

# Les rickettsioses: moyens diagnostiques

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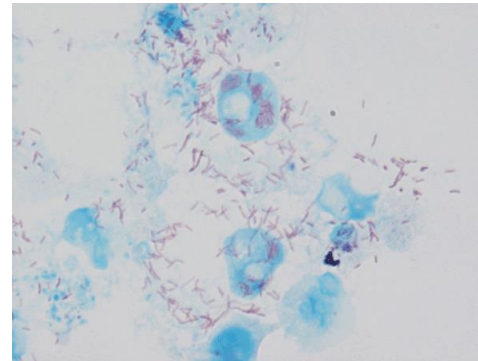


# HISTORIQUE

- ⊕ Fièvre boutonneuse méditerranéenne: Conor et Bruch (SPE) 1910.
- ⊕ Institut Pasteur de Tunis



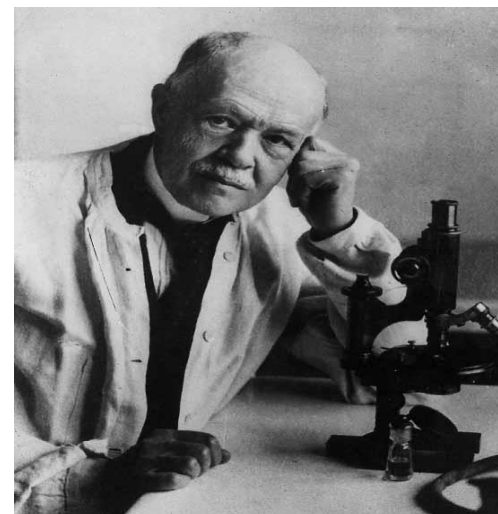
Conor, Institut Pasteur de Tunis



**Fièvre Boutonneuse méditerranéenne (FBM)**

# Charles Nicolle

1928 Reçoit le prix Nobel de médecine: le rôle du poux dans la transmission du typhus épidémique



Institut Pasteur de Tunis

# Plan

- Introduction
- données cliniques, épidémiologiques
- Moyens diagnostiques:
  - Sérologie
  - Biologie moléculaire
  - Autres méthodes diagnostiques « nouvelles »
  - Algorithme diagnostique
- Conclusion

# Rickettsioses



**Maladies infectieuses réémergentes**

**Répandues dans le monde entier**

**Causées par des micro-organismes pathogènes**

***Rickettsia***

**Transmises par une diversité d'arthropodes hématophages**

# *Rickettsia*

- Des petits coccobacilles à Gram négatif
- coloration de Gimenez à la fuchsine basique
- Se divisent par scission binaire
- Se cultivent sur des tissus

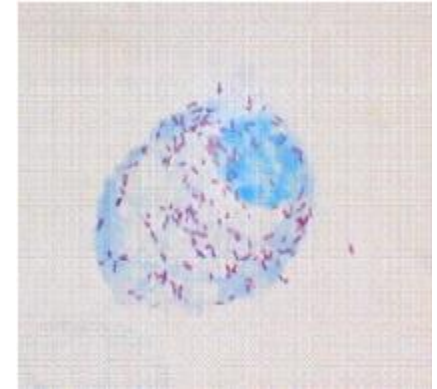
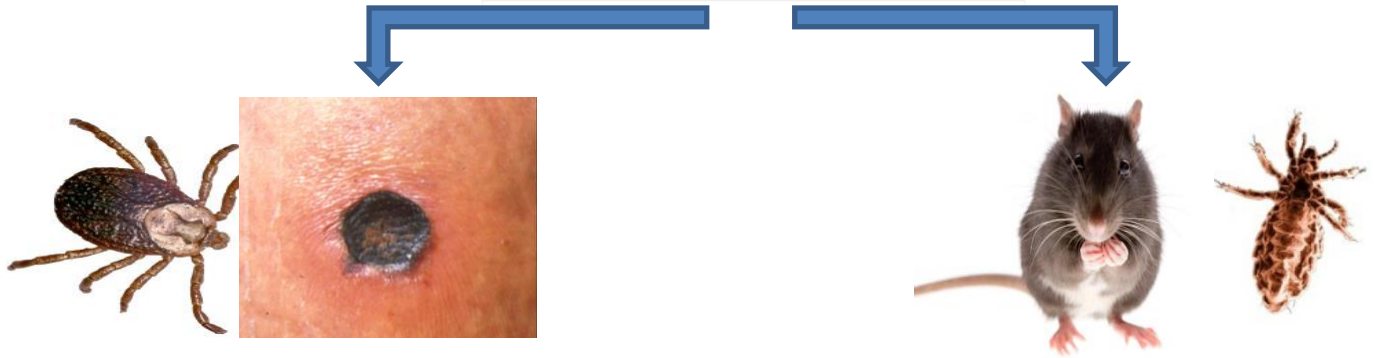


Figure 1 : *Rickettsia* à l'intérieur d'une cellule endothéliale humaine. Coloration de Gimenez.

