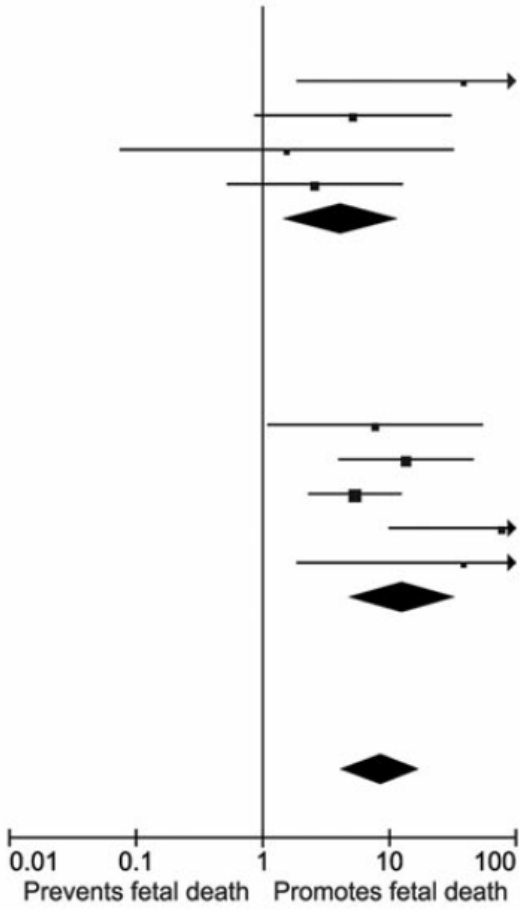
Million M, Roblot F, Carles D, D'Amato F, Protopopescu C, Carrieri MP, Raoult D.

Clin Infect Dis. 2014 Apr 18

A meta-analysis of 136 Q fever pregnancies, including 4 new cases and 7 population-based serological studies, revealed significant increases in fetal death and malformation after Q fever during pregnancy. This poor obstetric outcome is prevented by antibiotic treatment.

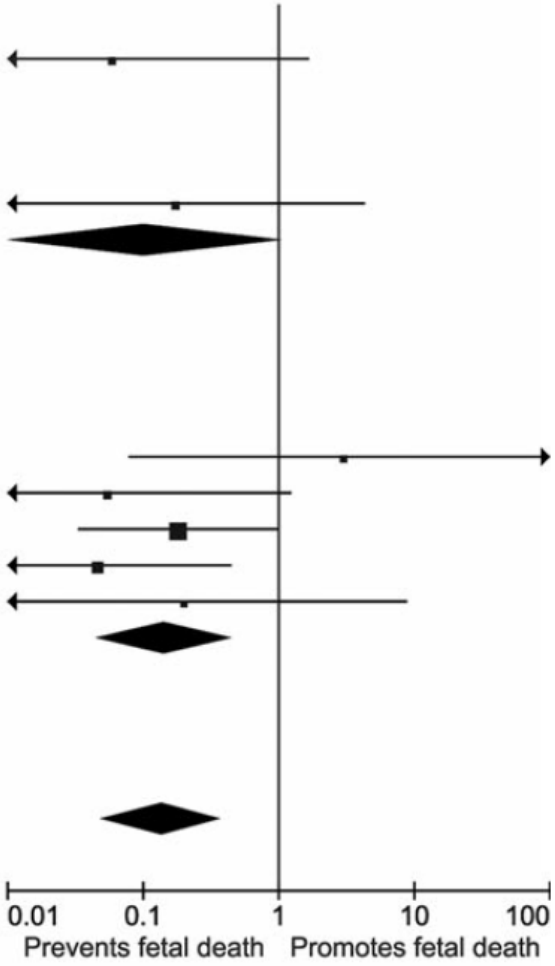
A Untreated Q fever and fetal death

	Untreated Q fever Events	Total	General population[a]	Odds Ratio, 95% CI	Year
Outside the French NRC for Q fever					
Dindinaud (France)	2	2		38.61 [1.85, 804.29]	1991
5 untreated case reports[b]	2	5		5.15 [.86, 30.81]	1993
Munster (Netherlands)	0	2		1.54 [.07, 32.17]	2012
Nielsen (Denmark)	2	8		2.57 [.52, 12.75]	2014
Subtotal (95% CI)		**17**		**4.22 [1.49, 11.97]**	
Total events	6				
Heterogeneity: I^2 = 0% (P = .41)					
Test for overall effect: Z = 2.71 (P = .007)					
French NRC for Q fever					
Stein (France)	2	4		7.72 [1.09, 54.82]	1998
Raoult (France)	7	11		13.51 [3.96, 46.17]	2002
Carcopino (France)	9	22		5.35 [2.29, 12.51]	2007
Angelakis (France)	10	11		77.23 [9.89, 603.28]	2012
Million (France)	2	2		38.61 [1.85, 804.29]	2014
Subtotal (95% CI)		**50**		**12.66 [4.88, 32.79]**	
Total events	30				
Heterogeneity: I^2 = 45% (P = .12)					
Test for overall effect: Z = 5.22 (P < .00001)					
Total (95% CI)		**67**		**8.62 [4.21, 17.63]**	
Total events	36				
Heterogeneity: I^2 = 32% (P = .16)					
Test for overall effect: Z = 5.90 (P < .00001)					
Test for subgroup differences: I^2 = 56.9% (P = .13)					



