SYMPOSIUM 10TH MAY 2007

BELGIUM

"Blood culture-negative endocarditis"

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Modified Duke criteria for diagnosis of infective endocarditis (IE)

Major criteria

Blood culture

Single positive culture for *C burnetti* or antibody titre against phase I >1 in 800

Endocardial involvement

(i) oscillating intracardiac mass on valve *or* supporting structure, or in the path of regurgitant jets, or on implanted material, in the absence of an alternative anatomical explanation, *or*

(ii) abscess, *or*

(iii) new partial dehiscence of prosthetic valve.

New valvular regurgitation (worsening of changing or pre-existing murmur not sufficient)

Minor criteria

Predisposing cardiac condition or intravenous drug use

Fever (temperature ≥38°C)

Vascular factors—major arterial emboli, septic pulmonary infarct, mycotic aneurysms, intracranial haemorrhage, conjonctival haemorrhage, Janeway's lesions

Immunological factors: glomerulonephritis, Osler nodes, Roth spots, rheumatoid factor

Microbiology—positive blood cultures, but not meeting major criteria; serological evidence of active infection with plausible microorganisms‡

Echocardiogram consistent with disease but not meeting major criteria§

Li JS, *et al*. Proposed modifications to the Duke criteria for the diagnosis of infective endocarditis. Clin Infect Dis, 2000;30(4):633-8.

Modified Duke criteria for diagnosis of infective endocarditis (IE)

Diagnosis

Definite

- Pathology or bacteriology of vegetations, major emboli, or intracardiac abscess specimen, *or*
- Two major criteria, *or*
- One major and three minor criteria, *or*
- Five minor criteria

Possible¶

- One major and one minor criterion, *or*
- Three minor criteria

Rejected

- Firm alternative diagnosis, *or*
- Resolution of syndrome after ⩽4 days of antibiotherapy, *or*
- No pathological evidence at surgery or autopsy after ⩽4 days of antibiotherapy
- Does not meat criteria mentioned above

Li JS, et al. Proposed modifications to the Duke criteria for the diagnosis of infective endocarditis. Clin Infect Dis, 2000 ;30(4):633-8.

Etiologic Agents in Infective Endocarditis

Agent	% of Cases
Streptococci	60 – 80
Viridans streptococci	30 – 40
Enterococci	5 – 18
Other streptococci	15 – 25
Staphylococci	20 – 35
Coagulase - positive	10 – 27
Coagulase - negative	1 – 3
Gram - negative aerobic bacilli	1.5 – 13
Fungi	2 – 4
Miscellaneous bacteria	< 5
Mixed infections	1 – 2
Culture - negative	< 5 – 24

Table 65-6, In Principles and Pratice of Infectious Diseases, Mandell, GL. et al. Ed, 2000, 5th ed., vol1: 1-1534

Diagnostic strategy for IE

1. Blood culture
2. Blood culture negative endocarditis
3. Serology
4. PCR on blood or valve
5. Valve histology

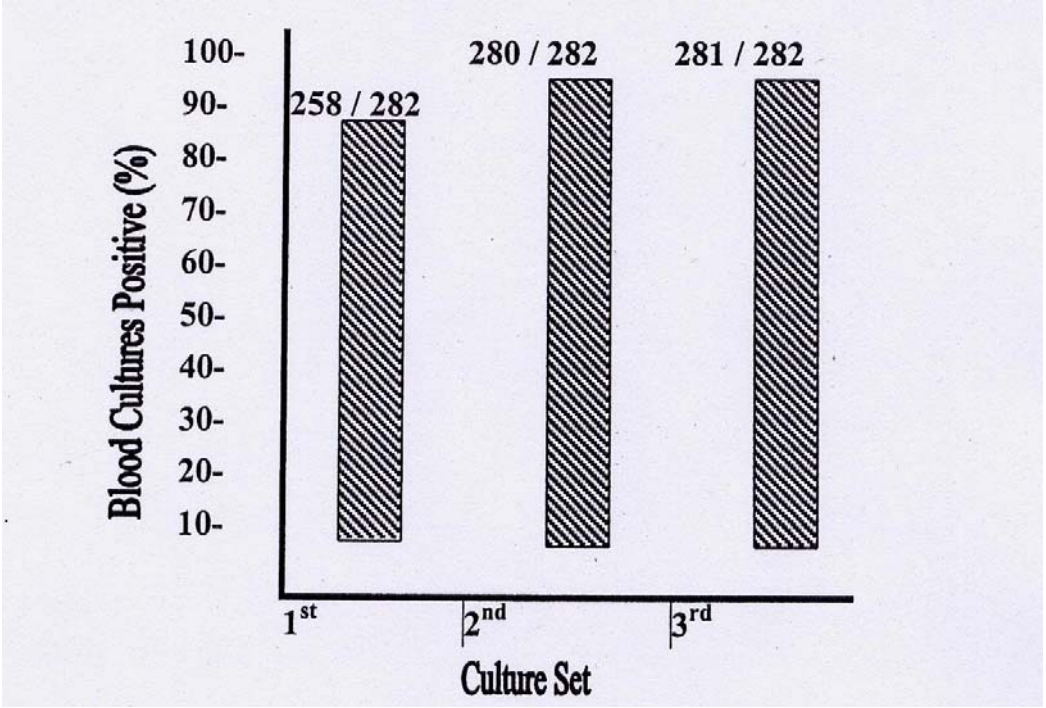
Blood culture

- **Blood cultures:**

  2 thirds of cases positive in IE

- **The first in 95%**
- **The 2 firsts in 98%**

Werner *et al*, JAMA, 1967



- **Then 3 samples are enough**

Blood culture

Volume and periodicity of blood culture

TABLE 1. Culture yield by volume and periodicity

No. of bacteremic episodes tested	Initial vol cultured (ml)	No. of episodes detected	Subsequent vol cultured (ml)	No. of additional episodes detected	Interval between cultures	Yield added by extra vol cultured (%)	*P*	95% confidence interval (%)
184	20	148	20	35	Simultaneous	19	<0.0001	13–25
30	20	24	20	5	10 min to 2 h apart	17	0.0313	2–31
72	20	55	20	12	2 to 24 h apart	17	<0.0003	7–26
210	20	161	20	42	Anytime within 24 h	20	<0.0001	14–26
51	20	36	40	12	Anytime within 24 h	24	<0.0003	10–37
51	40	43	20	5	Anytime within 24 h	10	0.0313	1–18

Sensitivity:

– **40 to 60 ml at any moment**

– **5 days incubation enough to detect HACECK bacteria**

Effects of volume and periodicity on blood cultures, Li J. *et al*., JCM, 1994.

Baron EJ, et al. Prolonged incubation and extensive subculturing do not increase recovery of clinically significant microorganisms from standard automated blood cultures. Clin Infect Dis 2005;41(11):1677-80.

Blood culture

Antibiotics prescribed before sampling

– **Significantly lower positivity rate from 100% to 88%**

Pazin *et al*., Arch Intern Med, 1982

– **Specifically streptococci are very susceptible to prior antibiotic administration**

Diagnostic strategy for IE

1. Blood culture
2. Blood culture negative endocarditis
3. Serology
4. PCR on blood or valve
5. Valve histology

Blood Culture Negative IE

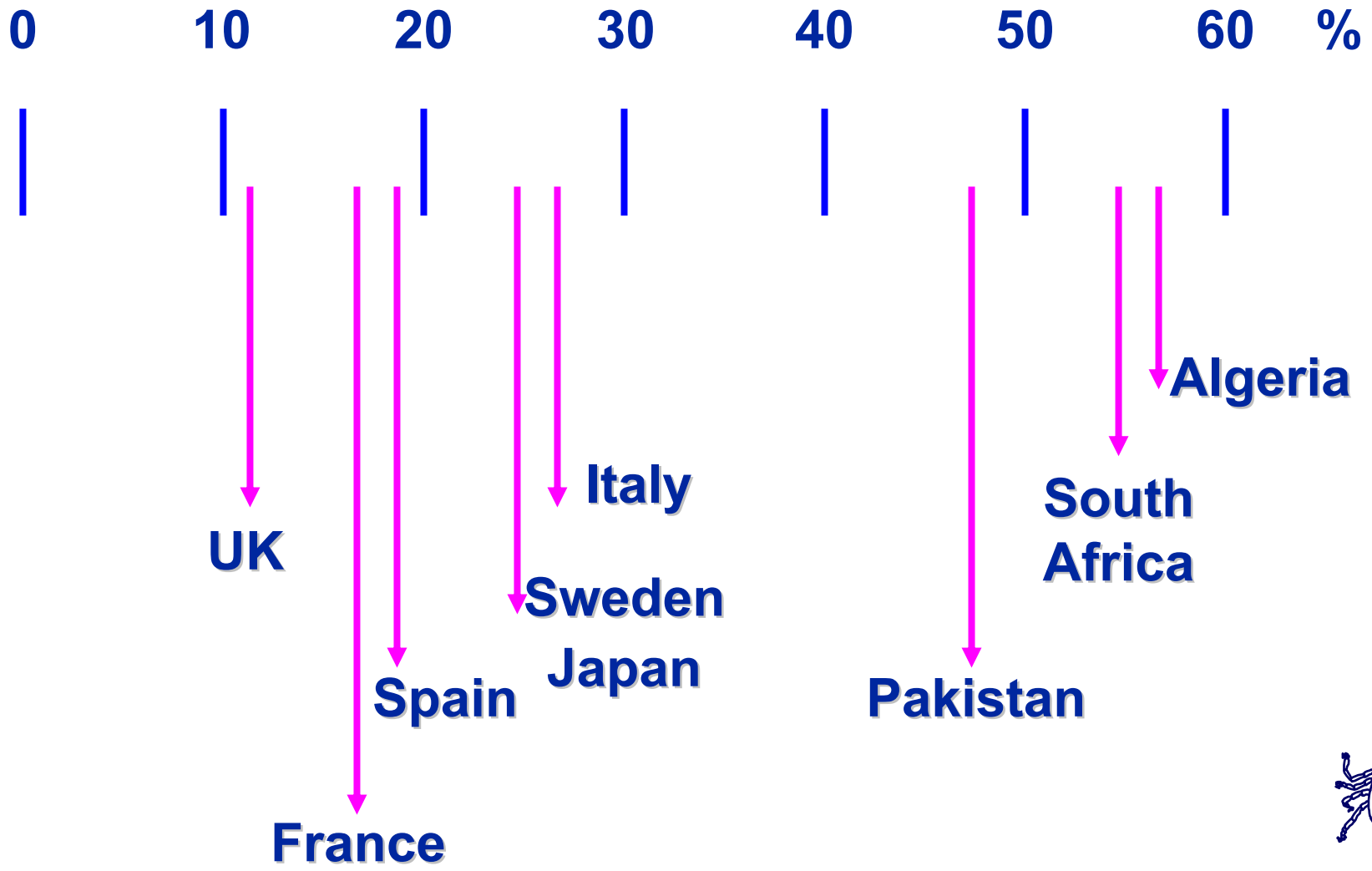
Bibliography Search - Strategy: PubMed 2006