*Rickettsia*



SFG

TG

Vecteurs

Tiques+++  
Puces (*R. felis*)  
Mîtes (*R. akari*)

Poux (*R. prowazekii*)  
Puces (*R. typhi*)

T° de croissance

35°

32°

%GC

32-33

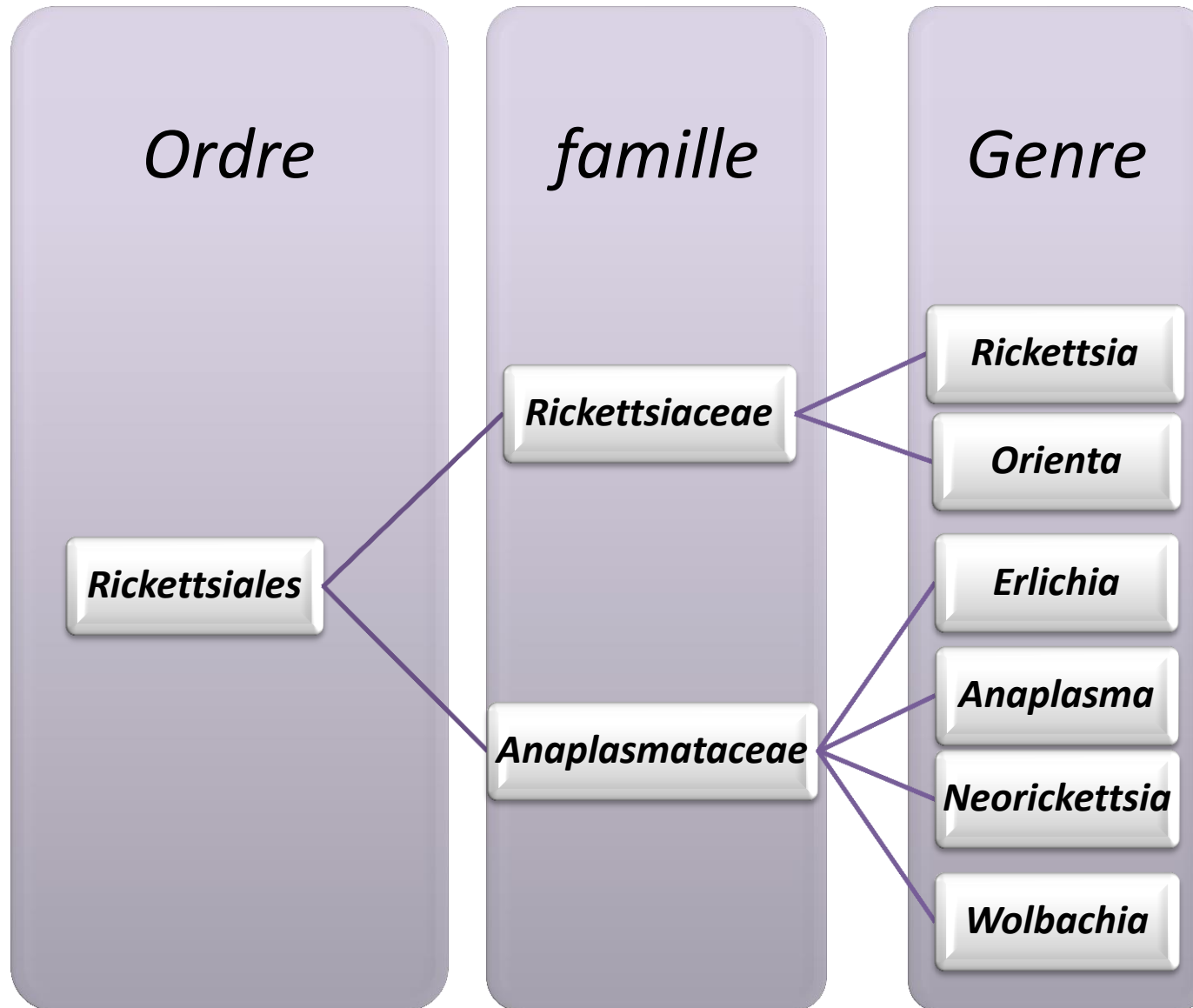
29

Mobilité intracellulaire

+

.





**Figure 2 :** Classification de l'ordre des *Rickettsiales*.

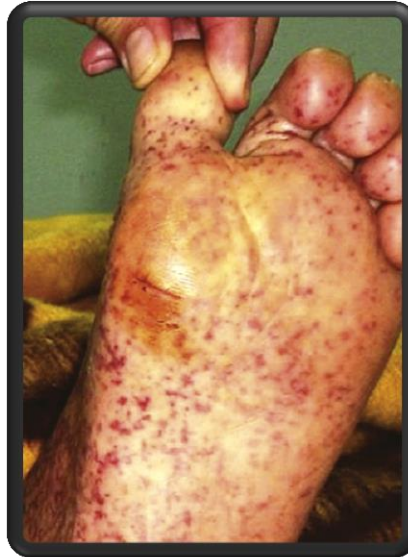
## Les tableaux cliniques

## FBM

- ❖ Fièvre 100%
- ❖ Éruption cutanée
- ❖ Incubation 1-16 jours



Eruption cutanée  
maculopapuleuse

*R. Conorii conorii*

Escarre, tache noire

# Typhus murin

**Tableau 1 :** Fréquence des différents signes cliniques au cours du typhus murin

<b>Signes cliniques</b>	<b>Fréquence (%)</b>
Fièvre	98-100
Céphalées	41-90
Rash	20-80
Arthralgies	40-77
Hépatomégalie	24-29
Toux	15-40
Diarrhée	5-40
Splénomégalie	5-24
Piqûre d'insecte	0-39
Nausées/ vomissements	3-48
Douleurs abdominales	11-60
Confusion	2-13

