



# BEST Of : Bactéries intracellulaires

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## Molecular diagnosis of human granulocytic anaplasmosis

*J Stephen Dumler<sup>1</sup> and Philippe Brouqui*

Journal : **Expert Rev Mol Diagn**  
Impact factor : **(2004)**

**Box 1. Summary of recommendations for the diagnosis of HGA (10).**

**Clinical and laboratory features**

- Acute febrile illness
- Geographic region with known Ixodes ticks that bite humans
- Disease during mid-April through late October (Northern hemisphere)
- Characteristic laboratory findings (thrombocytopenia, leukopenia, elevations in serum transaminase activities)

**Laboratory diagnostics**

- Acute and convalescent serum for antibody tests
- Peripheral blood smear examination for morulae
- PCR on acute-phase EDTA-anticoagulated blood (not serum or plasma)
- Blood collected before doxycycline therapy, preferably within the first 7 days of illness
- Blood tested promptly or refrigerated for less than 48 h prior to testing
- DNA preparation using standardized method simultaneously with known positive control (low level to assure adequate sensitivity) and negative control blood samples
- If positive control blood sample is not available, blood spiked with *Anaplasma phagocytophilum* in cultured cells (usually HL-60 cells) may be substituted
- Various PCR targets are appropriate: *ms* (16S rRNA gene) is most often used but *mip2* (*hge-44* or *p4*) or *ankA* (*gpank1*) may be more sensitive
- All negative samples should be tested for potential PCR inhibition (internal control, such as *Gapdh* or  $\beta$ -globin)
- All attempts to confirm a positive PCR with another independent method (serology, peripheral blood smear, culture, two independent gene targets) should be made

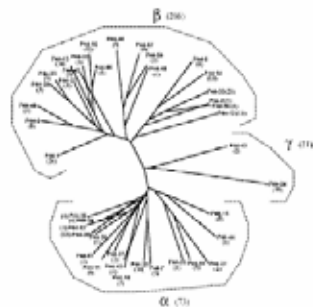
**Table 1. Relative sensitivities of diagnostic tests for human granulocytic ehrlichiosis at various intervals after onset of disease manifestations.**

Delay after onset of disease (days)	Diagnostic test			
	Blood smear	Culture	PCR	Serology
0-7 days	Medium	Medium	Medium	-
8-14 days	Low	Low	Low	Medium
14-30 days	-	-	Low	High
30-60 days	-	-	-	High
>60 days	-	-	-	High

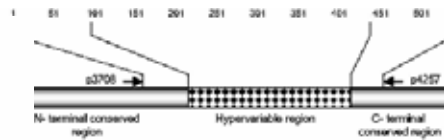


**Sequence Analysis of p44 Homologs Expressed by *Anaplasma phagocytophilum* in Infected Ticks Feeding on Naive Hosts and in Mice Infected by Tick Attachment**

Suleyman Felek,<sup>1</sup> Sam Telford III,<sup>2</sup> Richard C. Falco,<sup>2</sup> and Yasuko Rikihisa<sup>1\*</sup>



**FIG. 3.** Phylogenetic analysis of deduced amino acid sequences of 40 different p44 transcript species found expressed in mice and/or ticks. Amino acid sequences were aligned with the Clustal V program, and the tree was constructed by the neighbor-joining method. cDNA clone frequencies (of a total of 300 clones sequenced) for each p44 species are indicated in parentheses.



**Background :**

Le gène p44 (*Msp2*) est exprimé de façon variable dans les tiques, les cellules en culture, chez la souris, le cheval, l'homme ....

**Question :**

Comment est contrôlé l'expression de ce ( ces ) gène ( s ) ?

**Réponse :**

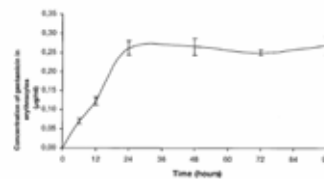
Il existe des paralogs différents de *msp2* chez la souris et chez la tique, mais pas de mosaïque ou de chimère mais une variation de la séquence dans les extrémités de la région conservée. La région hypervariable est stable. Il existe donc un panel de P44 présent dans le génome.