KEY WORDS : BCNE (English) - (Humans)

- 87 papers in 5 years
- 20 from our team
- 8 reporting PCR
- *Bartonella* : 18
- Q fever : 6
- Fungi : 4 (*Candida* : 2, *Histoplasma* : 2
- *Abiotrophia* / *Granuticella* : 2
- Coagulase negative staph : 2
- *Finegoldia magna* : 2
- *Streptococcus*, *Capnogtaphaga*, *Hemophilus*, Whipple's disease, *Salmonella*, *Mycoplasma*, *Chlamydia* : 1

B.C.N.E. Ratio

In different countries



Brouqui P, Raoult D. New insight into the diagnosis of fastidious bacterial endocarditis. FEMS Immunol Med Microbiol. 2006,

Table 3. Prevalence of bacterial agents involved in culture-negative endocarditis until 2004 as detected by a search in Medline® with the following keyword (''bacterial name'', endocarditis) and no restrictions

Bacteria	Agent characteristics	No. of published cases (Medline® 2004)
Coxiella burnettii	SIC Gram negative	419
Bartonella sp.	FIC Gram negative	120
Brucella sp.	FIC Gram negative bacilli	120
Abiotrophia spp.	EX Gram positive cocci	110
Actinobacillus actinomycetemcomitans	EX Gram negative bacilli	102
Haemophilus aphrophilus	EX Gram negative bacilli	78
Cardiobacterium hominis	EX Gram negative bacilli	78
Corynebacterium diphtheriae	EX Gram negative bacilli	67
Haemophilus parainfluenzae	EX Gram negative bacilli	68
Listeria monocytogenes	EX Gram positive bacilli	68
Erysipelothrix rhusiopathiae	EX Gram positive bacilli	52

common

rare

Brouqui P, Raoult D. New insight into the diagnosis of fastidious bacterial endocarditis. FEMS Immunol Med Microbiol. 2006,

(Cont)

Neisseria sp.	EX Gram negative cocci	< 50
Gemella sp.	EX Gram negative cocci	< 50
Mycoplasma sp.	Epicellular no Gram stain	< 50
Campylobacter sp.	EX Gram negative bacilli	< 50
Pasteurella	EX Gram negative bacilli	< 50
Mycobacterium sp.	FIC Ziehl positive bacilli	< 50
Legionella sp.	FIC Gram negative bacilli	< 50
Whipple's disease bacillus	FIC Gram negative bacilli	< 50
Francisella tularensis	FIC Gram negative bacilli	< 50
Yersinia sp.	FIC Gram negative bacilli	< 50

SIC, strict intracellular; FIC, facultative intracellular; EX, extracellular.

Very rare

Blood Culture Negative Endocarditis

	Lamas & Eykin, Heart,2003	Hoen *et al.* CID, 1995	Raoult *et al.* (definite IE) 1994-2002
Number	63	88	60
C. burnetii	8	7(35 tested)	22
Bartonella	6	-	5
Other fastidious bacteria (Chlamydia, Mycoplasma, T. whipplei)	1	2	1
Valve culture or PCR bacteria	6	5	8
Valve culture yeast	3	-	2
Other tests	7	1	3
No organisms	32 (51%)	70 (80%)	21(35%)
- Antibiotic	42		
- Before testing			18
- Abscess			2
- Infected Pace Maker			1

Blood Culture Negative Endocarditis

	Nb (%)	Serology	Culture	PCR
C. burnetii	167 (48 %)	167	61	41
Bartonella	100 (30 %)	99	23	47
T. whipplei	2 (1%)	0	1	2
M. hominis	1	0	0	1
G. elegans	1	0	0	1
L. pneumophila	1	1	0	0
Streptococcus sp.	4	0	0	4

Marseille collection of 348
BCN I.E./ 1983-2001

Houpikian P, Raoult D. Blood culture-negative endocarditis in a reference center: etiologic diagnosis of 348 cases. Medicine (Baltimore). 2005;84(3):162-73.

Diagnostic strategy for BCNE

1. Serology
2. PCR on blood or valve
3. Valve histology

The KIT

- **OBJECTIVES :**
  - STANDARDIZE AND TAG SAMPLES
  - DIMINISH % OF BCNI
  - DIMINISH NUMBER OF SAMPLES
  - DIMINISH DELAY FOR STARTING TREATMENT

The KIT

- **Biological diagnostic of I.E. based on 3 blood culture sampled early before treatment**
- **Serological testing of:**
  - **Rheumatoid factor**
  - ***Coxiella burnetii***
  - ***Bartonella* sp.**
  - ***Brucella* (in endemic countries)**

The KIT

Usual: 3 blood sampling T

If negative: 24 hours

3 new blood sampling

As many as 24 blood sampling when negative in 7days.

Then serology in 10 - 70% of cases

3 blood sampling

+

- serology

- rheumatoid factor

4 hours

The KIT D. Raoult *et al*. J. Clin. Microbiol.

- 3 blood culture (in 4 hours)
- Serology testing
  - *Bartonella* (cut of ≥ 800 IFA)
  - *Coxiella burnetii* (cut of ≥ 800 IgGI -IFA)
  - *Aspergillus* (ELISA)
  - *Brucella* sp. (≥ 160 IFA)
  - *Legionella pneumophila* (≥ 256 IFA)
- Rheumatoid factor detection

The KIT

- 1383 patients tested in Marseilles from 1994 to 2002
- Classified according to modified Duke Criteria
- Definite in 291 (21%)
- Possible in 131 (11%)
- Etiological diagnosis in definite case:
  - by blood culture 79%
  - serology 10%
  - cardiac valve testing

TABLE 3. Definite IE final diagnosis obtained with IE diagnostic kit: microbiological data

Method of detection and causative organism	No. of cases (%)
Blood culture	348 (81.5)
Staphylococcus aureus	78
Streptococcus bovis	67
Viridans streptococci	50
Coagulase-negative staphylococcus	50
Enterococcus faecalis	28
Escherichia coli	10
Other enterococci	8
Enterococcus durans	3
Enterococcus faecium	2
Enterococcus spp.	2
Enterococcus avium	1
HACEK group	8
Actinobacillus actinomycetemcomitans	5
Haemophilus aphrophilus	1
Haemophilus parainfluenzae	1
Cardiobacterium hominis	1
Streptococcus agalactiae	6
Candida spp.	6
Streptococcus pneumoniae	5
Actinobacillus spp.	5
Gemella spp., group G streptococcus, *Enterobacter cloacae*, and *Corynebacterium* spp.	3 each
Acinetobacter spp., *Abiotrophia defectiva*, and *Campylobacter fetus*	2 each
Neisseria sicca, *Ralstonia picketii*, *Pseudomonas aeruginosa*, *Chryseomonas*, *Klebsiella pneumoniae*, *Proteus mirabilis*, *Peptostreptococcus* spp., *Propionibacterium acnes*, and *Listeria monocytogenes*	1 each
Serology	34 (8)
Coxiella burnetii	26
Bartonella spp.	5
Legionella pneumophila	2
Aspergillus spp.	1
Valve analysis	15 (3.5)[a]
Culture	
Aspergillus spp., *Acremonium* spp., *Escherichia coli*, and *Propionibacterium acnes*	1 each
PCR	
Viridans streptococci	3
Streptococcus bovis	2
Granulicatella elegans, *Mycoplasma hominis*, *Streptococcus pneumoniae*, *Streptococcus anginosus*, *Streptococcus agalactiae*, and *Cardiobacterium hominis*	1 each
No etiology found	30 (7)
Total no. of IE cases	427

[a] The number of cases where the organism was detected by culture was 4 (0.9%); the number of cases detected by PCR was 11 (2.6%).

Raoult D, et al. Contribution of systematic serological testing in diagnosis of infective endocarditis. J Clin Microbiol. 43: 5238-42.

TABLE 4. Results of biological tests for suspected cases of IE, according to modified Duke criteria

Positive test	Total (1,998)	Duke classification (no. of cases)		
		Definitive IE (427)[a]	Possible IE (261)[a]	Rejected IE (1,310)
Blood culture	432	348	20	64
Major criterion	262	239	12	11
Minor criterion	170	109	8	53
Serology	57	34 (30)	2 (2)	21
C. burnetii major	33	26 (22)	1 (1)	6
Bartonella (titers of ≥400)	5	5 (5)	0	0
Legionella	11	2 (2)	0	9
Aspergillus	1	1 (1)	0	0
Chlamydia	2	0	1 (1)	1
Mycoplasma pneumoniae	4	0	0	4
Brucella	1	0	0	1
Rheumatoid factor	164	48 (8)	20 (17)	96
Total no. of upgraded cases		38	19	

[a] Values in parentheses are numbers of cases upgraded in the Duke criteria by test results.

Serology	34 (8)
Coxiella burnetii	26
Bartonella spp.	5
Legionella pneumophila	2
Aspergillus spp.	1
Valve analysis	15 (3.5)[a]
Culture	
Aspergillus spp., *Acremonium* spp., *Escherichia coli*, and *Propionibacterium acnes*	1 each
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Total no. of IE cases	427

[a] The number of cases where the organism was detected by culture was 4 (0.9%); the number of cases detected by PCR was 11 (2.6%).

The KIT

Resulted in

- **Upgraded classification in 50 patients**
  - **31 possible - definite**
  - **19 rejected - possible**
- **Etiological diagnosis of 30 cases (including a double infection Streptococcus - *C. burnetii*)**

We believe that systematic serological testing save time and money

Diagnostic strategy for BCNE

1. Serology
2. PCR on blood or valve
3. Valve histology

Serology and IE

		Causative agent		
Should be tested		***C. burnetii***	***B. henselae***	***B. quintana***
	Bartonella henselae **(≥ 800 using MIF)**	+	++	++
	Bartonella quintana **(≥ 800 using MIF)**	+	++	++
	Coxiella burnetii **(≥ 800 using MIF)**	+++	+/-	+/-
May be				
	Brucella	-	-	-
	Legionella pneumophila	+/-	-	-
	Chlamydia pneumoniae	-	++	++

Serology - Q fever IE

La Scola B, et al. Serological cross-reactions between *Bartonella quintana*, *Bartonella henselae*, and *Coxiella burnetii*. J Clin Microbiolgy, 1996; 34:2270-74.