## Les autres rickettsioses

**Tableau 2** : Caractéristiques cliniques de certaines espèces de rickettsies

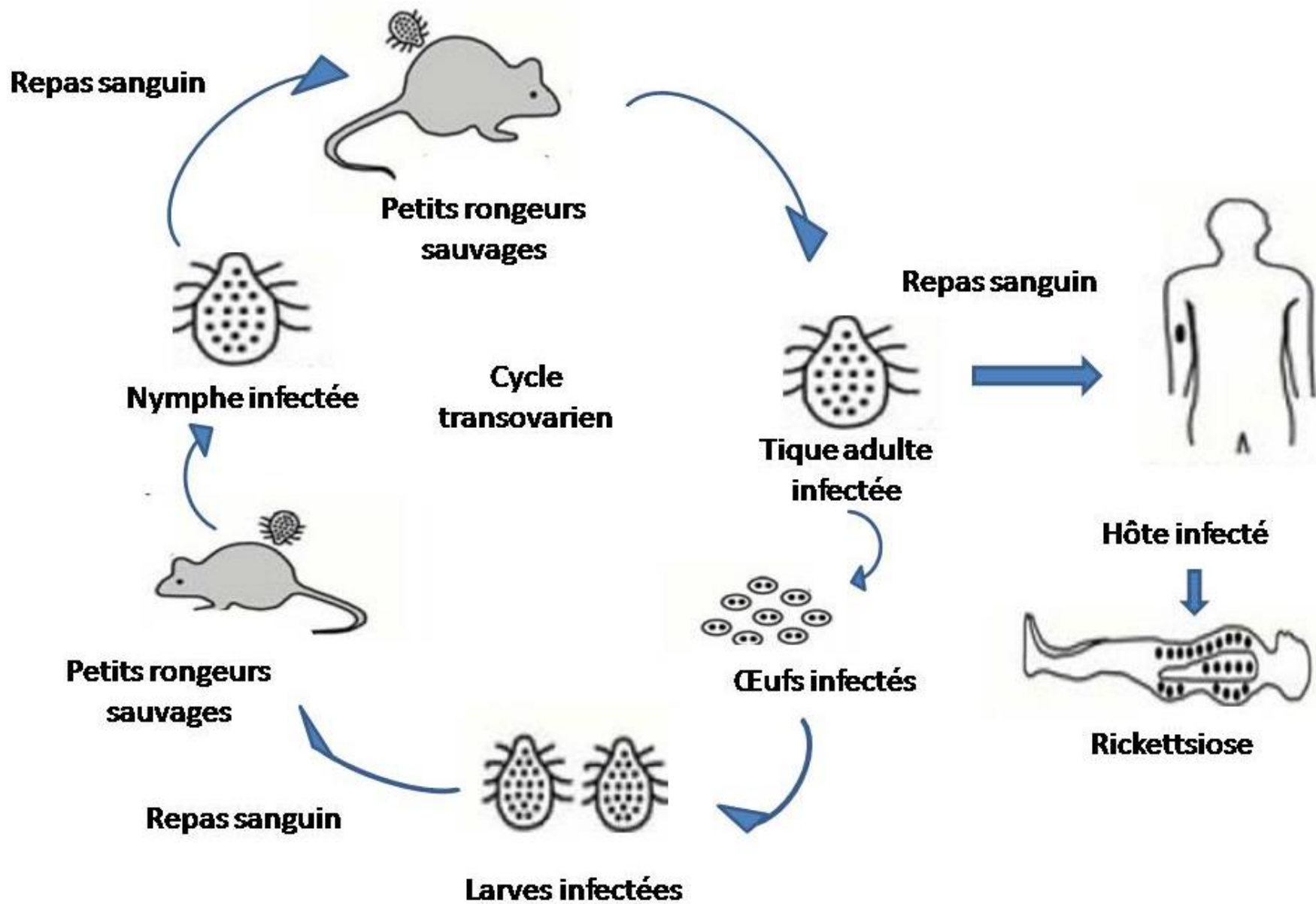
Espèce	Maladie	Vecteur	Caractéristique
<i>R. conorii</i> subsp <i>israelensis</i>	la fièvre boutonneuse d'Israël	<i>Rh. sanguineus</i>	Formes sévères
<i>R. conorii</i> subsp <i>caspia</i>	la fièvre d'Astrakhan	<i>Rhipicephalus pumilio</i>	Conjonctivites, céphalées et myalgies
<i>R. conorii</i> subsp <i>indica</i>	du typhus à tique d'inde	<i>Rh. sanguineus</i>	Rash purpurique
<i>R. felis</i>	La fièvre boutonneuse à puce	<i>Ctenocephalides felis</i>	Rash maculopapulaire
<i>R. africae</i>	La fièvre à tique africaine	<i>Amblyomma</i>	Escarres multiple
<i>R. slovaca</i>	SENLAT	<i>Dermacentor</i>	Escarre du cuir chevelu+ adénopathie
<i>R. helvetica</i>	Fièvre non éruptive	<i>Ixodes</i>	
<i>R. rickettsii</i>	Fièvre pourprée des montagnes rocheuses	<i>Dermacentor</i>	Formes sévères
<i>R. prowazekii</i>	Typhus épidémique	<i>Pediculus humanis corporis</i>	Formes sévères