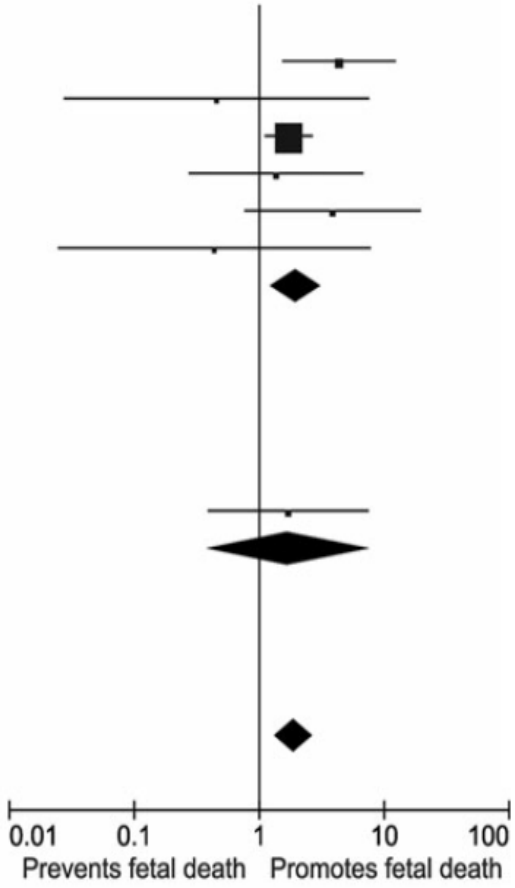
B Antibiotics for the prevention of fetal death associated with Q fever

	Treated Events	Treated Total	Untreated Events	Untreated Total		
Outside the French NRC for Q fever						
12 case reports	0	8	2	4	0.06 [.00, 1.67]	1988
Dindinaud (France)	0	0	2	2	Not estimable	1991
Marrie (Canada)	0	1	0	1	Not estimable	1993
Munster (Netherlands)	0	3	0	2	Not estimable	2012
Nielsen (Denmark)	0	7	2	8	0.17 [.01, 4.31]	2014
Subtotal (95% CI)		**19**		**17**	**0.10 [.01, 1.05]**	
Total events	0		6			
Heterogeneity: I^2 = 0% (P = .65)						
Test for overall effect: Z = 1.92 (P = .05)						
French NRC for Q Fever						
Stein (France)	1	1	2	4	3.00 [.08, 115.34]	1998
Raoult (France)	0	5	7	11	0.05 [.00, 1.24]	2002
Carcopino (France)	2	18	9	22	0.18 [.03, .99]	2007
Angelakis (France)	6	19	10	11	0.05 [.00, .45]	2012
Million (France)	1	2	2	2	0.20 [.00, 8.82]	2014
Subtotal (95% CI)		**45**		**50**	**0.15 [.05, .46]**	
Total events	10		30			
Heterogeneity: I^2 = 2% (P = .39)						
Test for overall effect: Z = 3.29 (P = .001)						
Total (95% CI)		**64**		**67**	**0.14 [.05, .38]**	
Total events	10		36			
Heterogeneity: I^2 = 0% (P = .63)						
Test for overall effect: Z = 3.85 (P = .0001)						
Test for subgroup differences: I^2 = 0% (P = .79)						