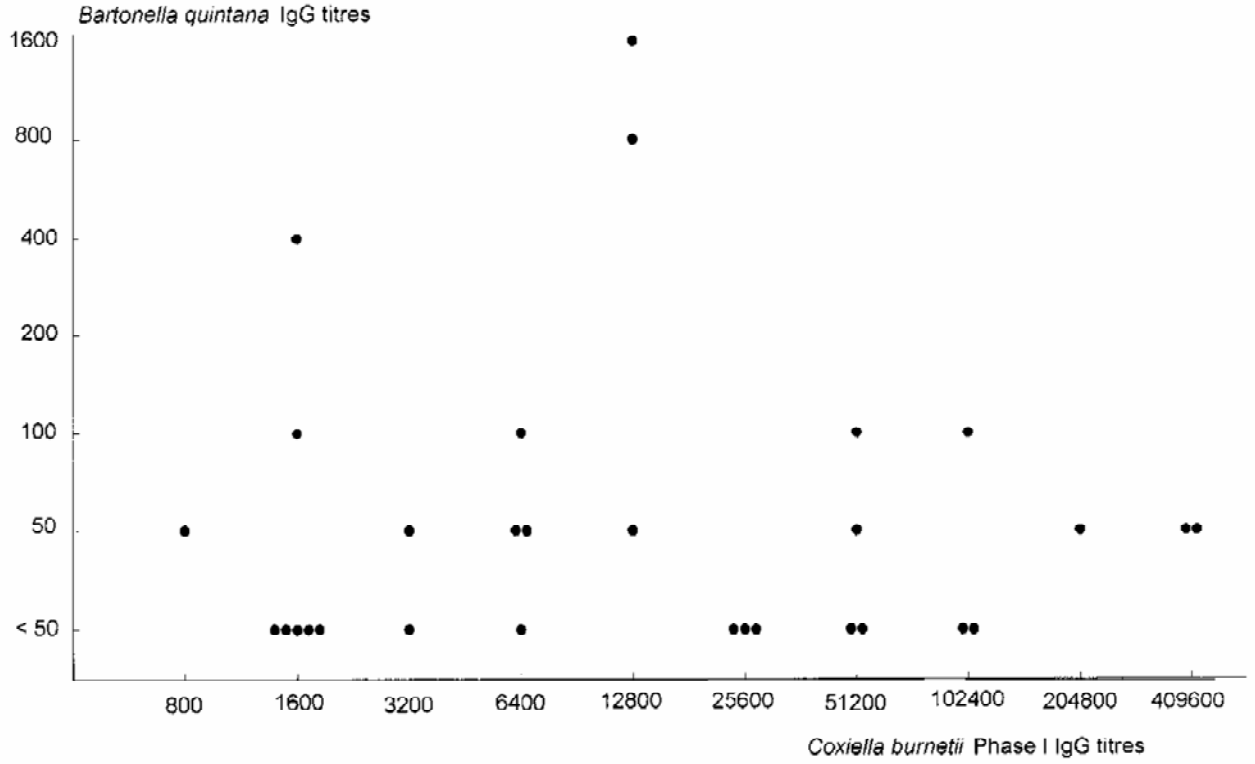


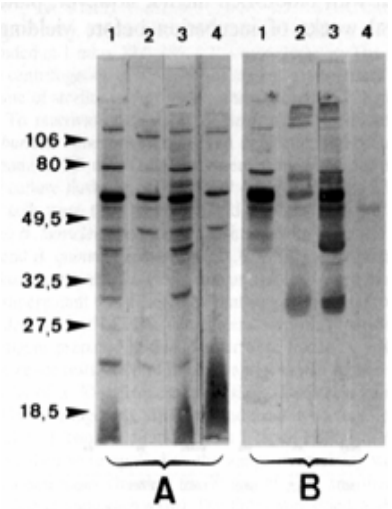
FIG. 2. *C. burnetii* phase I IgG titers and *B. quintana* IgG titers for patients with chronic Q fever before treatment.

Serology

Blood Culture Negative Endocarditis

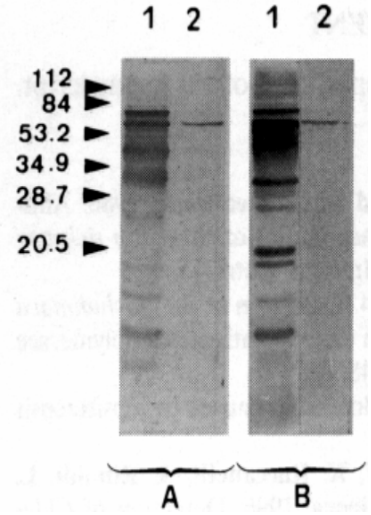
Bartonella

- High level antibodies. Some level for *B. henselae* and *B. quintana.*



Bartonella and *C. burnetii*

- Cross reactions between *Bartonella, Chlamydia,* and *Coxiella burnetii* agents of culture-negative endocarditis
  -> cross adsorption, western bloting



Bartonella and *Chlamydia*

Serology

JOURNAL OF CLINICAL MICROBIOLOGY, Sept. 1997, p. 2283–2287 Vol. 35, No. 9
0095-1137/97/$04.00+0

Serological Cross-Reactions between *Bartonella* and *Chlamydia* Species: Implications for Diagnosis

M. MAURIN,[1] F. EB,[2] J. ETIENNE,[3] AND D. RAOULT[1]*

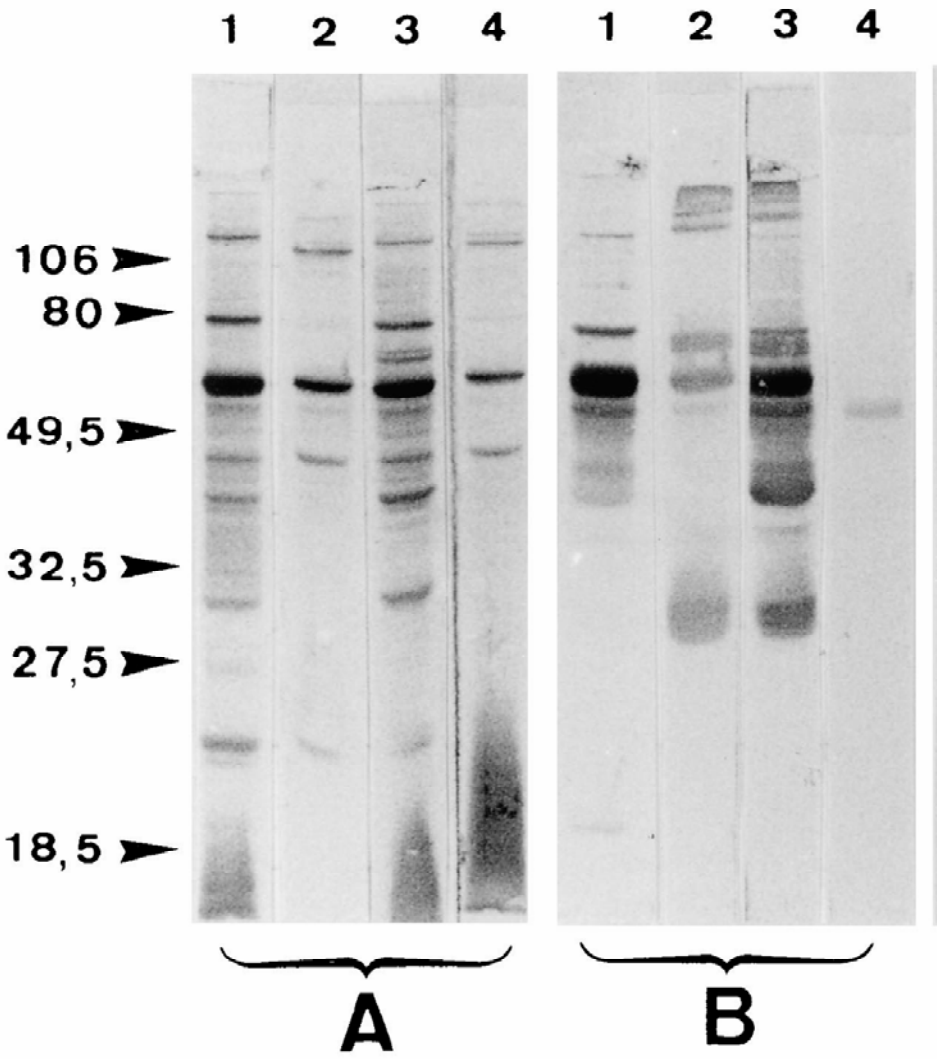
Unité des Rickettsies, CNRS UPRESA 6020, Université de la Méditerranée, Faculté de Médecine, 13385 Marseille,[1] Centre Hospitalier Universitaire d'Amiens, 80054 Amiens, Cedex 1,[2] and Centre Hospitalier Universitaire Edouard Herriot, 69437 Lyon Cedex 03,[3] France

TABLE 1. Clinical and epidemiological data and outcomes for the eight patients previously reported as having *Chlamydia*-related endocarditis[a]

Patient no./age (yr)/ yr of diagnosis[b]	Previous defect	Infected valve	Homeless	Alcoholic	Valvular replacement	Outcome
1/45/1984	No	Ao	Yes	Yes	Yes	Cure
2/56/1988	RF	Ao	No	No	Yes	Cure
3/39/1990	RF	Ao	No	Yes	Yes	Cure
4/26/1985	RF	Mt	No	No	Yes	Unknown
5/35/1985	AB	Ao	No	Yes	Yes	Death
6/45/1989	No	Ao + Mt	No	Yes	Yes	Cure
7/39/1987	M	Ao + Mt	Yes	Yes	Yes	Death
8/36/1989	No	Ao	Yes	Yes	Not possible	Death

[a] Abbreviations: RF, rheumatic fever; AB, aortic bicuspid; M, murmur; Ao, aortic; Mt mitral.
[b] All patients were males.

Serology



La Scola B, et al. Serological cross-reactions between *Bartonella quintana*, *Bartonella henselae*, and *Coxiella burnetii*. J Clin Microbiolgy, 1996; 34:2270-74.

Diagnostic strategy for BCNE

1. Serology
2. PCR on blood or valve
3. Valve histology

RISKS

Vertical contamination (previous amplifications)

MEASURES

- **Prevention**
  - Use separate, dedicated, and controlled rooms
  - Wearing gloves, caps and coats
  - PCR performed in closed system
  - Avoiding positive control (suicide-PCR)

  - Uracil-DNA-glycosylase/dUTP use
- **Detection**
  - Running one negative control for 5 samples

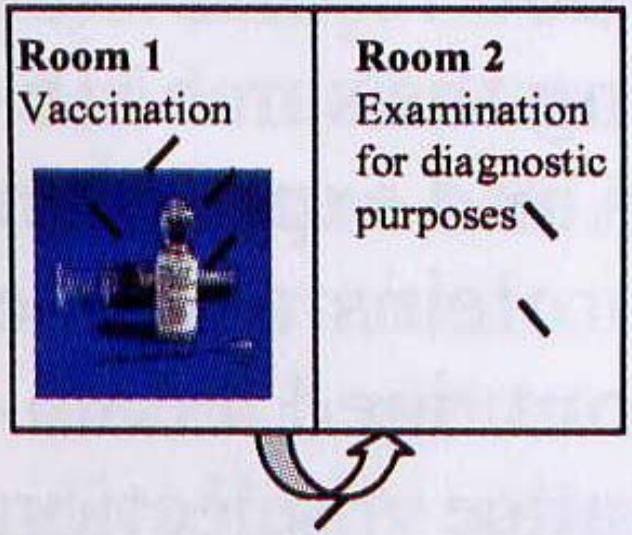
Horizontal contamination (carry-over)