## ✦ Maladies vectorielles:

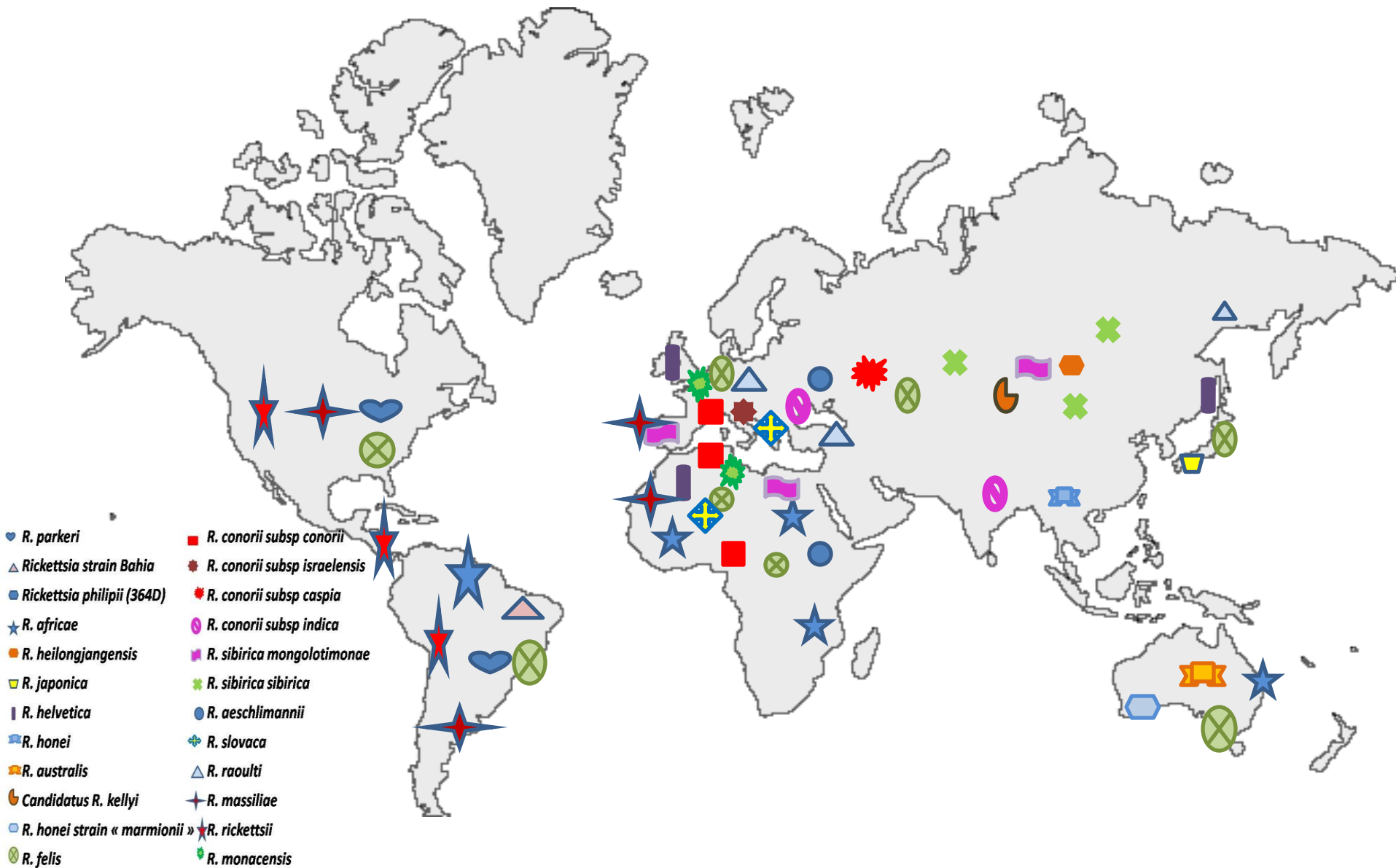
Fréquence et transmission  Activité et répartition des vecteurs

## ✦ Réservoir:

- Animaux : rongeurs, mammifères, oiseaux
- Vecteurs: TIF et TTO
- Homme: hôte accidentel














**Figure 3 :** Cycle de transmission et de maintien de l'infection à *Rickettsia* dans la nature

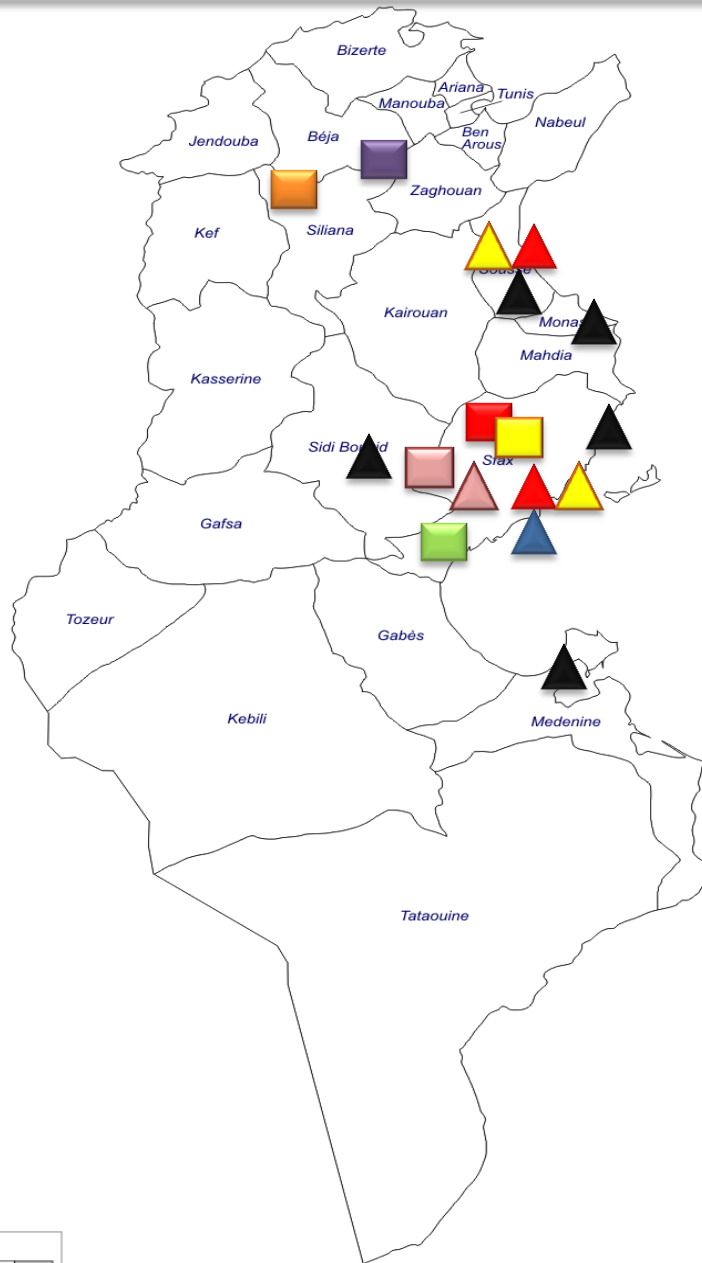


**Figure 5 :** Répartition mondiale des différentes espèces de rickettsies du SFG décrites comme pathogènes pour l'Homme.