C Positive *Coxiella burnetii* serology and fetal death

	Positive serology		Negative serology			
	Events	Total	Events	Total		
Outside the French NRC for Q fever						
Langley (Canada)	4	104	41	4483	4.33 [1.52, 12.33]	2003
vander Hoek (Netherlands)	0	49	24	1125	0.45 [.03, 7.58]	2011
Quijada (Spain)	70	108	203	392	1.72 [1.10, 2.67]	2011
Nielsen (Denmark)	2	169	6	687	1.36 [.27, 6.79]	2013
Eyigor (Turkey)	10	12	26	46	3.85 [.76, 19.56]	2013
Munster (Netherlands)	0	183	6	1046	0.44 [.02, 7.78]	2013
Subtotal (95% CI)		**625**		**7779**	**1.97 [1.22, 3.19]**	
Total events	86		306			
Heterogeneity: I^2 = 11% (P = .35)						
Test for overall effect: Z = 2.76 (P = .006)						
French NRC for Q fever						
Rey (France)	2	16	736	9493	1.70 [.39, 7.49]	2000
Subtotal (95% CI)		**16**		**9493**	**1.70 [.39, 7.49]**	
Total events	2		736			
Heterogeneity: Not applicable						
Test for overall effect: Z = 0.70 (P = .48)						
Total (95% CI)		**641**		**17272**	**1.89 [1.31, 2.72]**	
Total events	88		1042			
Heterogeneity: I^2 = 0% (P = .47)						
Test for overall effect: Z = 3.42 (P = .0006)						
Test for subgroup differences: I^2 = 0% (P = .85)						



Then a little bit competition

- New definition then
- Q fever and pregnancy
- Prophylaxis of endocarditis
- New culture medium

Then a little but of discovery

Multiplexed whole bacterial antigen microarray, a new format for the automation of serodiagnosis: the culture-negative endocarditis paradigm. Gouriet F, Samson L, Delaage M, Mainardi JL, Meconi S, Drancourt M, Raoult D. Clin Microbiol Infect. 2008 Dec;14(12):1112-8

- Lymphoma caused by *C.burnetii*
- French Guyana
  - New strain
  - New reservoir
  - New mechanism of pathogenicity
- New treatments?

Reduction in incidence of Q fever endocarditis: 27 years of experience of a national reference center.

Edouard S, Million M, Royer G, Giorgi R, Grisoli D, Raoult D.

J Infect. 2014 Feb;68(2):141-8.

OBJECTIVES:

We conducted an observational study to evaluate the impact of our antibioprophylaxis protocols implemented in 2000, on the incidence of Q fever endocarditis diagnosed in our French reference center between 1985 and 2011.

METHODS:

Endocarditis was diagnosed according to modified Duke Criteria, serological and PCR results. Our prophylaxis recommendations consist of a systematic echocardiography and an antibioprophylaxis in patients with acute Q fever and risk factors for developing endocarditis.

RESULTS:

Over the last 27 years, we diagnosed 4231 acute Q fever and 818 endocarditis. Despite a significantly increased number of acute Q fever diagnoses and the use of systematic PCR testing of valves allowing serendipitous Q fever endocarditis diagnoses, we observed a decline of Q fever endocarditis. The number of cases has decreased from 316, which represents 18% of newly diagnosed cases of Q fever between 1998 and 2004, to 225, which corresponds to 11% of the cases diagnosed between 2005 and 2011.

CONCLUSION:

We believe that this decrease was a result of our strategies for prophylaxis. If this assumption is true, we may have prevented more than 150 cases of Q fever endocarditis in France over the past 10 years.

Evolution from acute Q fever to endocarditis is associated with underlying valvulopathy and age and can be prevented by prolonged antibiotic treatment.

Million M, Walter G, Thuny F, Habib G, Raoult D.

Clin Infect Dis. 2013 Sep;57(6):836-44

BACKGROUND:

The prevention of Q fever endocarditis through the use of systematic echocardiography and antibiotic prophylaxis in patients with acute Q fever and valvulopathy has never been validated in a cohort study.

METHODS:

From 2007 to 2012, all patients followed at the French National Referral Center for acute Q fever were included in a cohort study. The prevention of endocarditis included a systematic transthoracic echocardiography (TTE) and a 12-month course of doxycycline and hydroxychloroquine prophylaxis in patients with significant valvulopathy. Transesophageal echocardiography (TEE) was performed in patients with a negative TTE and a rapid rise of phase I immunoglobulin G titers.

RESULTS:

Seventy-two patients were included with a median follow-up time of 22 months. A valvulopathy was identified in 31 patients (43%), being previously unknown in 24 (33%) and diagnosed only upon TEE or a second TTE in 7 (10%). The major determinants associated with endocarditis were age (hazard ratio [HR], 1.07; 95% confidence interval [CI], 1.006-1.13; P = .03), aortic regurgitation (HR, 10.2; 95% CI, 3.2-32.2; P < .001), and mitral regurgitation (HR, 4.78; 95% CI, 1.4-16.0; P = .01). Antibiotic prophylaxis was highly effective (HR, 0.002; 95% CI, .00-.77; P = .04) for the 31 patients with valvulopathy.