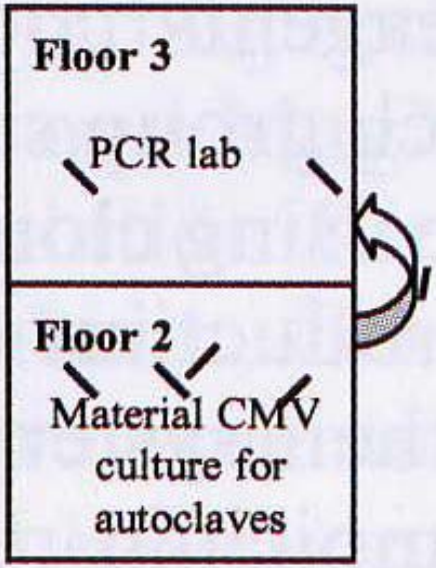
- **Prevention**
  - PCR performed in closed system
- **Detection**
  - Running one negative control for 5 samples
  - Use as a positive control the same bacterium species as those searched but which are not usual pathogens
  - Sequencing all the amplicons

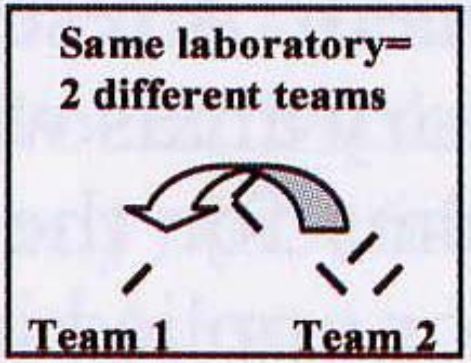
Water and Reagent's contamination

- **Prevention**
  - Digestion with restriction enzymes
- **Detection**
  - Use Mix as negative control
  - Confirm PCR using a second gene

Neighboring contamination





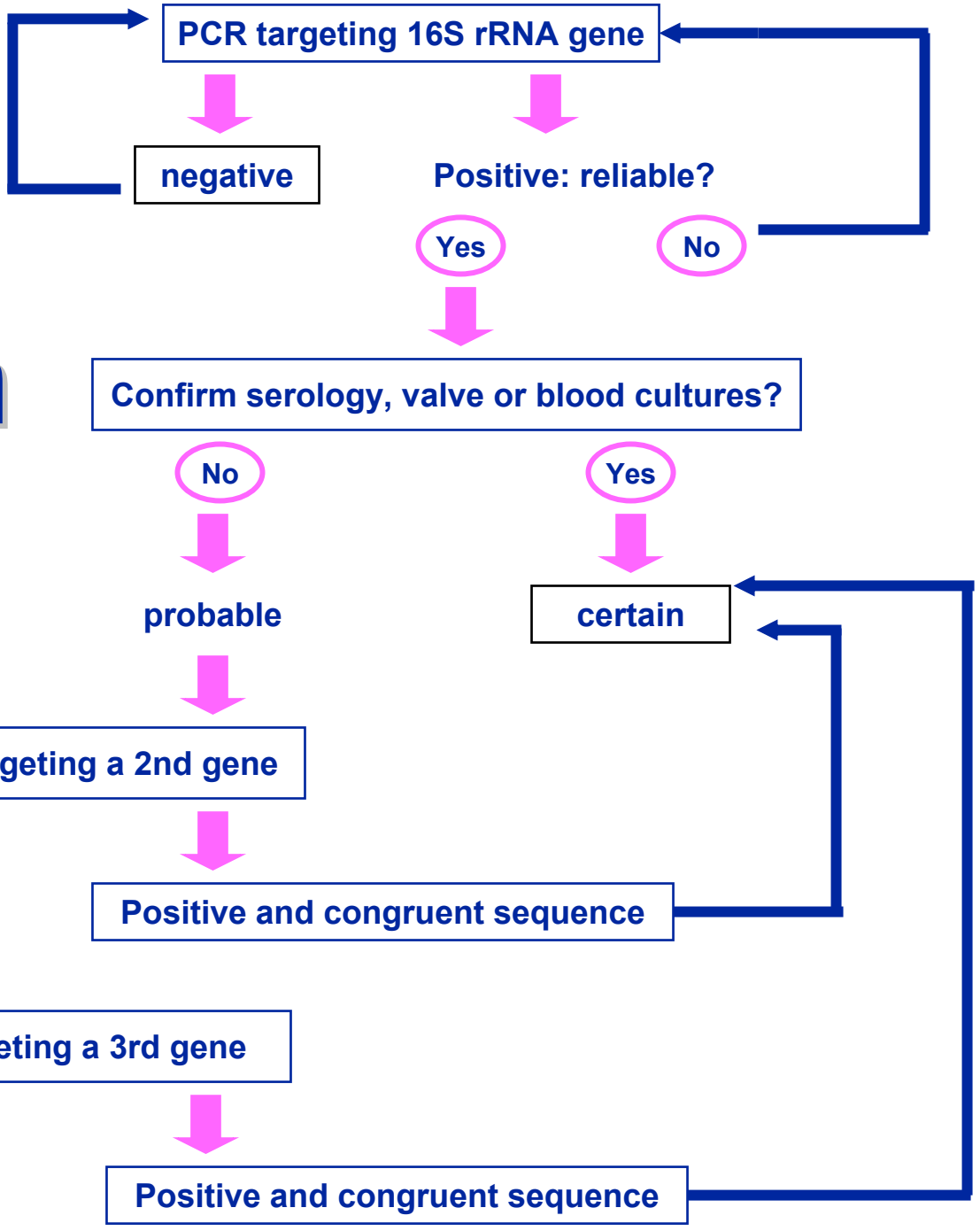


- **Prevention**
  - Communication between teams to be sure that the basic rules are applied
  - Close the laboratory
- **Detection**
  - Running one negative control for 5 samples

Valve

Valves sampling is essential

PCR on valve


PCR targeting 16S rRNA gene
negative
Positive: reliable?
Yes
No
Confirm serology, valve or blood cultures?
No
Yes
probable
certain
PCR targeting a 2nd gene
negative
Positive and congruent sequence
PCR targeting a 3rd gene
negative
Positive and congruent sequence

Greub G, et al. Diagnosis of infectious endocarditis in patients undergoing valve surgery.

Am J Med. 2005;118:230-238.

Table 3 Sensitivity, specificity, positive and negative predictive values of histology, PCR and culture performed on valvular samples taken from patients with and without infectious endocarditis

Diagnostic approach	Sensitivity	Specificity	Positive predictive value	Negative predictive value
Histology*,†	63% (62/98)	100% (118/118)	100% (62/62)	115/143 (82.5%)
PCR after interpretation‡,§	61% (64/105)	100% (118/118)	100% (64/64)	74% (118/159)
Valve culture§	13% (14/105)	98% (116/118)	87% (14/16)	56% (116/207)

*Using stringent criteria (ie, the presence of vegetation, microorganisms, and/or valvular inflammation with a predominance of polymorphonuclear cells).

†Assessed on valves taken from 98 patients with definite infectious endocarditis (modified Duke criteria[14] calculated prior to valve surgery) and from 118 patients without infectious endocarditis.

‡Amplification, sequencing and interpretation of the results according to Figure 1.

§Assessed on 223 valvular samples taken from 118 patients without infectious endocarditis and from 105 patients with definite infectious endocarditis. (Seven patients with possible infectious endocarditis were reclassified as definite infectious endocarditis based on histological examination of valvular samples).

Greub G, et al. Diagnosis of infectious endocarditis in patients undergoing valve surgery.

Am J Med. 2005;118:230-238.

Table 5 Bacterial identification obtained by sequencing DNA amplified from 68 valve samples

	PCR positive with diagnosis initially established by	
Bacterial identification	serology or blood culture	valve analysis
Staphylococcus aureus	11	1
Coagulase-negative Staphylococci	4	0
Streptococcus bovis	12	2*
Streptococcus sp. (other than S. bovis)	15	2
Enteroccocus faecium	1	0
Enterococcus faecalis	6	0
Bartonella quintana	2†	0
Coxiella burnetii	3†	0
Mycoplasma hominis	0	1‡
Granulicatella sp.	0	1
Abiotrophia sp.	1	0
Actinobacillus sp.	1	0
Pasteurella sp.	1	0
Escherichia coli	1	0
Enterobacter sp.	1	0
Candida albicans	1	0
Aspergillus sp.	0	1
Total	60	8

*Both patients presented a discordant positive blood culture.

†Blood culture negative, serology positive.

‡Later confirmed by serology.

PCR of the valve in blood culture negative

	Bosshard	Lang	Breitkopf	Gauduchon	Millar	Greub	Benslimani	Houpikian	Grijalva	Podgladen	
Total	3	1	12	3	4	12	17	96	7	5	160 (100%)
"S. viridans"		1	8		1	2	3	2	3		20 (13%)
"S. bovis"	1		1	1		2		2		1	8 (5%)
Enterococcus			1								1(1%)
Abiotrophia-Granuticatella						1	1	2			4 (3%)
HACCEK	1						2				3 (2%)
Coag. Neg. Staph.			1	1							2 (1%)
Propionibacterium			1								1 (1%)
Bartonella sp.			1		3	2	10	47		4	67 (42%)
Coxiella burnetii				1		3		41			45 (28%)
T. whipplei	1							2	1		4 (3%)
Mycoplasma hominis								1			2 (1%)
Fungi						1	1		3		5 (3%)

423 patients series

Diagnostic: 312 (74%)

Serology

- Q fever: 187
- *Legionella*: 1
- *Bartonella*: 57

63

PEER + PCR on valve

- *Bartonella*: 6
- *Streptococci*: 19
- *T. whipplei*: 10
- *Fungi*: 8
- *Diphteroides*: 4
- *S. aureus*: 4
- *S. epidermidis*: 2
- HACEK: 3
- Others: 10

No Diagnostic: 111 (26%)

Marseille 2002 - 2007

Brouqui P, Raoult D. New insight into the diagnosis of fastidious bacterial endocarditis. FEMS Immunol Med Microbiol.

2006;47: 1-13.

Table 4. Usefulness of polymerase chain reaction in culture-negative endocarditis

Microorganism identified by PCR on valve	Frequency	Commentary
Streptococci	+++	In patient receiving antibiotic
Fastidious streptococci	++	Therapy
Tropheryma whippelii	++	
Mycoplasma	+	
Mycobacterium spp.	+	
Bartonella spp.	++	When serology is not performed
Coxiella burnetii	++	When serology is not performed

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Streptococcus pneumoniae Endocarditis: Persistence of DNA on Heart Valve Material 7 Years after Infectious Episode

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We amplified by PCR and sequenced *Streptococcus pneumoniae rpoB* from DNA of the cardiac valve of a man who had presented with pneumococcal endocarditis 7 years earlier. Histopathologically, the valve did not show evidence of endocarditis. This case raises the question of persistence of DNA without any evidence of infection.