## Répartition des rickettsies en Tunisie

Vecteur Homme

- |   |   |                               |
|---|---|-------------------------------|
|    |  | <i>R. conorii conorii</i>     |
|    |  | <i>R. conorii israelensis</i> |
|    |  | <i>R. felis</i>               |
|   |  | <i>R. aeschlimannii</i>       |
|   |  | <i>R. typhi</i>               |
|   |   | <i>R. massiliae</i>           |
|  |   | <i>R. helvetica</i>           |
|  |   | <i>R. monacensis</i>          |



**Figure 6:** répartition des différentes espèces de *Rickettsia* rapportées chez les patients et les vecteurs



Diagnostic?



# Diagnostic microbiologique

Diagnostic  
biologique  
directe



La sérologie



Diagnostic  
biologique



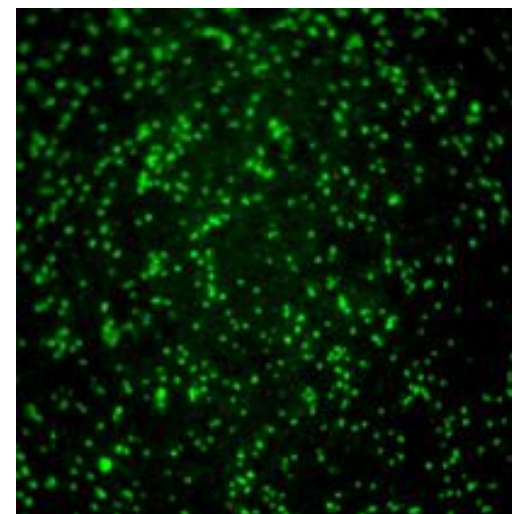
# Sérologie



# La sérologie

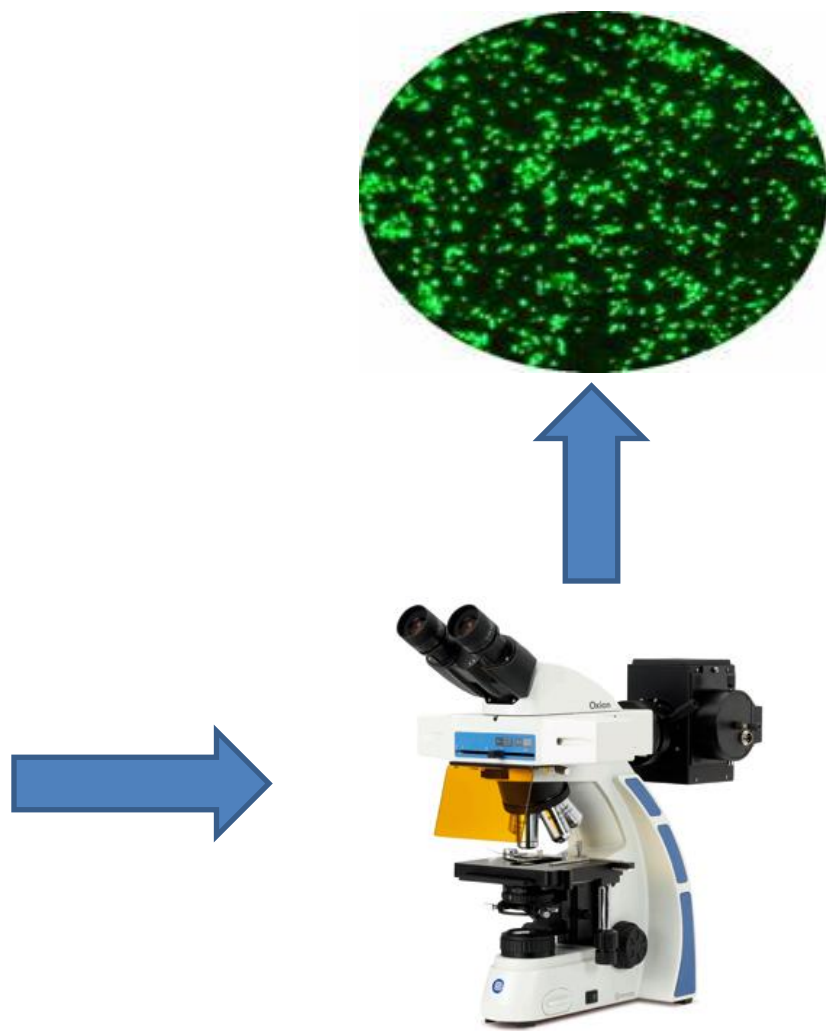
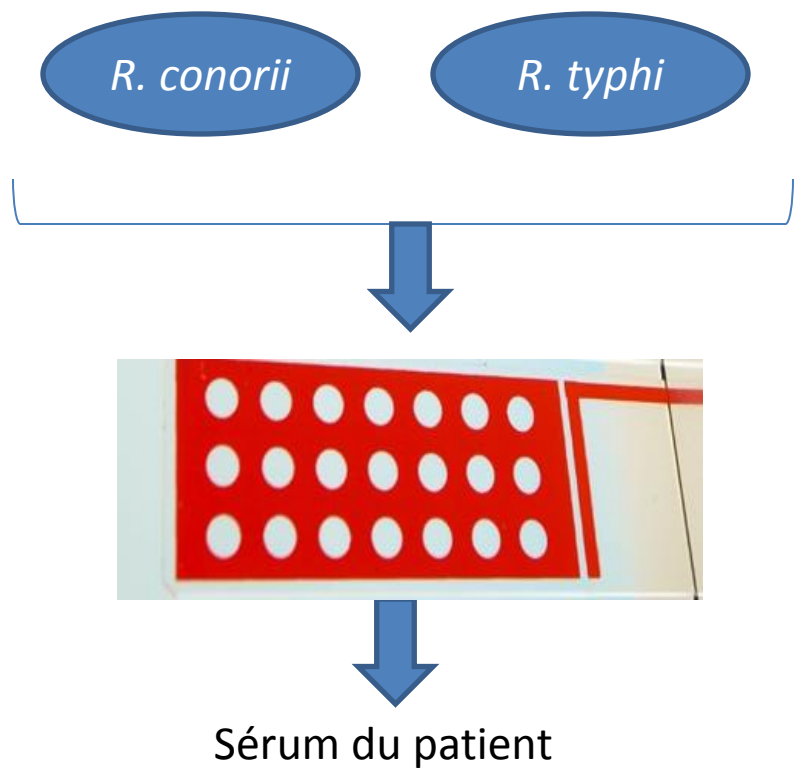
## Micro-immunofluorescence (MIF) :