CONCLUSIONS:

Acute Q fever could be associated with an increased prevalence of valvulopathy. The evolution from acute Q fever to endocarditis is associated with age and valvulopathy and can be entirely prevented by antibiotic prophylaxis. Although the name "chronic Q fever" suggests otherwise, rapid evolution (<1 month) was observed.

Then a little bit competition

- New definition then
- Q fever and pregnancy
- Prophylaxis of endocarditis
- New culture medium

Then a little but of discovery

Multiplexed whole bacterial antigen microarray, a new format for the automation of serodiagnosis: the culture-negative endocarditis paradigm. Gouriet F, Samson L, Delaage M, Mainardi JL, Meconi S, Drancourt M, Raoult D. Clin Microbiol Infect. 2008 Dec;14(12):1112-8

- Lymphoma caused by *C.burnetii*
- French Guyana
  - New strain
  - New reservoir
  - New mechanism of pathogenicity
- New treatments?

Host cell-free growth of the Q fever bacterium *Coxiella burnetii*

Anders Omsland[a], Diane C. Cockrell[a], Dale Howe[a], Elizabeth R. Fischer[b], Kimmo Virtaneva[c], Daniel E. Sturdevant[c], Stephen F. Porcella[c], and Robert A. Heinzen[a,1]

[a]Coxiella Pathogenesis Section, Laboratory of Intracellular Parasites, [b]Electron Microscopy Unit, and [c]Genomics Unit, Research Technology Section, Research Technology Branch, Rocky Mountain Laboratories, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Hamilton, MT 59840

Edited by Emil C. Gotschlich, The Rockefeller University, New York, NY, and approved January 22, 2009 (received for review November 26, 2008)

The inability to propagate obligate intracellular pathogens under axenic (host cell-free) culture conditions imposes severe experimental constraints that have negatively impacted progress in understanding pathogen virulence and disease mechanisms. *Coxiella burnetii, the causative agent of human Q (Query) fever, is an* obligate intracellular bacterial pathogen that replicates exclusively in an acidified, lysosome-like vacuole. To define conditions that support *C. burnetii growth, we systematically evaluated the organism's* metabolic requirements using expression microarrays, genomic reconstruction, and metabolite typing. This led to development of a complex nutrient medium that supported substantial growth (approximately 3 log10) of *C. burnetii in a 2.5% oxygen* environment. Importantly, axenically grown *C. burnetii were* highly infectious for Vero cells and exhibited developmental forms characteristic of in vivo grown organisms. Axenic cultivation of *C.burnetii will facilitate studies of the organism's pathogenesis and* genetics and aid development of Q fever preventatives such as an effective subunit vaccine. Furthermore, the systematic approach used here may be broadly applicable to development of axenic media that support growth of other medically important obligate intracellular pathogens.

Proc Natl Acad Sci U S A. 2009 March 17; 106(11): 4430–4434.

JCM
Journals.ASM.org

Cell Extract-Containing Medium for Culture of Intracellular Fastidious Bacteria

Sudhir Singh, Malgorzata Kowalczewska, Sophie Edouard, Carole Eldin, Céline Perreal, Pascal Weber, Said Azza, Didier Raoult
Aix Marseille Université, URMITE, UM63, CNRS 7278, IRD 198, INSERM 1095, Marseille, France

The culture of fastidious microorganisms is a critical step in infectious disease studies. As a proof-of-concept experiment, we evaluated an empirical medium containing eukaryotic cell extracts for its ability to support the growth of *Coxiella burnetii*. Here, we demonstrate the exponential growth of several bacterial strains, including the *C. burnetii* Nine Mile phase I and phase II strains, and *C. burnetii* isolates from humans and animals. Low-oxygen-tension conditions and the presence of small hydrophilic molecules and short peptides were critical for facilitating growth. Moreover, bacterial antigenicity was conserved, revealing the potential for this culture medium to be used in diagnostic tests and in the elaboration of vaccines against *C. burnetii*. We were also able to grow the majority of previously tested intracellular and fastidious bacterial species, including *Tropheryma whipplei*, *Mycobacterium bovis*, *Leptospira* spp., *Borrelia* spp., and most putative bioterrorism agents. However, we were unable to culture *Rickettsia africae* and *Legionella* spp. in this medium. The versatility of this medium should encourage its use as a replacement for the cell-based culture systems currently used for growing several facultative and putative intracellular bacterial species.