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0095-1137/05/$08.00+0 doi:10.1128/JCM.43.1.163–167.2005

PCR Detection of Bacteria on Cardiac Valves of Patients with Treated Bacterial Endocarditis

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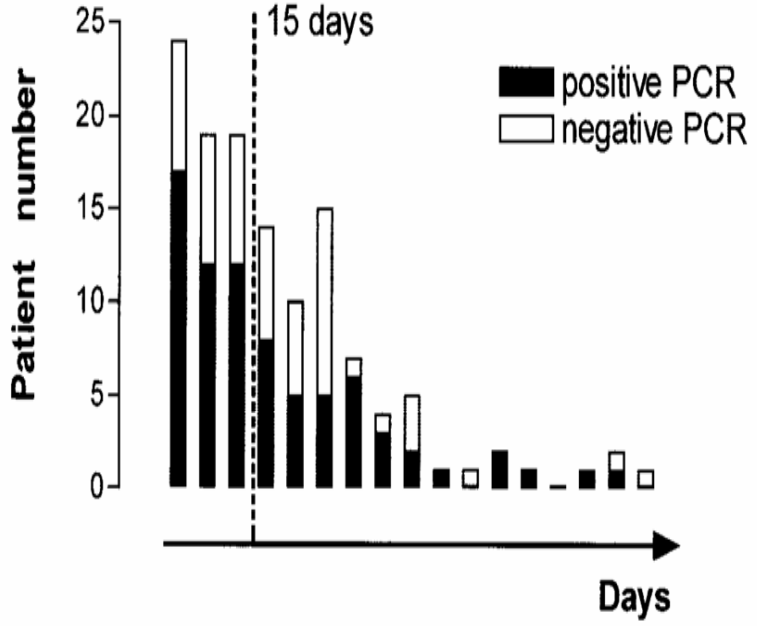


TABLE 3. Long-term persistence of bacterial DNA in patients who had completed antibiotic treatment for IE at the time of surgery[a]

Case/age/sex[b]	Bacterium	Involved valve	Delay between diagnosis and surgery
1/55/M	*Staphylococcus pneumoniae*	Aortic bioprosthesis	7 yr
2/69/F	*S. bovis*	Aortic, native	167 days
3/80/M	*S. bovis*	Mitral, native	730 days
4/39/M	*Enterococcus faecium*	Aortic bioprosthesis	850 days
5/36/M	*Streptococcus gordonii*	Aortic, native	45 days
6/33/M	*Bartonella quintana*	Aortic homograft	224 days
7/70/F	*Streptococcus sanguinis*	Mitral bioprosthesis	545 days

[a] All patients had no clinical or histological signs of IE, and valve cultures were sterile.
[b] M, male; F, female.

PCR on blood

Septifast ® Roche and I.E.: We tested 63 patients

- 19 blood culture positive (8 + Septifast ® Roche)
- 20 treated blood culture positive (3 + Septifast ® Roche):15%
- 12 EIHN not included (0)
- 10 infection on intracardiac device (0)

May be useful in EIHN who received antibiotic treatment.

Problems : - Volume

- Spectrum (no HACEK, *Coxiella*, *Bartonella*)
- Interpretation (low level Spectro, *S. epidimidis*)

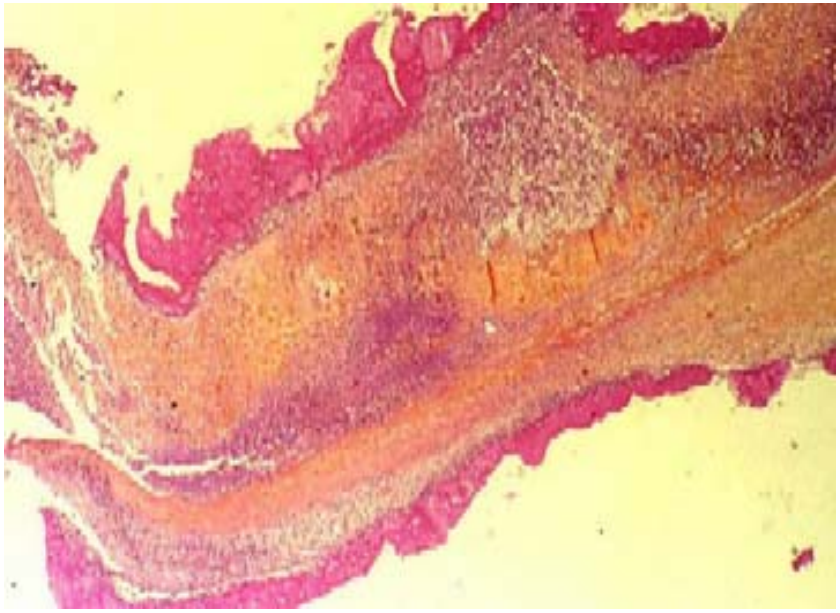
Diagnostic strategy for BCNE

1. Serology
2. PCR on blood or valve
3. Valve histology

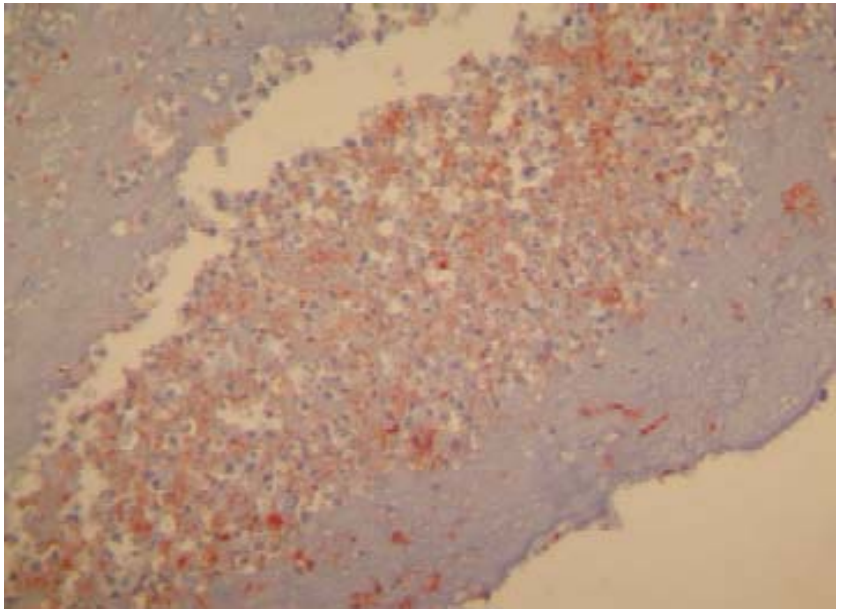
Valve Histology

- Only vegetation, microorganisms and valvular inflammation including polymorphonuclears have a 100% specificity
- Gold standard (definite criterion)
- Inflammation may be considered a major criterion
- Immunohistochemistry help to diagnose etiology

Valve Histology



Infective endocarditis (HPS)