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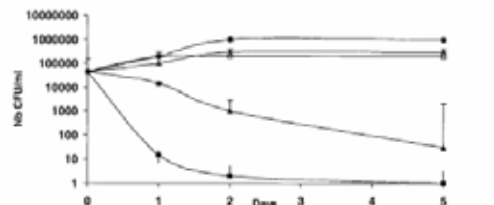


**Culture and Antibiotic Susceptibility of *Bartonella quintana* in Human Erythrocytes**

Jean-Marc Rolain,<sup>1</sup> Max Maurin,<sup>2</sup> Marie-Noëlle Mallet,<sup>1</sup> Daniel Parzy,<sup>2</sup> and Didier Raoult<sup>1\*</sup>



**FIG. 4.** Kinetics of persistence of gentamicin in human erythrocytes determined by HPLC. Gentamicin was used at 9 µg/ml in medium that was changed each day.



**FIG. 3.** Bactericidal effects of rifampin at 1 µg/ml (○) and 6 µg/ml (▲) and gentamicin at 1 µg/ml (□) and 6 µg/ml (■) on *B. quintana* in human erythrocytes. ●, growth control. Nb, number.

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### Recommendations for Treatment of Human Infections Caused by *Bartonella* Species

J. M. Robain,<sup>1</sup> P. Brouqui,<sup>1,2</sup> J. E. Koehler,<sup>3</sup> C. Maguina,<sup>4</sup> M. J. Dolan,<sup>5</sup> and D. Raoult<sup>1,2\*</sup>

TABLE 6. Guidelines and recommendations for the treatment of infections caused by *Bartonella* species<sup>a</sup>

Disease	Regimen for <sup>b</sup> :		Strength of recommendation	Reference(s)
	Adults	Children		
Typical CSD	No recommendation	No recommendation	BI	56, 84
	For patients with extensive lymphadenopathy, consider azithromycin at 500 mg p.o. on the first day and 250 mg p.o. on days 2 to 5 as a single daily dose	For patients with extensive lymphadenopathy, consider azithromycin at 10 mg/kg p.o. on day 1 and 5 mg/kg p.o. on days 2 to 5 as a single daily dose		
Retinitis	Doxycycline at 100 mg p.o. BID for 4-6 wk and rifampin at 300 mg p.o. BID for 4-6 wk	Unknown	AII	56, 84
Trench fever or chronic bacteremia with <i>B. quintana</i>	Doxycycline at 200 mg p.o. QD for 4 wk and gentamicin 3 mg/kg i.v. QD for 2 wk	Unknown	AI	36
BA <sup>b</sup>	Erythromycin at 500 mg p.o. QID for 3 mo	Erythromycin ethylsuccinate p.o. at a total of 40 mg/kg/day in four divided doses (maximum total daily dose, 2 g/day) for 3 months	AII	58
	Or doxycycline at 100 mg p.o. BID for 3 mo		AII	58
PH <sup>c</sup>	Erythromycin at 500 mg p.o. QID for 4 mo	Erythromycin ethylsuccinate p.o. at 40 mg/kg total/day in four divided doses (maximum total daily dose, 2 g/day) for 4 mo	AII	58
	Or doxycycline at 100 mg p.o. BID for 4 mo		AII	58




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### Recommendations for Treatment of Human Infections Caused by *Bartonella* Species

J. M. Robain,<sup>1</sup> P. Brouqui,<sup>1,2</sup> J. E. Koehler,<sup>3</sup> C. Maguina,<sup>4</sup> M. J. Dolan,<sup>5</sup> and D. Raoult<sup>1,2\*</sup>