JCM 2013; 51 (8):2599-607

Then a little bit competition

- New definition then
- Q fever and pregnancy
- Prophylaxis of endocarditis
- New culture medium

Then a little but of discovery

Multiplexed whole bacterial antigen microarray, a new format for the automation of serodiagnosis: the culture-negative endocarditis paradigm. Gouriet F, Samson L, Delaage M, Mainardi JL, Meconi S, Drancourt M, Raoult D. Clin Microbiol Infect. 2008 Dec;14(12):1112-8

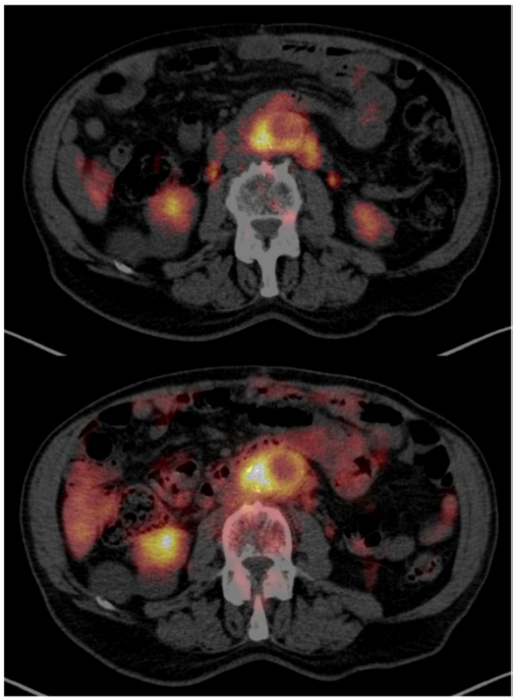
- Lymphoma caused by *C.burnetii*
- French Guyana
  - New strain
  - New reservoir
  - New mechanism of pathogenicity
- New treatments?