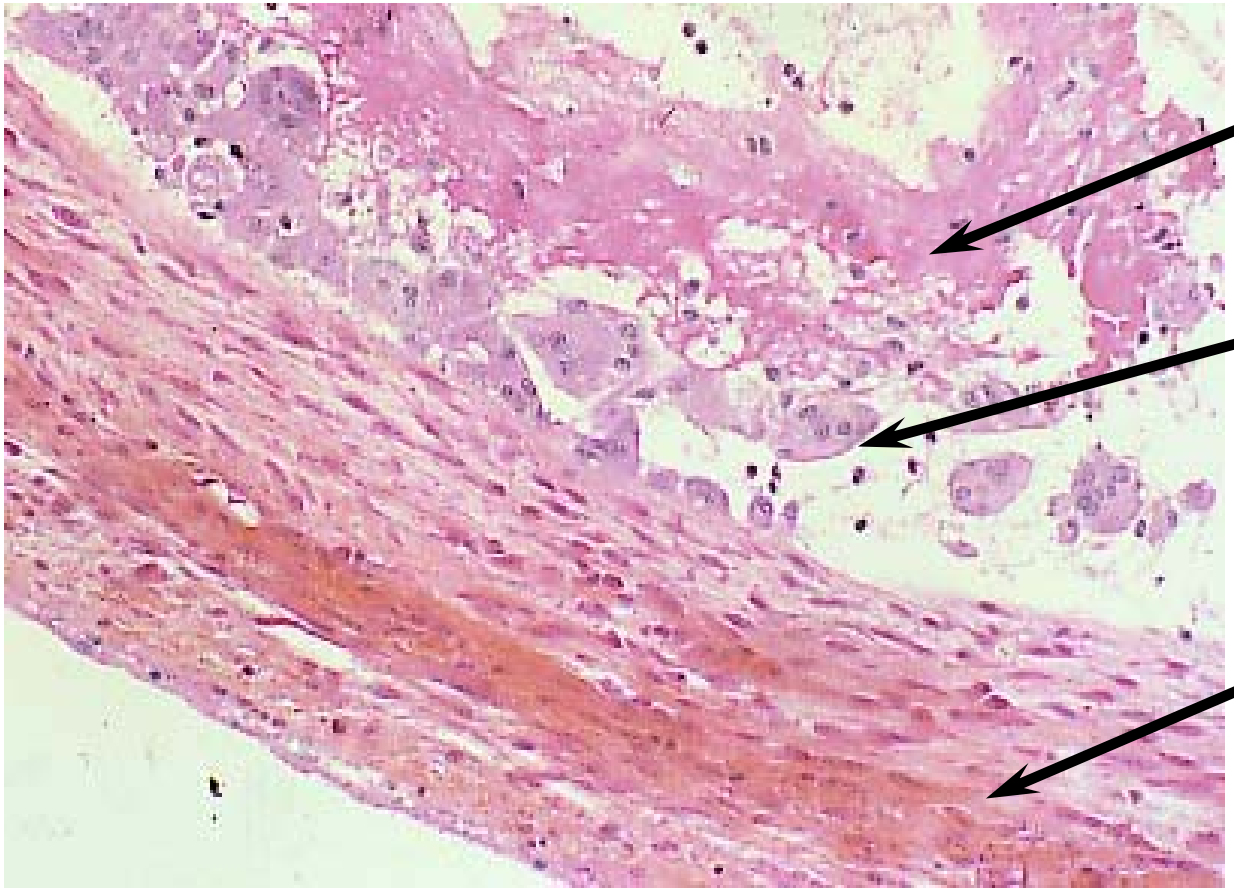
Immunohistochemistry: CD15

Vegetation

True IE

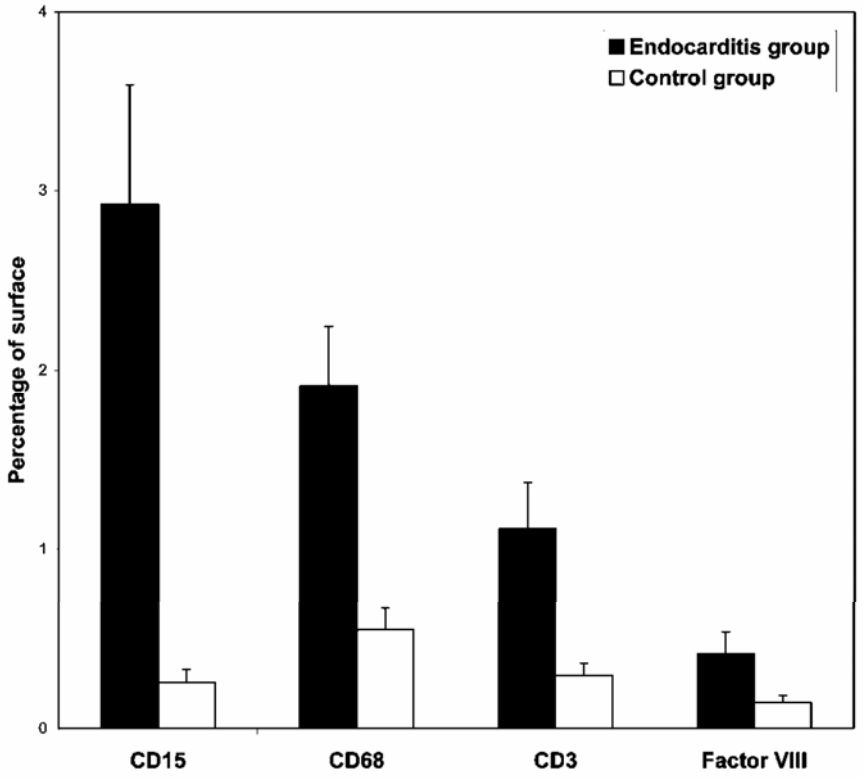
Polymorphonuclears

Valve Histology



Valvular bioprosthesis
Non-infective degenerative lesions

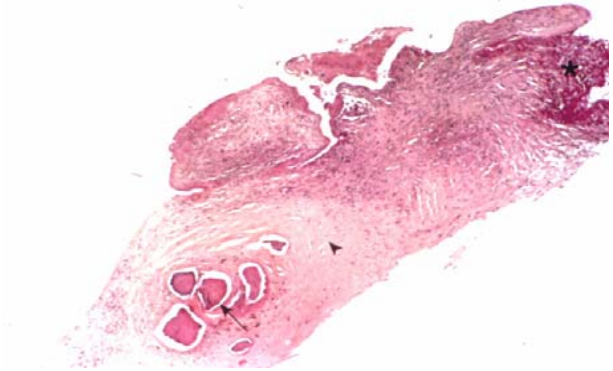
- Only 70% of patients with mechanic valve, histologically proven IE, have vegetations



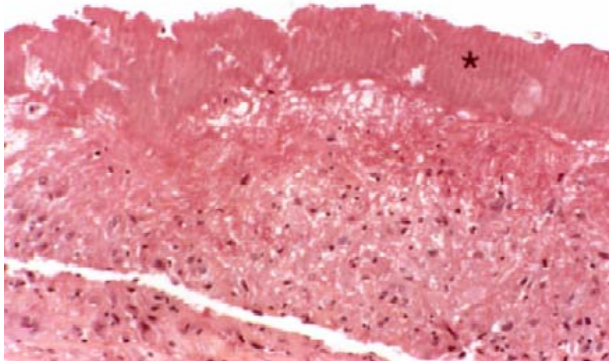
Lepidi H, et al. Quantitative histological examination of mechanical heart valves. Clin Infect Dis. 2005;40:655-661

Valve Histology

Lepidi H. Am J Clin Pathol, 2000.



Hematoxylin-eosin-saffron



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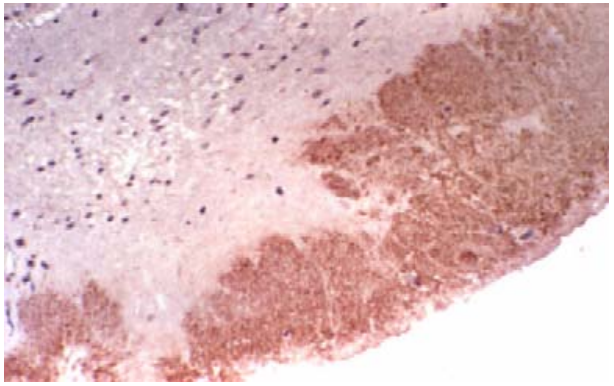
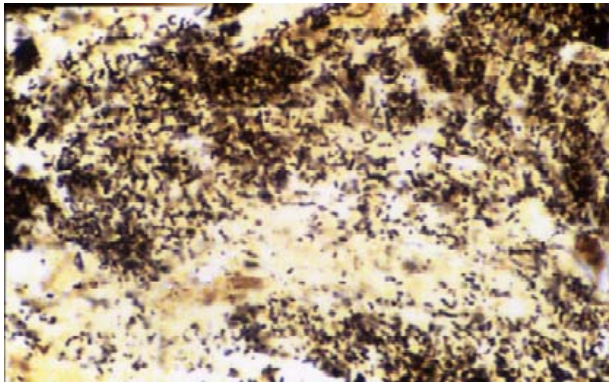


Immunohistochemistry



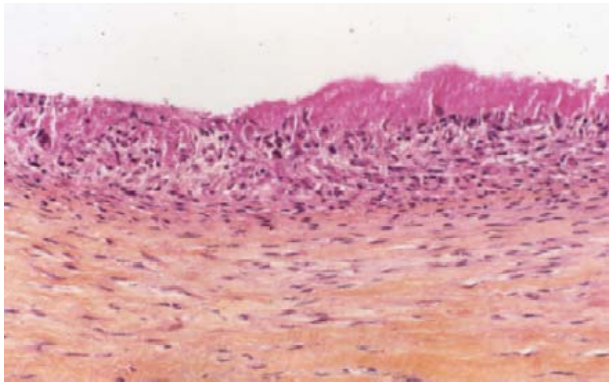
Warthin-Starry staining

Bartonella

Valve Histology

Lepidi H. J Infect Dis, 2003.

Hematoxylin-eosin-saffron



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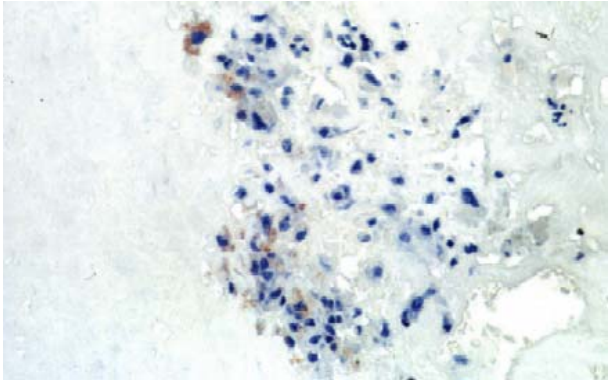
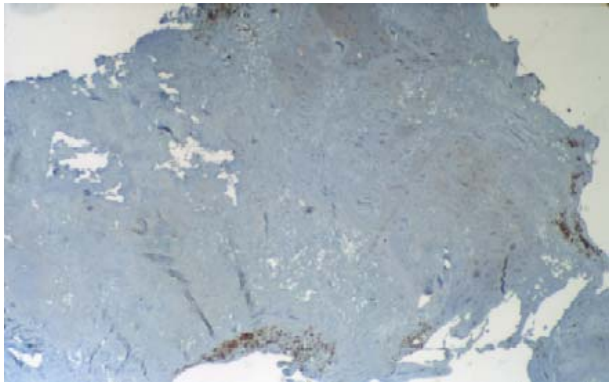


Immunohistochemistry

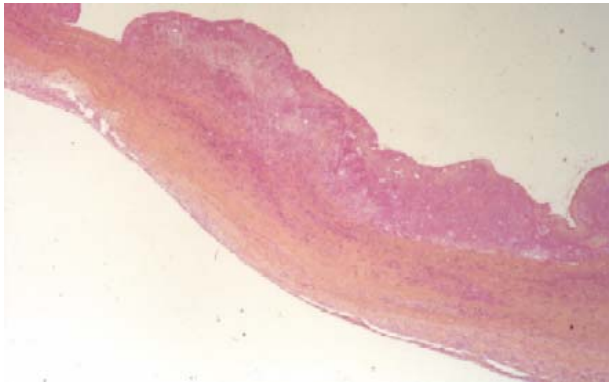


CD 68

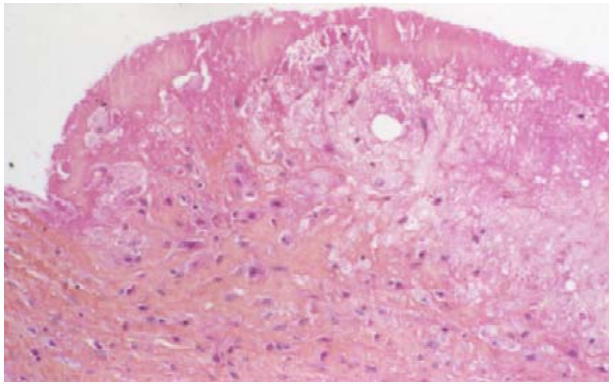
Coxiella burnetii

Valve Histology

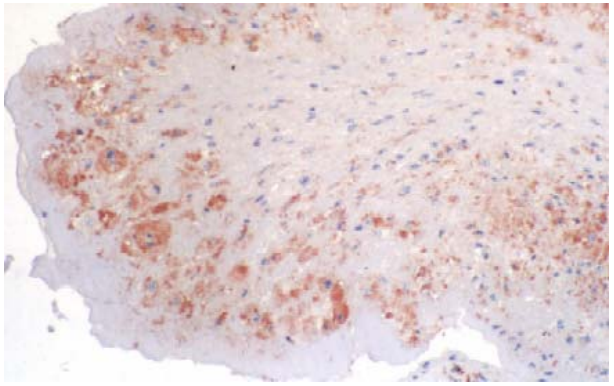
Lepidi H. J Infect Dis, 2004.



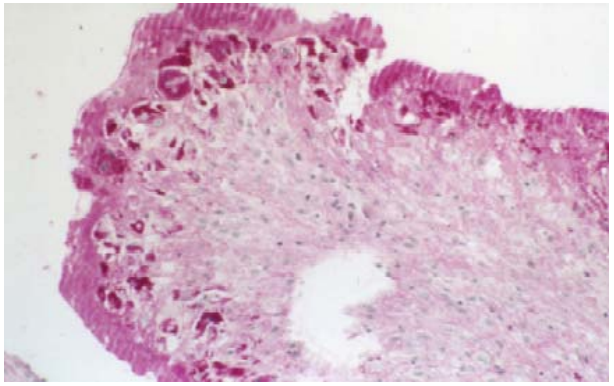
Hematoxylin-eosin-saffron



Hematoxylin-eosin-saffron



Immunohistochemistry



PAS staining

Whipple

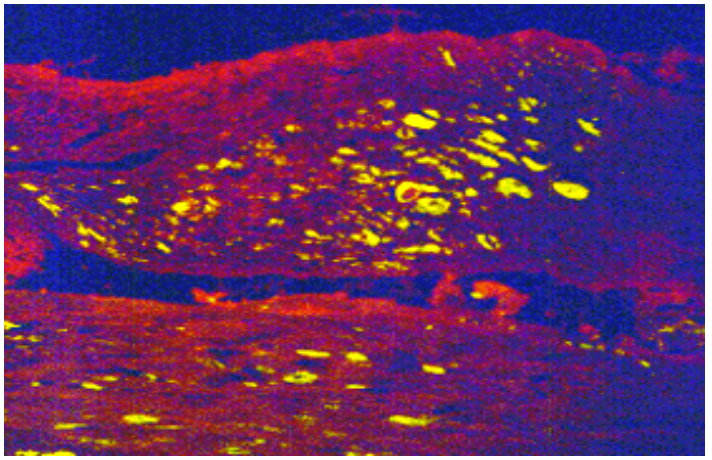
Valve

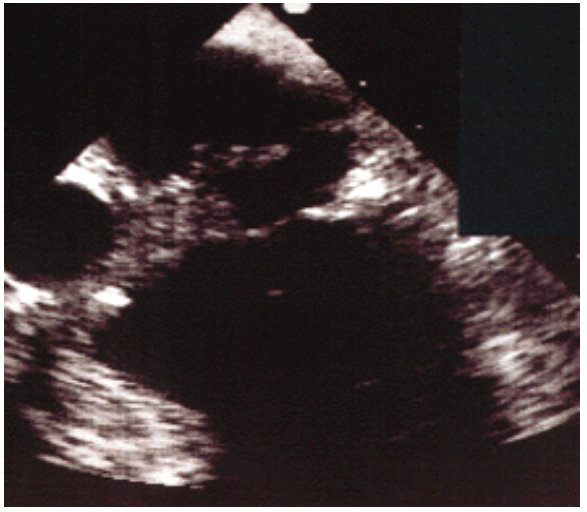
- Culture - low sensitivity
- Histology - not evaluated
- PCR - is it a new duke criteria?