Endocarditis	Suspected <i>Bartonella</i> , culture negative: Gentamicin at 3 mg/kg/day i.v. for 14 days and ceftriaxone at 2 g i.v. or i.m. QD for 6 wk with or without doxycycline at 100 mg p.o. or i.v. BID for 6 wk	Unknown	AII	83
			BII	83
Endocarditis	Documented <i>Bartonella</i> , culture positive: Doxycycline at 100 mg p.o. BID for 6 wk and gentamicin at 3 mg/kg/day i.v. for 14 days <sup>d</sup>		BII	83
			BII	83
Carrion's disease Oroya fever	Chloramphenicol at 500 mg p.o. or i.v. QID for 14 days and another antibiotic (a beta-lactam is preferred)  Or ciprofloxacin at 500 mg p.o. BID for 10 days	Chloramphenicol at 50-75 mg/kg/day p.o. or i.v. divided into four doses for 14 days and another antibiotic (a beta-lactam is preferred)  Or ciprofloxacin in children 7-12 years 250 mg p.o. BID for 10d	AII	67
			BIII	— <sup>d</sup>
Verruga peruana	Rifampin at 10 mg/kg/day p.o. for 14 days  Or streptomycin at 15-20 mg/kg/day i.m. for 10 days	Rifampin at 10 mg/kg/day p.o. for 14 days (maximum total daily dose of 600 mg/day)	AII	67
			AII	67

<sup>a</sup> Abbreviations: BID, twice a day; QD, once a day; QID, four times a day.

<sup>b</sup> Longer treatment for HIV-infected and other immunocompromised patients (AII) (56).

<sup>c</sup> If gentamicin cannot be given, replace it with rifampin at 300 mg p.o. twice daily.

<sup>d</sup> Maguina, unpublished.



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Journal : **J Clin Microbiol**  
 Impact factor : **3.96**

### Aneruptive Fever Associated with Antibodies to *Rickettsia helvetica* in Europe and Thailand†

Pierre-Edouard Fournier,<sup>1</sup> Caroline Allombert,<sup>1</sup> Yupin Sappatamongkol,<sup>2</sup> Giuseppe Caruso,<sup>2</sup> Philippe Brouqui,<sup>1</sup> and Didier Raoult<sup>1\*</sup>

- 2 patients diseased with perimyocarditis (Sweden) diagnostic with PCR in cardiac tissues
- 9.2 % seroprevalence in forest workers in Northern France
- 1 case published of unexplained fever (France)
- *Ixodes ricinus*



Patient	Age <sup>b</sup>	Sex	Geographic area	Month of onset	Fever <sup>a</sup>	Head-ache <sup>a</sup>	Myal-gia <sup>a</sup>	Arthral-gia <sup>a</sup>	Conjunc-tivitis <sup>a</sup>	Report of tick bite (location) <sup>c</sup>	Inoculation eschar (location) <sup>c</sup>	Cutaneous rash <sup>c</sup>	Treatment <sup>d</sup>	Recov-ery <sup>e</sup>
1	70	M	Northern Italy	May	+	+	-	+	-	+	+	-	No treatment	-
2	58	M	Northern Italy	May	+	+	+	+	-	+	-	-	No treatment	-
3	50	F	Northern Italy	May	+	+	+	+	-	-	-	-	No treatment	-
4	37	M	Northeastern France	August	+	+	+	+	-	-	-	-	No treatment	-
5	63	F	Northeastern France	June	+	+	+	+	-	+	-	-	No treatment	-
6	27	M	Northeastern Thailand	January	+	+	+	+	-	-	-	-	Doxycycline	-
7	43	M	Northeastern Thailand	December	+	+	+	-	+	-	-	-	Cefotaxime	-
8	57	M	Northeastern Thailand	October	+	+	+	-	+	-	-	-	Doxycycline	-
Total (%)					6M, 2F	8 (100)	8 (100)	6 (75)	5 (62)	2 (25)	4 (50)	1 (12.5)	0	8 (100)

<sup>a</sup> +, positive criterion; -, negative criterion.  
<sup>b</sup> The mean age of the patients was 50.6 ± 14.2 years.