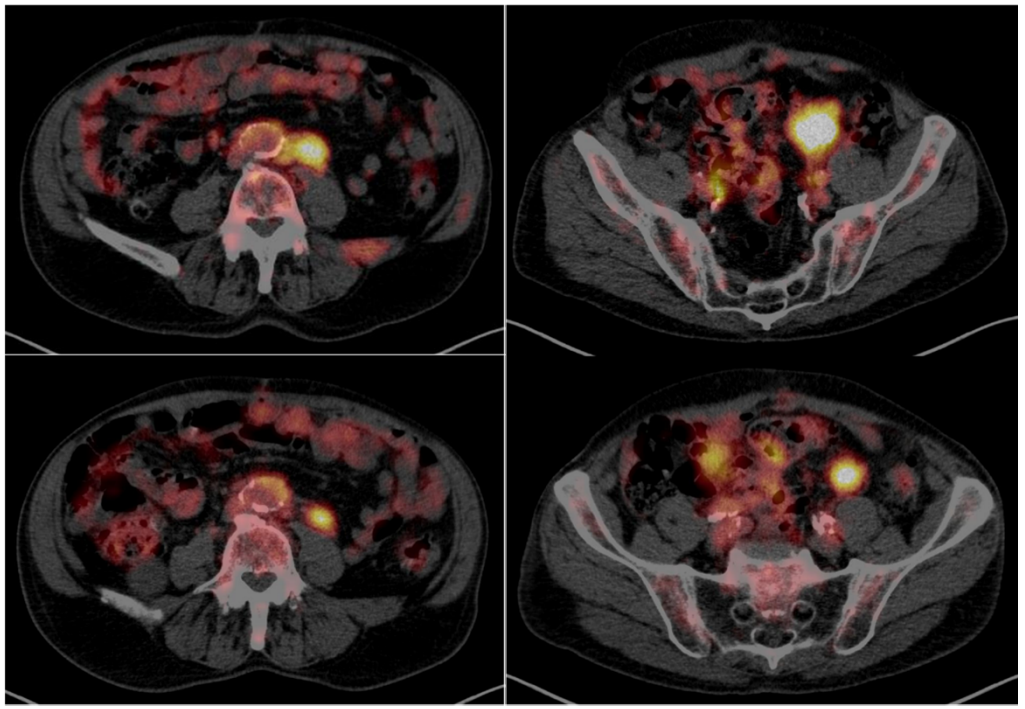
Coxiella burnetii and B-Cell Lymphoma

Triggering patient

After several months of antibiotics

Before antibiotics



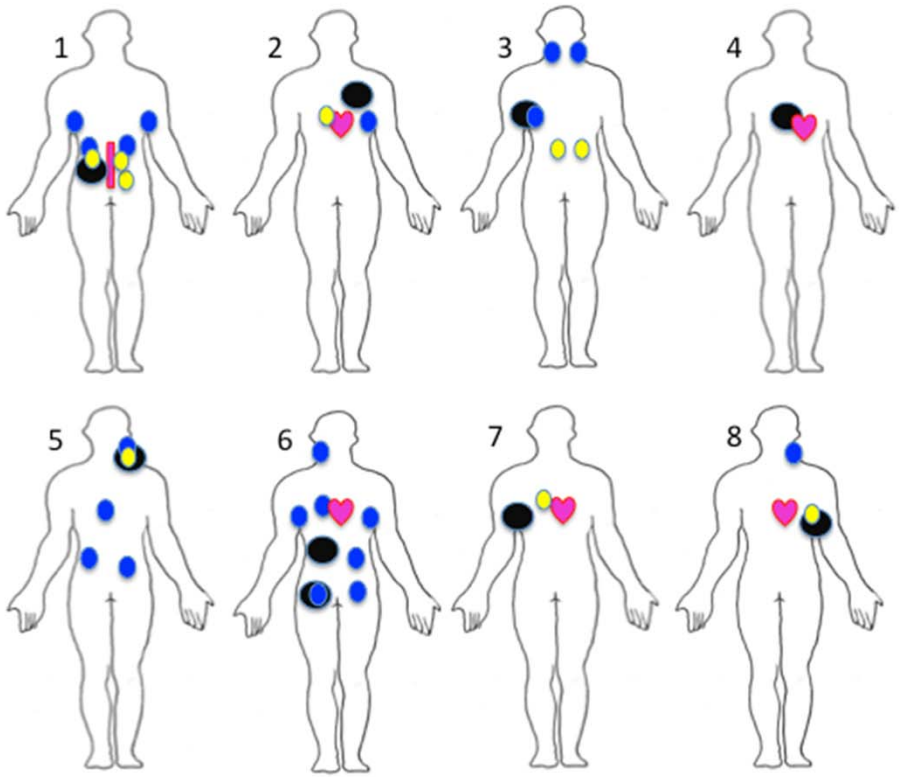


Hypermetabolism associated with Q fever vascular infection decreased

BUT

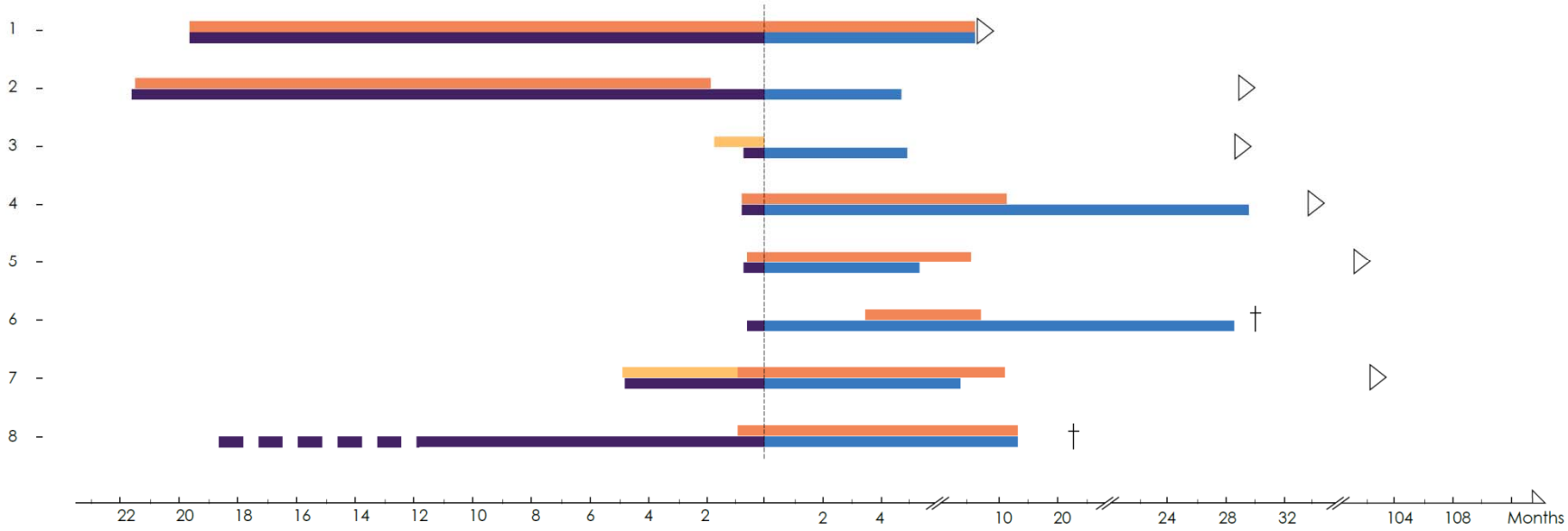
Hypermetabolism of associated lymph nodes dramatically increased leading to B-cell lymphoma