- ✿ La méthode de référence
- ✿ Une méthode sensible et spécifique
- ✿ Accessible aux laboratoires de routines
- ✿ Le manque de standardisation
- ✿ Les réactions croisées observées entre les différentes espèces de rickettsies



# Sérologie

## Micro-immunofluorescence



## seuils de positivité

- IgM = 1/32
- IgG = 1/128

## Critères de positivité

- les IgM sont positives à des taux supérieurs à 1/32
- séroconversion entre deux sérums
- Elévation significative des IgG

## Diagnostic d'espèce

**Titres (IgM +IgG)espèce A**  
 **$\geq$**   
**2 Titres (IgM +IgG)espèce B**

TRANSACTIONS OF THE ROYAL SOCIETY OF TROPICAL MEDICINE AND HYGIENE (1995) 89, 266–268

## Seroepidemiological survey of rickettsial infections among blood donors in central Tunisia

Amel Omezzine Letaief<sup>1,3</sup>, Saloua Yacoub<sup>2</sup>, Hervé Tissot Dupont<sup>3</sup>, Christine Le Cam<sup>3</sup>, Liliane Ghachem<sup>2</sup>, Letaief Jemni<sup>1</sup> and Didier Raoult<sup>3\*</sup> <sup>1</sup>Service de Maladies Infectieuses and <sup>2</sup>Centre de Transfusion Sanguine, CHU F. Hached, 4000 Sousse, Tunisie; <sup>3</sup>Unité des Rickettsies, CNRS EP 70054, Faculté de Médecine, 27 Boulevard Moulin, 13385 Marseille, France

### Abstract

In this report we attempt to evaluate the prevalence of antibodies against *Rickettsia conorii*, *R. typhi*, *Coxiella burnetii*, and *Ehrlichia chaffeensis* in central Tunisia. Five hundred sera from blood donors, collected between March and June 1993, were tested for these 4 antibodies using an indirect immunofluorescence antibody assay (IFA). Nine percent of the sera had antibodies against *R. conorii* (IgG > 1:32) by IFA, and 8% by Western blotting; with IFA, 3.6% had antibodies to *R. typhi*, 26% to *C. burnetii* (> 1:50), and none to *E. chaffeensis*. Infection rates with *R. conorii* and *R. typhi* did not differ significantly between the sexes, but fewer young people had antibodies to *R. typhi*. A significantly higher prevalence of antibodies to *C. burnetii* was noted for males with no difference between age classes. No significant difference was detected between urban and rural areas. This study confirmed the endemicity of rickettsioses, and revealed a high seroprevalence of Q fever, in central Tunisia.

**Keywords:** Mediterranean spotted fever, typhus, Q fever, ehrlichiosis, *Rickettsia conorii*, *Rickettsia typhi*, *Coxiella burnetii*, *Ehrlichia chaffeensis*, seroprevalence, Tunisia



International Journal of Infectious Diseases (2005) 9, 331–334



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<http://intl.elsevierhealth.com/journals/ijid>

## Clinical and laboratory features of murine typhus in central Tunisia: a report of seven cases

A. Omezzine Letaïef<sup>a,\*</sup>, N. Kaabia<sup>a</sup>, M. Chakroun<sup>b</sup>,  
M. Khalifa<sup>a</sup>, N. Bouzouaia<sup>b</sup>, L. Jemni<sup>a</sup>

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Received 3 January 2004; received in revised form 11 August 2004; accepted 14 September 2004

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**Médecine et  
maladies infectieuses**

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Médecine et maladies infectieuses 43 (2013) 226–229

Original article

## Murine typhus in Tunisia: A neglected cause of fever as a single symptom<sup>☆</sup>

*Le typhus murin en Tunisie : cause négligée de fièvre isolée*

A. Znazen<sup>a,\*</sup>, B. Hammami<sup>b</sup>, A. Ben Mustapha<sup>c</sup>, S. Chaari<sup>a</sup>, D. Lahiani<sup>b</sup>, I. Maaloul<sup>b</sup>,  
 M. Ben Jemaa<sup>b</sup>, A. Hammami<sup>a</sup>