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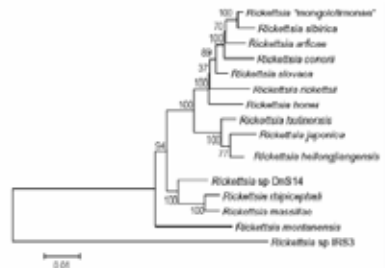


Journal : **Emerg Infect Dis**  
 Impact factor : **5.96**

### Acute Tick-borne Rickettsiosis Caused by *Rickettsia heilongjiangensis* in Russian Far East

Oleg Y. Mediannikov,<sup>1†‡</sup> Yuri Sidorov,<sup>1</sup> Leonid Ivanov,<sup>1</sup> Eugenia Mokretsova,<sup>1</sup> Pierre-Edouard Fournier,<sup>2</sup> Irina Tarasevich,<sup>1</sup> and Didier Raoult<sup>2</sup>

- First isolated from *Dermacentor salivarum* in 1982 in China
- 1992, serological diagnostic in 12 patients with fever, headaches, rash, eschar lymphadenopathy and conjunctivitis
- 1996 isolation of the bacteria from 7 patients



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Table 4. Epidemiologic, clinical and laboratory data of 13 patients with rickettsiosis

Feature or sign	Value (n = 13)
Sex, male/female	8/5
Age, y, mean	42 (13-66)
Mean period between onset and hospitalization, d	4.6
Mean stay at the hospital, d	9.7
Primary diagnosis of rickettsiosis at admission	9
History of tick bite	6
Incubation period, d, median (range)	5.5 (4-7)
Antibiotics taken before hospitalization	7
Chills	13
Myalgia	13
Headache	13
Dizziness	11
Myalgia, arthralgia	13
Nausea	2
Adenitis	13
Maculopapular rash	12
Rash appearance after onset of disease, d, median	1.6
Duration of rash, d, median (range)	5.5 (4-7)
Presence of eschar	12
Lymphadenopathy reported to the center	10
Subcutaneous lymphangitis, leading to regional lymph nodes	2
Hepatosplenomegaly	3
Splenomegaly	2
Neutrophilic leukocytosis	7
Leukocytosis at admission, $\times 10^9/\text{mm}^3$	6
Leukopenia at admission, $\times 10^9/\text{mm}^3$	2
Increased ESR ( $>11$ mm/h for men, $>20$ mm/h for women)	12
Thrombocytopenia, $<150,000/\text{mm}^3$	1
Prothrombin time (PT), s, $>14$	1
Increased AST activity, $>1.2$ times	6
Increased ALT activity, $>1.2$ times	2
Doxycycline treatment, 100 mg twice daily for 14 d	13

\* For additional definitions see Table 1 under corresponding text.

### Acute Tick-borne Rickettsiosis Caused by *Rickettsia heilongjiangensis* in Russian Far East

Olga V. Melnikova,<sup>1,2</sup> Tat. Shalobina,<sup>1</sup> Larisa Ivanova,<sup>1</sup> Ekaterina Melnikova,<sup>1</sup> Pavel Khramov,<sup>1</sup> Olga Tsvetkova,<sup>1</sup> and Igor Ruzicki<sup>1</sup>



Figure 3. Eschar and faint macular rash in patient 9.

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### *Rickettsia parkeri*: A Newly Recognized Cause of Spotted Fever Rickettsiosis in the United States

Christopher D. Paddock,<sup>1</sup> John W. Sumner,<sup>1</sup> James A. Comer,<sup>1</sup> Sherif R. Zaki,<sup>1</sup> Cynthia S. Goldsmith,<sup>1</sup> Jerome Goddard,<sup>1</sup> Susan L. F. McLellan,<sup>1</sup> Cynthia L. Tammings,<sup>1</sup> and Christopher A. DM<sup>1,2</sup>