Blood Culture Negative Endocarditis

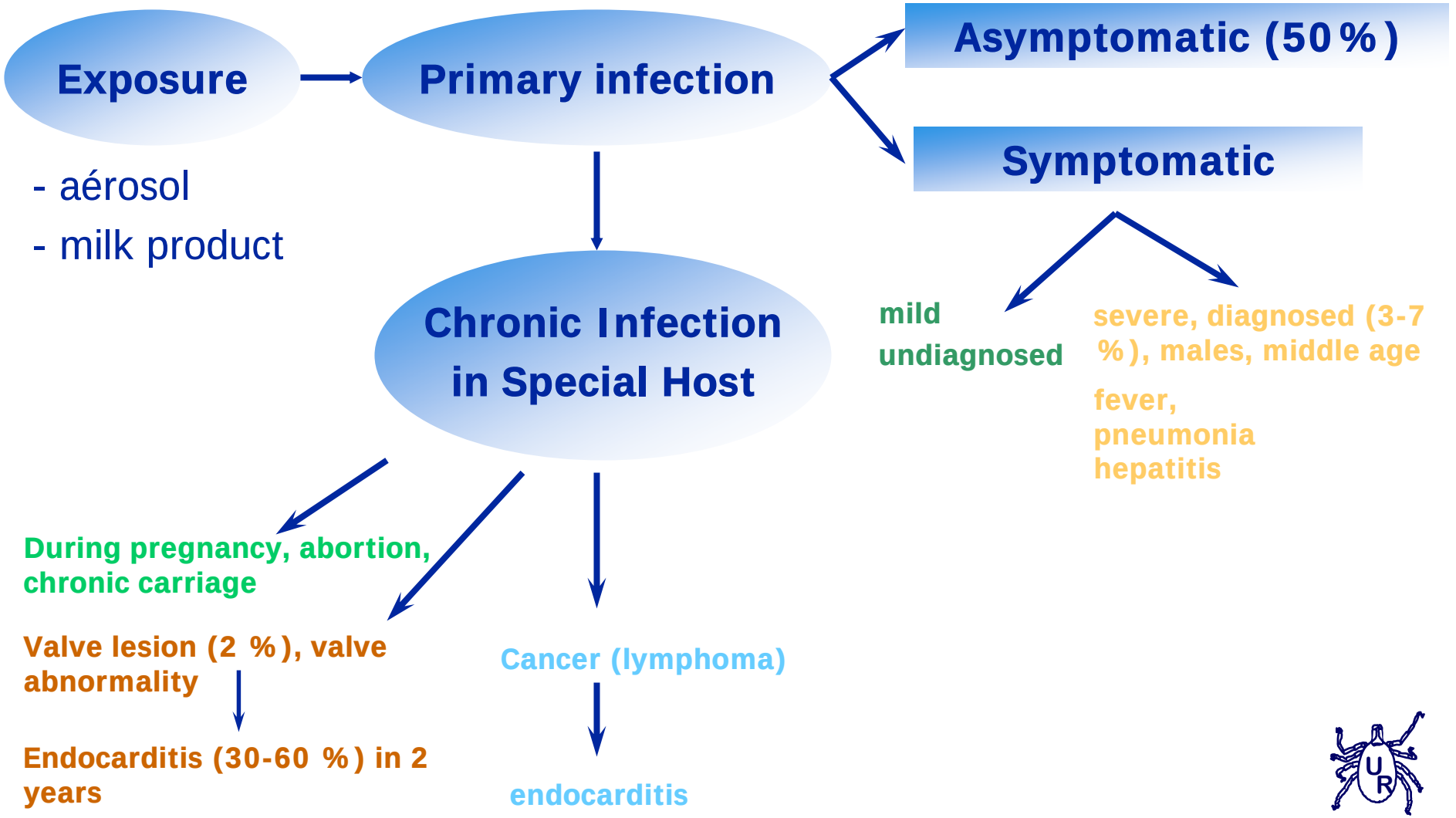
Q fever

- "Culture negative endocarditis"
- In patients with previously known valvulopathy +++
- In immunocompromised patients
- Atypical presentation (fever and vegetations are lacking)
- Mean diagnostic delay: 12 month
- First case of IE in males younger than 65 years with a prothesis in Marseille
- Prognosis: 23 % of cases are fatal
  - Marseille 1984 : 65 %
  - Marseille 2000 : 5 %





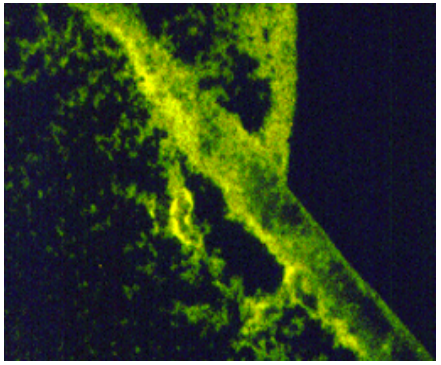
Pathophysiology

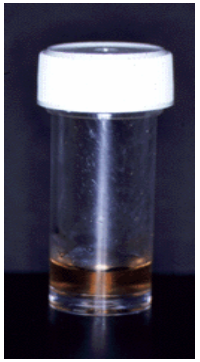


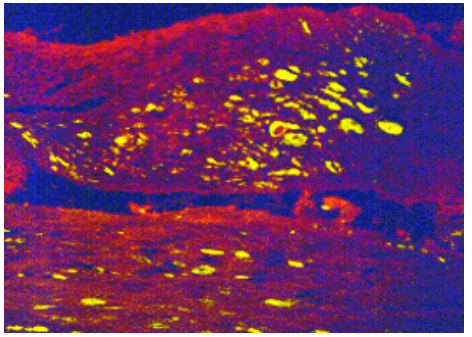
Blood Culture Negative Endocarditis

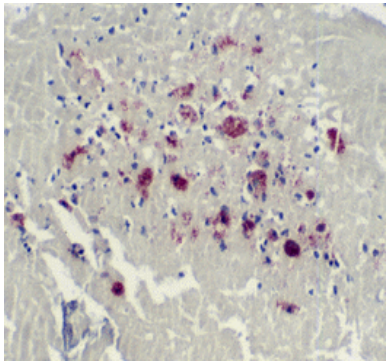
Q fever

- Serology: **Modified Duke endocarditis service criteria** **(IFA: IgG phase I ≥ 800; sensitivity 98 %, specificity 100 %)**
- Isolation (Shell vial technique): blood and heart valves
- Immunofluorescence or Immunohistochemistry: heart valves
- PCR



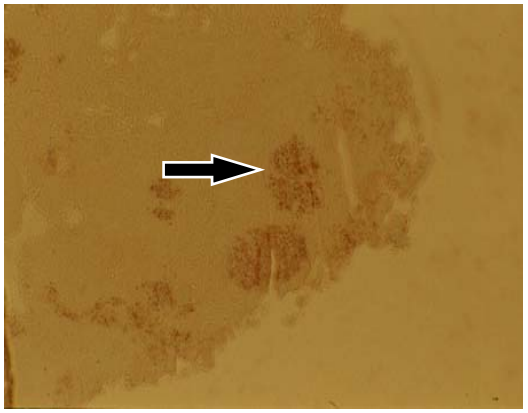






Blood Culture Negative Endocarditis

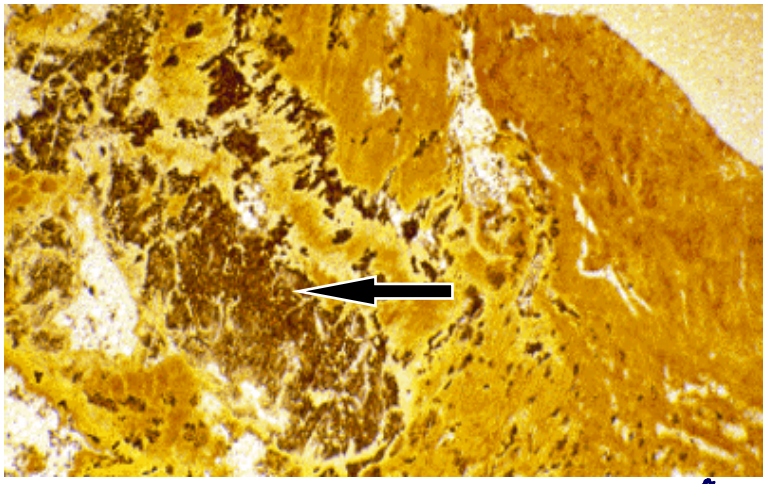
Bartonella



- *B. quintana, B. henselae, B. elizabethae*
- ~ 3 % of all endocarditis cases
- In France, Canada, UK (?)
- Rare in Northern Europe,

>10% in Northern Africa

- males > females
  aortic valve > mitral valve
  extensive valve damage



Znazen A. et al. high prevalence of *Bartonella quintana* endocarditis in sfax, Tunisia. Am J Trop Med Hyg. 2005;72: 503-7.