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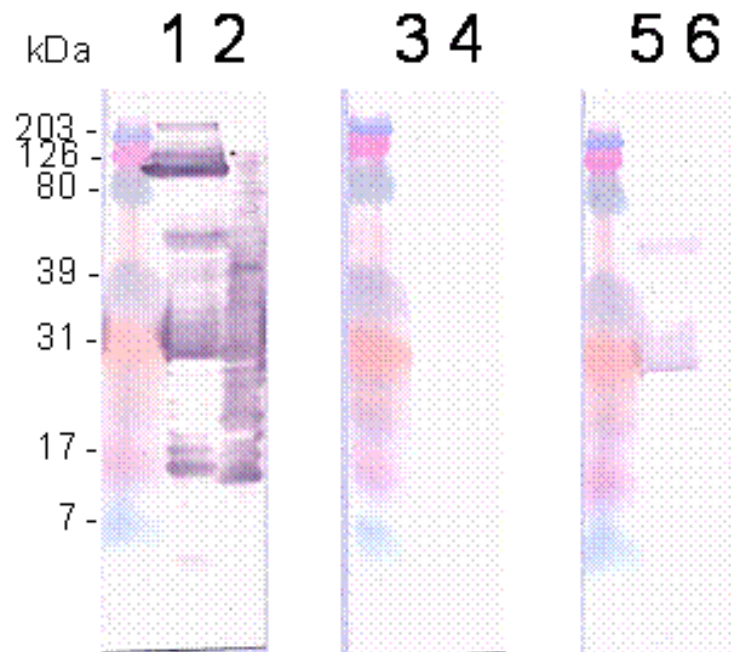
Available online 22 April 2013

**Tableau 3:** Concordance entre la clinique et la sérologie chez les patients ayant eu un 2<sup>ème</sup> sérum (les données de l'étude multicentrique Tunisie 2012-2014).

	Nombre	Diagnostic clinique		
		FBM/RICK /Intracellulaire	Virose	Autre
Sérologie positive	121 (61%)	116 (71,2%)	5 (14,3%)	
Sérologie négative	78 (39%)	47(28,8%)	30 (85,7%)	1
	199	163	35	1

# La sérologie

## Immunoblot +/- cross adsorption



**Western blot** avant (1,2) et après adsorption croisée avec *R. typhi* (3, 4) et *R. felis* (5, 6). Pistes 1, 3, 5 : antigène *R. typhi* ; pistes 2, 4, 6 : antigène *R. felis*.

Emerging Infectious Diseases • www.cdc.gov/eid • Vol. 12, No. 1, January 2006

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DISPATCHES

# ***Rickettsia felis* Infection, Tunisia**

**Abir Znazen,\*† Jean-Marc Rolain,\*  
Nader Hammami,† Adnane Hammami,†  
Mounir Ben Jemaa,‡ and Didier Raoult\***

We report, for the first time, serologic evidence of *Rickettsia felis* and *R. aeschlimannii* infections acquired in Tunisia from 1998 to 2003. We found that most patients with antibodies against both *R. conorii* and *R. typhi* had serologic evidence of *R. felis* infection.

---

## Characterization of Rickettsial Diseases in a Hospital-Based Population in Central Tunisia

Naoufel Kaabia and Amel Letaief

*Infectious Diseases Unit, Farhat Hached, University Hospital Sousse – Tunisia*

In Tunisia, 2 rickettsial groups, spotted fever group and typhus group, have been described since the beginning of the 20th century. Mediterranean spotted fever (MSF), also known as Boutonneuse fever, caused by *Rickettsia conorii* and transmitted by the dog tick *Rhipicephalus sanguineus*, is the most frequent rickettsial infection observed. Its seroprevalence in our region is 9% among blood donors and 23% in hospitalized febrile patients. Typhus group rickettsioses, caused by *R. typhi* and *R. prowazekii*, are less frequently reported than in the 1970s. Only sporadic cases of typhus were reported in the last decade. However, *R. typhi* antibodies were present in 3.6% among healthy people and 40% in patients with acute fever of undetermined origin. In the unit of Infectious Diseases at Farhat Hached University Hospital in Sousse, during 2007, 5% of hospitalized patients had eruptive fever, and half of the cases met clinical criteria of MSF and/or were confirmed by rickettsial serology. The majority of cases (90%) were noted in hot seasons, and contact with domestic animals was found in 76%. The most common symptoms were fever (present in all cases), skin rash (in 85% to 98% of cases), and headache (in 69.5% of cases). The clinical triad (fever + rash + “tache noire”) was noted in 32 to 61%. Normal blood cells or leukopenia, cytolysis, and thrombopenia were the most frequent biological abnormalities. Complications and malignant forms of rickettsial infections were reported in 5% to 6% among hospitalized adult patients. When specific serology was performed, MSF was confirmed in 15% and we noted an emergence of murine typhus (MT) mistaken for *R. conorii* or viral infection. *Rickettsia felis* was identified in 1 patient, whereas 17% of cases remained undetermined. *Rickettsia conorii* Malish was identified by PCR in skin biopsies. Doxycycline was the antibiotic of choice for rickettsial infections; it was prescribed in the majority of patients, associated with fever defervescence, in a mean of 72 hours. The mean length of stay among hospitalized patients with rickettsial infections was 5.9 days. In conclusion, in our region, MSF and murine typhus are endemic. Doxycycline should be prescribed in patients with acute fever and skin rash, especially in hot seasons. These rickettsioses were characterized by benign prognosis. More skin biopsies are needed to identify other SFG rickettsies.