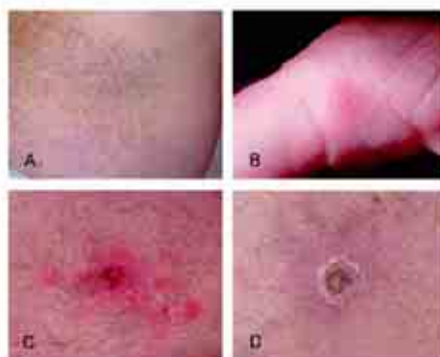


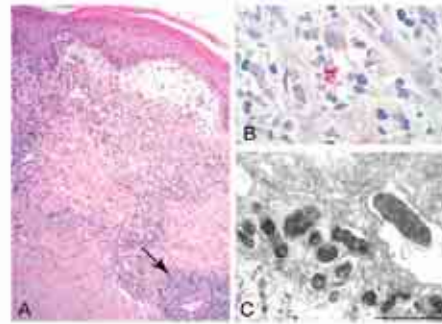
Figure 1. Cutaneous lesions in a patient infected with *Rickettsia parkeri*. A, Diffuse pink macular rash involving the abdomen. B, A small papule on the medial aspect of the first digit. C and D, Faint lesions located on the parital aspects of the right and left lower legs, respectively.

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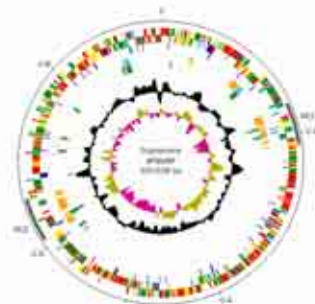
**Figure 2.** Histopathologic and immunohistochemical evaluation of a biopsy specimen from the margin of an eschar, and ultrastructure of *Rickettsia parkeri* (strain Paris2001) isolated in cell culture. A, Lymphovascular nodule (arrow) representing focus (arrowhead) showing the superficial and deep nuclei, and subepithelial fibrosis at the periphery of the eschar (represented grossly in figure 1) (hematoxylin and eosin stain, original magnification  $\times 25$ ). B, Immunohistochemical staining of 3% rickettsiae (red) in the cytoplasm of a cell in a focus of perivascular inflammation (immunohistochemical preparation with epithelial (top) red substrate and hematoxylin counterstain; original magnification  $\times 25$ ). C, Central (red) rod-shaped bacteria in the cytoplasm of a Vero E6 cell (an electron micrograph; arrow) (arrowhead and head-stroke stain; bar equals 1  $\mu$ m).

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## Sequencing and analysis of the genome of the Whipple's disease bacterium *Tropheryma whippelii*

Stephen D. Bentley, Matthias Melnick, Lee O'Murphy, Mark J. Palmer, Cyril H. Yang, Lynn D. Clover, Heide F. Nordberg, Guntay S. Bilen, Michael A. Quill, Daniel E. Harris, Axel von Hertzen, Anthea Quinn, Simon Foster, Robert Squares, Stephen Dougan, Bart C. Berrel, Julian Parkhill, David A. Hume



### Panel 1: General features of the *T. whippelii* genome

Size	925 038 bp
G+C content	46.3%
Coding sequences	784*
Coding content	84.4%
Average gene length	598 bp
rRNA	1 (16S–23S–5S)
tRNA	51
Other stable RNA	1

\*127 which are in a contig.

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**Culture of *Tropheryma whippelii* from Human Samples: a 3-Year Experience (1999 to 2002)**

Florence Fenollar,<sup>1</sup> Marie-Laure Bigé,<sup>1</sup> Valérie Gauduchon,<sup>2</sup> and Didier Raoult<sup>1\*</sup>