7 additional patients with Q fever and B-cell lymphoma



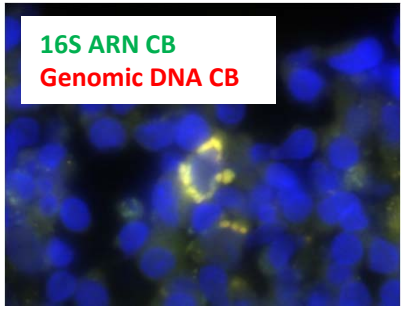
Patients presented Endocarditis (5), Vascular infection (1) or Lymphadenitis (2). Anatomical proximity was noted in 5/8 cases with a striking extra-nodal « pectoral » lymphoma following Q fever endocarditis (Patient 2).

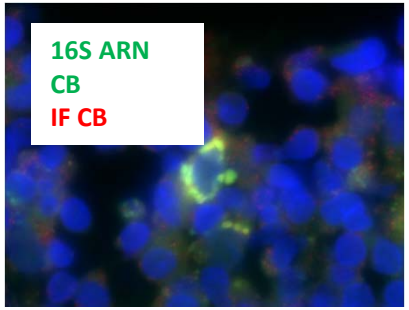
Temporal association between Q fever and B-cell Lymphoma diagnosis

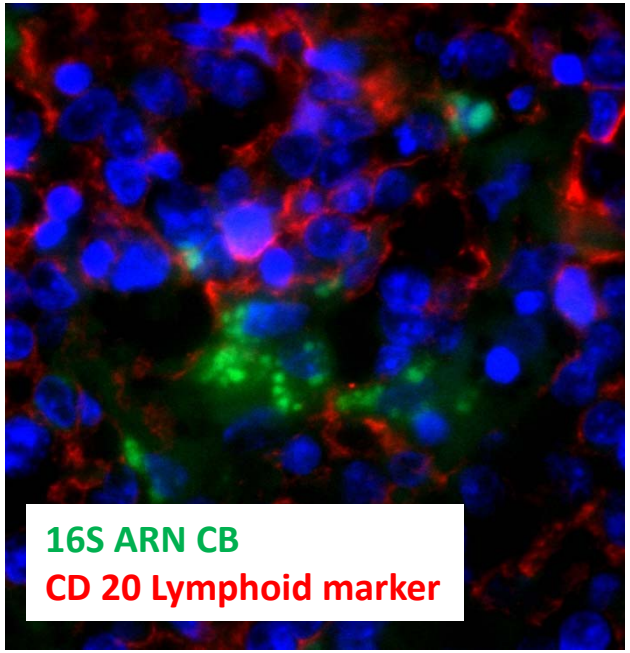


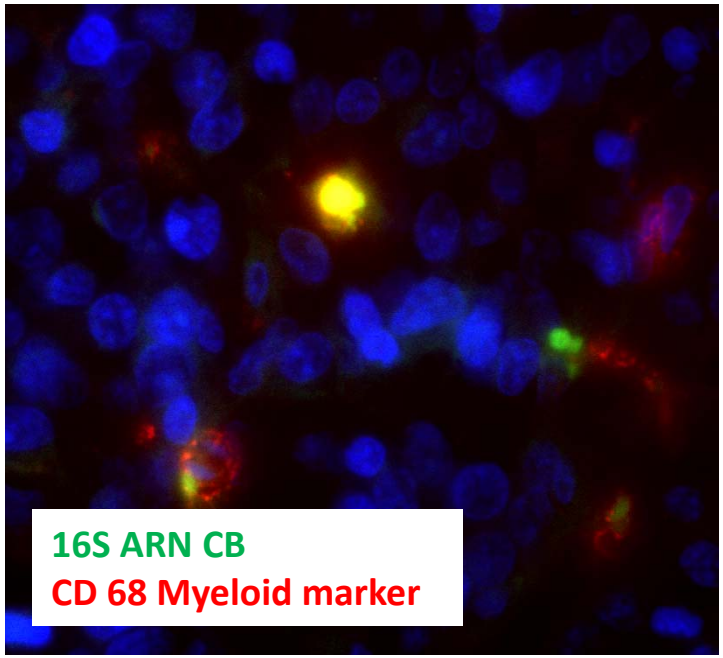
In all but 1 case Q fever diagnosis preceded Lymphoma diagnosis but in 2 cases an adenopathy was known before the Q fever diagnosis (1 cases with a previous adenopathy known for years).