TABLE 2

Results of serology, Western blot, PCR, antibiotic therapy, and outcome of patients with *B. quintana* endocarditis from cardiology service of CHU of Sfax, Tunisia

	Serology				LCN PCR					
Case/sex/age	*Chlamydia*	BQ	BH	WB	*rnpb* gene	*fur* gene	Final diagnosis	Antibiotic therapy	Operated	Outcome
1/M/44	+	6400	6400	BQ	BQ	BQ	BQ	Ampi + gena/ciflox	Yes	Recovery
2/F*	+	1600	3200	BQ	BQ	BQ	BQ			
3/M/46	+	1600	1600	BQ	Negative	BQ	BQ	Peni G + genta/cefo + ampi oflo	Yes	Recovery
4/F/56	+	6400	3200	BQ	Negative	BQ	BQ†	Peni G + genta/doxy + sxt	Yes	Recovery
5/M/31	+	3200	3200	BQ	BQ	BQ	BQ	No antibiotics	No	Death
6/M/44	+	6400	6400	BQ	BQ	BQ	BQ	Ampi + genta + amphoB	Yes	Recovery
7/M/48	+	3200	3200	BQ	Negative	BQ	BQ	Peni G + genta/cefo + oflo	Yes	Recovery
8/F/26	+	3200	1600	BQ	Negative	BQ	BQ	Cefo + genta	Yes	Recovery
9/M/44	+	3200	3200	BQ	Negative	BQ	BQ	Ampi + genta	No	LS
10/M/56	+	800	400	BQ	BQ	BQ	BQ	No antibiotics	No	LS
11/M/40	+	6400	3200	BQ	BQ	BQ	BQ	Ampi + genta	No	Recovery
12/M/20	+	3200	3200	BQ	BQ	BQ	BQ	Ampi + amik/oflo + vanco + amik	Yes	Recovery
13/M/20*	+	1600	1600	BH	Negative	Negative	BH			

Antibiotics: amik, amicacin; amphoB, amphotericin B; Ampi, ampicillin; doxy, doxycyclin; genta, gentamicin; oflo, ofloxacin; Peni G, penicillin G; vanco, vancomycin; sxt, cotrimoxazole. BH, *B. henselae;* BQ, *B. quintana; Cb, Coxiella burnetii.* LS, lost of sight; WB, Western blot.

* For patients 2 and 13, no data were available.

† Diagnosis of endocarditis due to *C. pneumoniae* was made by PCR performed on the valve.

Benslimani A, Fenollar F, Lepidi H, Raoult D. Bacterial zoonoses and infective endocarditis, Algeria. Emerg Infect Dis. 2005;11 : 216-224.

Table 2. Distribution of 110 infective endocarditis cases* diagnosed in Algeria using blood culture, cardiac valve culture, serologic testing, cardiac valve polymerase chain reaction (PCR), and PCR on serum samples

	Positive samples/tested samples					
Identified microorganisms	Blood culture (N = 110)	Cardiac valve culture (N = 38)	Serologic testing (N = 61)	Cardiac valve PCR (N = 38)	PCR on serum sample (N = 9)	Total
Streptococcus spp. and related genera	0/22	0/4	NP	7/0	NP†	24/0
Bartonella quintana	0/1‡	0/3	5/2	10/0	3/2	12/2
Staphylococcus spp.	2/10	0/3	NP	2/1	NP	11/3
HACEK§	0/4	0/0	NP	1/1	NP	5/1
Enterococcus spp.	1/1	0/0	NP	1/0	NP	2/1
Brucella melitensis	0/1	0/0	2/0	2/0	NP	2/0
Coxiella burnetii	0/0	0/0	2/0	0/0	1/NP	2/0
Corynebacterium spp.	0/2	0/1	NP	1/0	NP	2/0
Mycoplasma hominis	0/0	0/0	NP	1/0	NP	1/0
Enterobacteria spp.	1/1	0/0	NP	0/0	NP	1/1
Alcaligenes faecalis	0/1	0/0	NP	0/0	NP	1/0
Pseudomonas aeruginosa	0/1	0/0	NP	0/0	NP	1/0
Bacillus cereus	0/0	0/0	NP	1/0	NP	1/0
Candida spp.	0/0	0/1	NP	1/0	NP	1/0
	Negative samples for definite infective endocarditis/negative samples for possible infective endocarditis					
No etiology	10/25	2/7	8/20	2/7	NP/NP	

*77 definite and 33 possible.
†NP, not performed.
‡If we consider that *Bartonella quintana* was misidentified as *Haemophilus influenzae.*
§HACEK, *Haemophilus*, *Actinobacillus*, *Cardiobacterium*, *Eikenella*, *Kingella.*

Brouqui P, Raoult D. New insight into the diagnosis of fastidious bacterial endocarditis. FEMS Immunol Med Microbiol.

2006;47: 1-13.

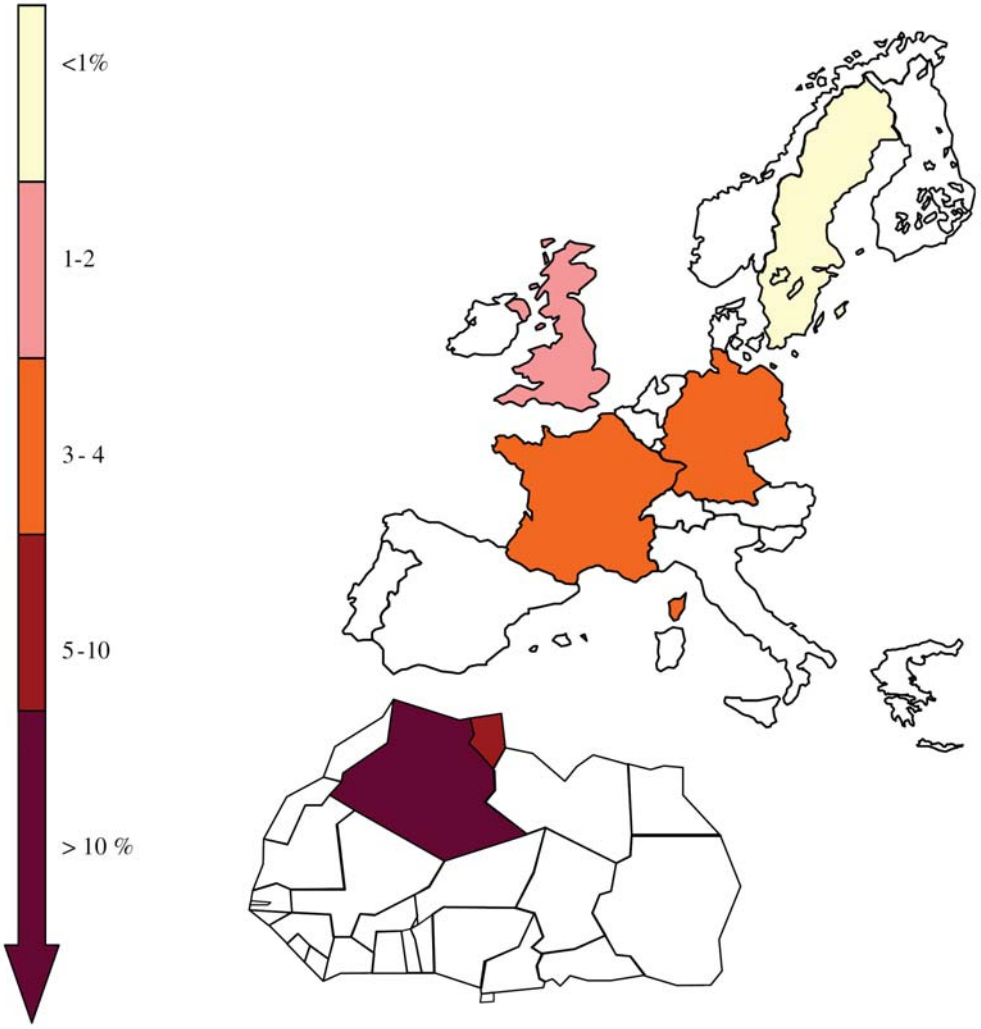


Fig. 1. Prevalence (%) of *Bartonella quintana* endocarditis in Europe and North Africa.

Blood Culture Negative Endocarditis

Whipple's disease

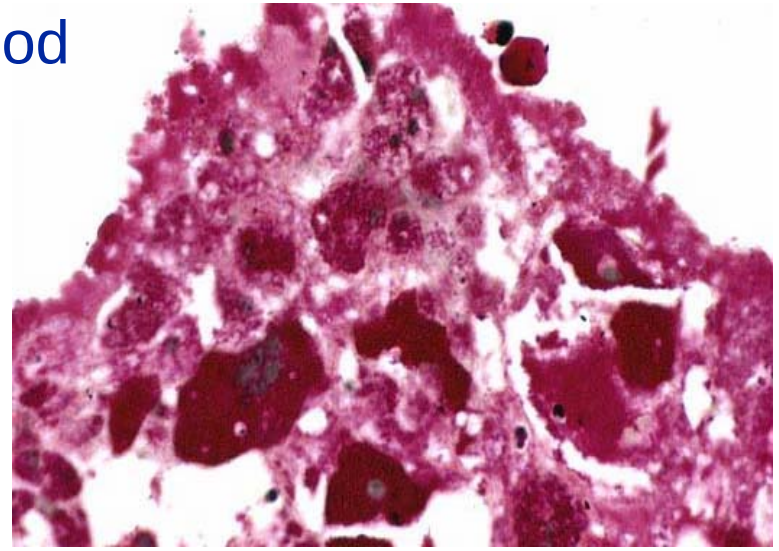
- 42 year old man
- IQ of 54, encephalitis during childhood
- Rheumatic Fever ?
- 1997 - severe aortic insuffisency

cardiac insuffisency

- May 1998 vegetation on mitral

valve at the echography

- No fever, no diarrhea
- Surgery of the valve
- No general Whipple's disease



PAS staining

Fenollar F, Puéchal X, Raoult D. Whipple's disease. New Engl J Med. 2007;356:55-66.

Table 2. Demographic and Clinical Features of Classic Whipple's Disease.*

Feature	Patients with Whipple's Disease
	no./total no. (%)
Male sex	770/886 (87)
Arthralgia or arthritis	244/335 (73)
Diarrhea	272/335 (81)
Weight loss	223/240 (93)
Fever	128/335 (38)
Adenopathy	174/335 (52)
Melanoderma	99/240 (41)
Neurologic signs†	33/99 (33)
Ocular signs†	6/99 (6)
Pleural effusion	26/190 (14)

Fenollar F, Puéchal X, Raoult D. Whipple's disease. New Engl J Med. 2007;356:55-66.

Feature	Value
Blood culture–negative endocarditis associated with *T. whipplei*[30-38]	
No. of patients	17
Male sex — no. (%)	14 (82)
Previous valvular disease — no. (%)	7 (41)
Acute rheumatic fever	3 (18)
Bicuspid aortic valve	2 (12)
Aortic bioprosthesis	2 (12)
Antecedent — no. (%)	12 (71)
Arthralgia or arthritis	8 (47)
Seronegative polyarthritis	2 (12)
Psoriatic arthritis	1 (6)
Myalgia	1 (6)
Interval between onset of symptoms and definite diagnosis — range (mean)	2 mo–20 yr (5 yr)
Involved valves — no. (%)	
Aortic	8 (47)
Mitral	4 (24)
Tricuspid	1 (6)
Aortic and mitral	3 (18)
Aortic and tricuspid	1 (6)
Fever — no. (%)	2 (12)
Cardiac vegetations — no. (%)	13 (76)
Congestive heart failure — no. (%)	10 (59)
Arterial emboli — no. (%)	10 (59)