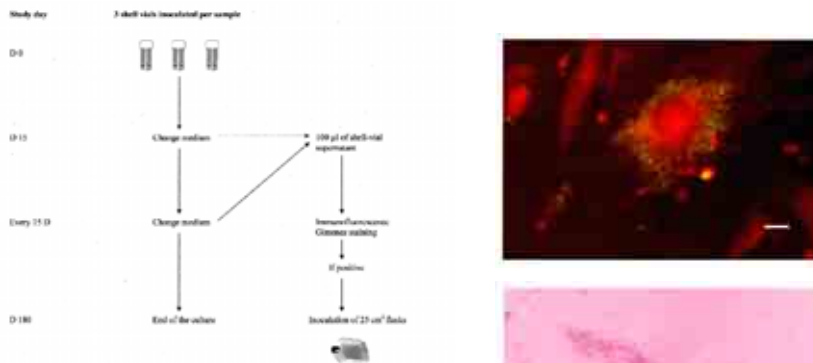
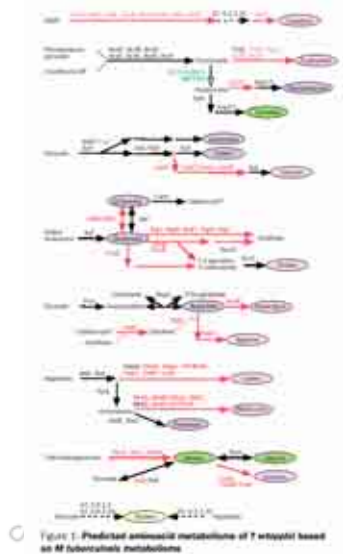


FIG. 1. Schematic representation of the procedure for isolation of *T. whippelii*.

- Décontamination des biopsies (digestives) n'empêche pas l'isolement
- Délai moyen d'isolement : 30 jours
- L'isolement est difficile sous vancomycine
- L'établissement de la souche plus facile si antibiothérapie < 7 jours

**Genome-based design of a cell-free culture medium for *Tropheryma whippelii***

Patricia Remeis, Nicolas Oropoulet, Hiroyuki Ogata, Bernard Le Scaëc, Guy Vestris, Jean-Michel Claverie, Didier Raoult



Voies métaboliques reconstruites par ordinateur avec les annotations du génome de *T. whippelii* et comparé à *M. tuberculosis*

- en Rose: voie métabolique manquante (absence de gène pour 9 AA)
- en Lila: voie métabolique partiellement déficiente (7AA)
- en Vert: voies métaboliques intactes (3AA)

**Culture axénique possible avec du milieu additionné en AA manquants**

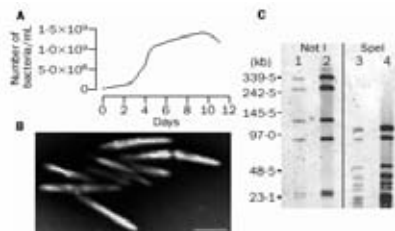


Figure 2: Time-course assessment of *T. whippelii* (strain Twist-Marseille CNCM I-2202) growth under axenic conditions.



### Antibiotic Susceptibility of *Tropheryma whippelii* in MRC5 Cells

Areen Boulos, Jean-Marc Rolain, and Didier Raoult\*  
Unité des Rickettsies, CNRS UMR 6028 IFR48, Faculté de Médecine, Université de la Méditerranée,  
13385 Marseille Cedex 05, France

TABLE 2. MICs of antibiotics active against *T. whippelii* as determined by Light Cycler assay

Antibiotic	MICs (µg/ml)		
	Twist	Endo-5	Slow
Penicillin G	1	0.5	0.5
Amoxicillin	0.5	1	1
Rifampin	0.5	1	2
Erythromycin	1	1	2
Clarithromycin	2	2	2
Telithromycin	0.25	0.5	0.5
Doxycycline	1	2	2
Sulfamethoxazole-trimethoprim	20.5	4/1	4/1
Teicoplanin	0.25	0.5	0.5
Vancomycin	10	10	10
Streptomycin	0.5	1	1
Gentamicin	4	8	8
Thiamphenicol	0.25	0.25	1
Chloramphenicol	1	1	2
Imipenem	0.5	10	10
Aztreonam	10	10	20
Cephalotin	10	10	20
Ceftriaxone	10	10	10
Colimycine	20	20	40
Levofloxacin	0.25	0.5	0.5
Ofloxacin	4	4	4
Ciprofloxacin	4	4	4
Hydroxychloroquine	2	4	8

#### *T. whippelii* sensible in cellulo (RT-PCR)

-Doxycycline, macrolides, kétolides, aminosides, pénicilline, rifampicine, teicoplanine, chloramphénicol, et cotrimoxazole