Coxiella burnetii multiplies persistently in myeloïd CD68+ but not lymphoïd CD20+ cells within lymphomatous material in 4 patients









Then a little bit competition

- New definition then
- Q fever and pregnancy
- Prophylaxis of endocarditis
- New culture medium

Then a little but of discovery

Multiplexed whole bacterial antigen microarray, a new format for the automation of serodiagnosis: the culture-negative endocarditis paradigm. Gouriet F, Samson L, Delaage M, Mainardi JL, Meconi S, Drancourt M, Raoult D. Clin Microbiol Infect. 2008 Dec;14(12):1112-8

- Lymphoma caused by *C.burnetii*

- French Guyana
  - New strain
  - New reservoir
  - New mechanism of pathogenicity
- New treatments?

Comparison between Emerging Q Fever in French Guiana and Endemic Q fever in Marseille, France.

Edouard S, Mahamat A, Demar M, Abboud P, Djossou F, Raoult D.

Am J Trop Med Hyg. 2014 May;90(5):915-9.

Q fever is an emergent disease in French Guiana. We compared the incidence clinical and serologic profiles between patients from Cayenne, French Guiana and Marseille in metropolitan France during a four-year period. The annual incidence of diagnosed acute Q fever was significantly higher in Cayenne (17.5/100,000) than in Marseille (1.9/100,000) ($P = 0.0004$), but not the annual incidence of endocarditis (1.29 versus 0.34/100,000). Most patients had fever (97%) and pneumonia (83%) in Cayenne versus 81% and 8% in Marseille ($P < 0.0001$ and $P < 0.0001$, respectively) but transaminitis was more common in patients from Marseille (54% versus 32%; $P < 0.0001$). The proportion of patients with cardiovascular infections was significantly lower in Cayenne (7%) than in Marseille (17%) ($P = 0.017$), although they showed a stronger immune response with higher levels of phase I IgG ($P = 0.024$). The differing epidemiology, clinical, and serologic responses of patients from Cayenne and Marseille suggest a different source of infection and a different strain of Coxiella burnetii.

Sloth as a putative reservoir of Q fever in Guiana
B.Davoust *et al*.EID in press

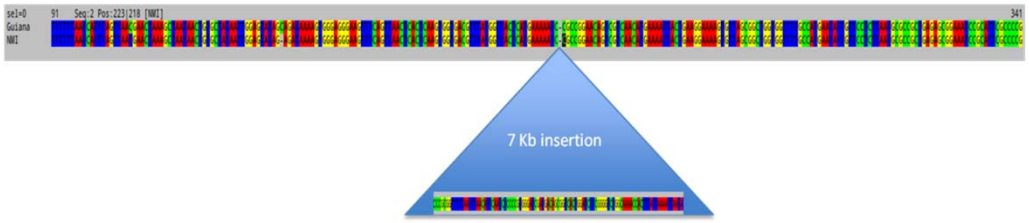
Unique clone of Coxiella burnetii causing severe Q fever, French Guiana.

Mahamat A, Edouard S, Demar M, Abboud P, Patrice JY, La Scola B, Okandze A, Djossou F, Raoult D.

Emerg Infect Dis. 2013 Jul;19(7):1102-4.

Acute Q fever is an emergent and severe disease in French Guiana. We obtained 5 Coxiella burnetii isolates from samples of patients from Cayenne and found an epidemic clone circulating in Cayenne. This clone has caused pneumonia and endocarditis and seems to be more virulent than previously described strains.

- Preliminary results : **deletion of 7 Kb** → 3 genes involved in the communication of the bacterium with the host cell



- Development of a system of real-time PCR to screen> 200 other strains of C. burnetii and 300 positive clinical specimens by PCR to detect other virulent strains can potentially be responsible for future epidemics

Then a little bit competition

- New definition then
- Q fever and pregnancy
- Prophylaxis of endocarditis
- New culture medium

Then a little but of discovery

Multiplexed whole bacterial antigen microarray, a new format for the automation of serodiagnosis: the culture-negative endocarditis paradigm. Gouriet F, Samson L, Delaage M, Mainardi JL, Meconi S, Drancourt M, Raoult D. Clin Microbiol Infect. 2008 Dec;14(12):1112-8

- Lymphoma caused by *C.burnetii*
- French Guyana
  - New strain
  - New reservoir
  - New mechanism of pathogenicity

- New treatments?

Effect of omeprazole on vacuole size in Coxiella burnetii-infected cells.
Botelho-Nevers E, Singh S, Chiche L, Raoult D.
J Infect. 2013 Mar;66(3):288-9.

Lovastatin, but not pravastatin, limits in vitro infection due to Coxiella burnetii.
Botelho-Nevers E, Espinosa L, Raoult D, Rolain JM.
J Antimicrob Chemother. 2008 Oct;62(4):845-7