-Doxycycline et hydroxychloroquine bactéricide (Cf *C. burnetii*)

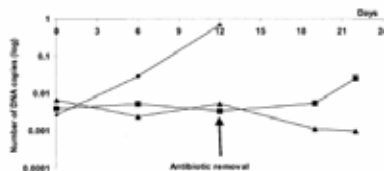


FIG. 3. Growth kinetics of *T. whippelii* Tein strains treated for 12 days with either doxycycline alone or doxycycline plus hydroxychloroquine. Antibiotics were removed after 12 days and samples were re-inoculated in fresh confluent MRC5 cells. Regrowth of the bacterium was determined by quantitative PCR. Symbols: ■, growth control; ●, doxycycline (2 µg/ml); ▲, doxycycline (2 µg/ml) plus hydroxychloroquine (1 µg/ml).

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### Correlation between Serum Doxycycline Concentrations and Serologic Evolution in Patients with *Coxiella burnetii* Endocarditis

J. M. Rolain, M. N. Mallet, and D. Raoult

•Traitement des endocardites à *C. burnetii* : Doxycycline et hydroxychloroquine pendant 18 mois.

•Moins de 5% de rechutes

•L'arrêt du traitement est déterminé par IgG 1<800 et IgA 1<50

•Malgré cela il y a des patients qui n'arrivent pas à ces critères.

•Il existe des rechutes ?

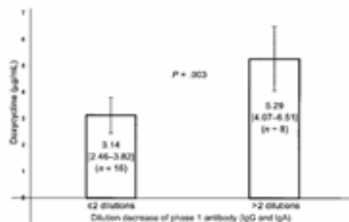


Figure 2. Serum doxycycline concentration and dilution decrease of phase 1 antibody (IgG and IgA) after 1 year of treatment with doxycycline (200 mg/day orally) and hydroxychloroquine. The first group (<=2 dilutions) corresponds to patients for whom the no. of reciprocal dilutions decrease of phase 1 antibody titers was <=2 dilutions, whereas the second group corresponds to patients for whom the no. of reciprocal dilutions was >2 dilutions.

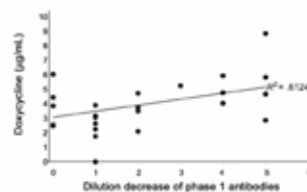


Figure 1. Correlation between serum doxycycline concentration and dilution decrease of phase 1 antibody (IgG and IgA) after 1 year of treatment with doxycycline (200 mg/day orally) and hydroxychloroquine.

CXRS





## Culture of *C. burnetii* from the dental pulp of experimentally infected guinea pigs

G rard Aboudharam, Michel Drancourt, Didier Raoult\*

Dental pulp has recently been proposed as a preferred sample to detect nucleic acid from pathogen in human remains. This has been applied to the detection of HIV in modern times [1,2] and *Yersinia pestis* in middle age and XVIIIth century in human remains [3,4]. We have previously shown that the DNA of *Coxiella burnetii*, the agent of Q fever and a potential bioweapon, could be detected in the dental pulp of experimentally infected guinea pig [5]. Here we show that *C. burnetii* could be cultivated from some dental pulp samples and therefore we propose that dental pulp may be considered equivalent to a small blood sample for recovery of pathogenic agents.

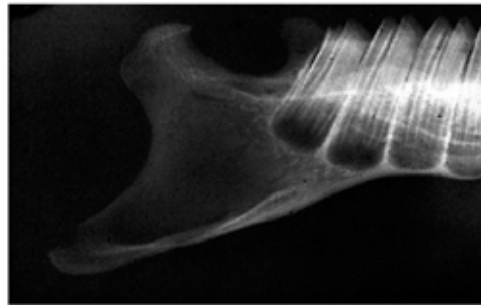


Fig. 1. Radiology of the guinea pig mandibula showing the large dental pulp in the teeth.

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## Effect of Sex on *Coxiella burnetii* Infection: Protective Role of 17 -Estradiol

Marc Leone,<sup>1,2</sup> Am lie Honstetter,<sup>1</sup> Hubert Lepid,<sup>1</sup> Christian Capo,<sup>1</sup> Francis Bayard,<sup>1</sup> Didier Raoult,<sup>1</sup> and Jean-Louis Mege<sup>1</sup>

Fi vre Q plus souvent symptomatique chez l'homme que chez la femme

La s ropr valence est la m me

R le des hormones sexuelles dans l'expression de la fi vre Q ?

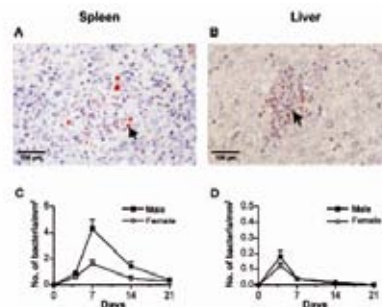


Figure 3. *Coxiella burnetii* load in mouse tissues. Male and female mice were infected with *C. burnetii* and killed at 0, 7, 14, and 21 days post-infection. Spleen and liver tissues were removed, and the spleen (left) and the liver (right) A and B. Representative histographs of tissue from female mice of the 7 after infection (original magnification,  $\times 400$ ). C and D. Mean  $\pm$  SD no. of bacteria/mg; also see the average of 5 mice/time point.

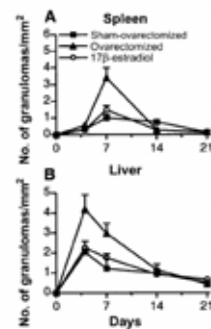


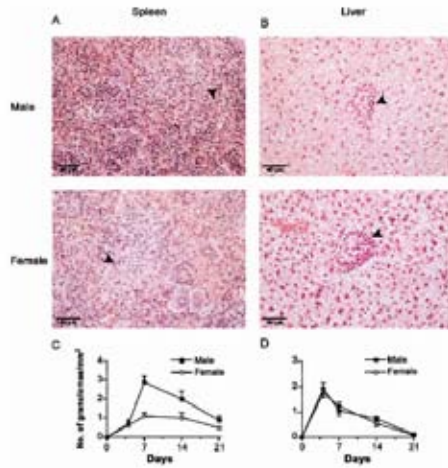
Figure 4. Granuloma counts in ovariectomized female mice. Mice were or were not ovariectomized, and 17 -estradiol was administered to ovariectomized mice. Then they were infected with *Coxiella burnetii* and killed at different times. Granulomas were revealed by histochemical analysis in the spleen (A) and the liver (B). Data are the mean  $\pm$  SD no. of granulomas/mg and are the average of 5 mice/time point.



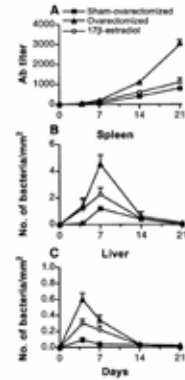
# Effect of Sex on *Coxiella burnetii* Infection: Protective Role of 17 $\beta$ -Estradiol

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**Figure 2.** Granuloma expression in mouse tissues. Male and female mice were infected with *Coxiella burnetii* and killed at different times. Granulomas (arrowheads) were revealed by 7-alkaline-oxidase in the spleen (left) and the liver (right). C and D. Representative micrographs of tissues (original magnification,  $\times 200$ ). C and D. Mean  $\pm$  SD no. of granulomas/mm<sup>2</sup>; data are the average of 5 mice/time point.



**Figure 3.** *Coxiella burnetii* load in ovariectomized female mice. Mice were or were not ovariectomized, and 17 $\beta$ -estradiol was administered to ovariectomized mice. Then they were infected with *C. burnetii* and killed at different times. A. Titer of circulating antibodies (Ab) to *C. burnetii* data are the mean  $\pm$  SD of 5 mice/time point. B and C. Bacteria were revealed by immunostaining in the spleen (B) and the liver (C). Data are the mean  $\pm$  SD no. of bacteria/mm<sup>2</sup> and are the average of 5 mice/time point.

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