**Key words:** *Rickettsia conorii*; *Rickettsia typhi*; *Rickettsia prowazekii*; *Rickettsia felis*; epidemiology; Tunisia



# Détections des rickettsies



# Diagnostic biologique direct

Biopsies cutanées



Sang EDTA



LCR

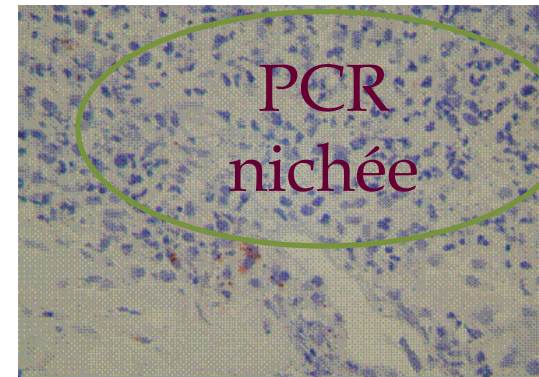


# Différentes Techniques de détection des Rickettsies

**Culture cellulaire**

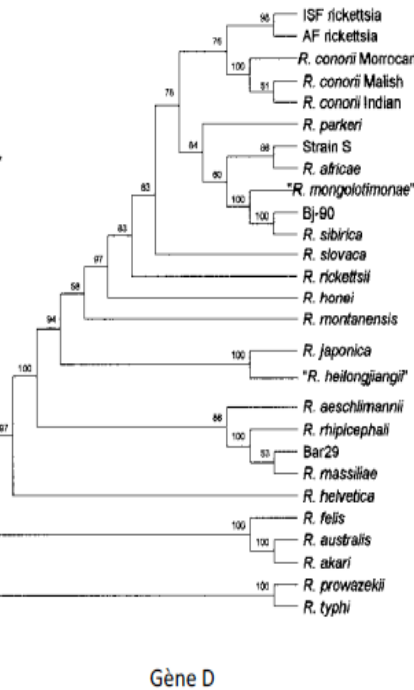
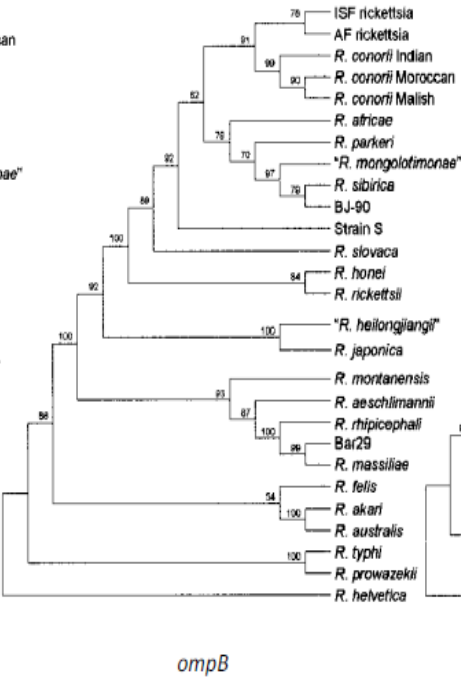
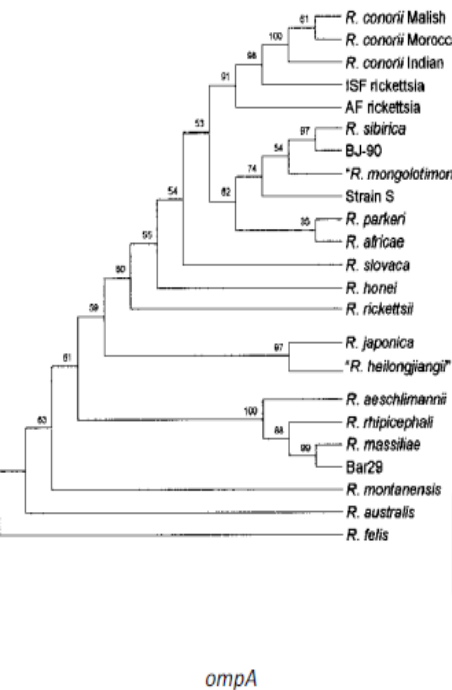
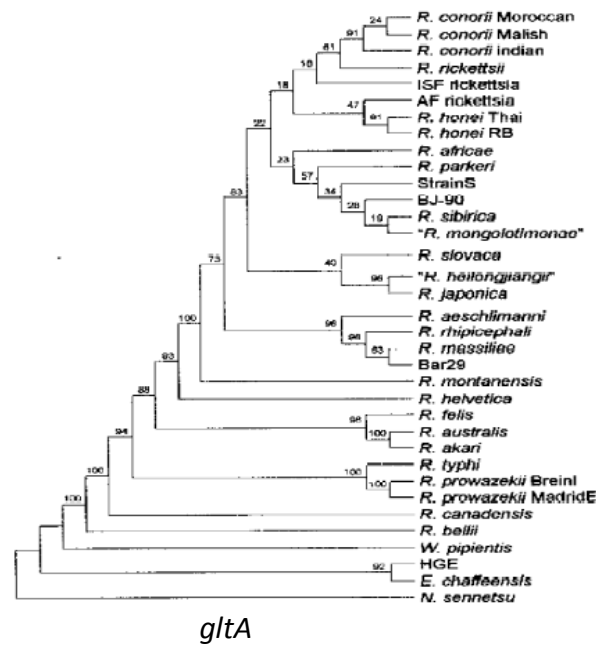
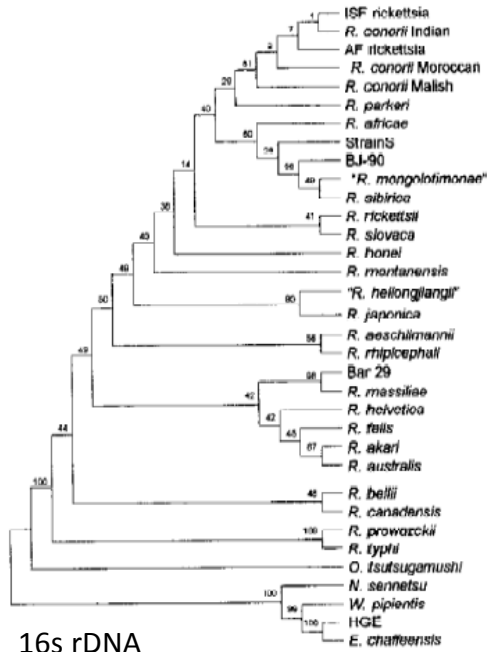


**Immunohistochimie**



**Biologie moléculaire**

PCR en  
temps réel



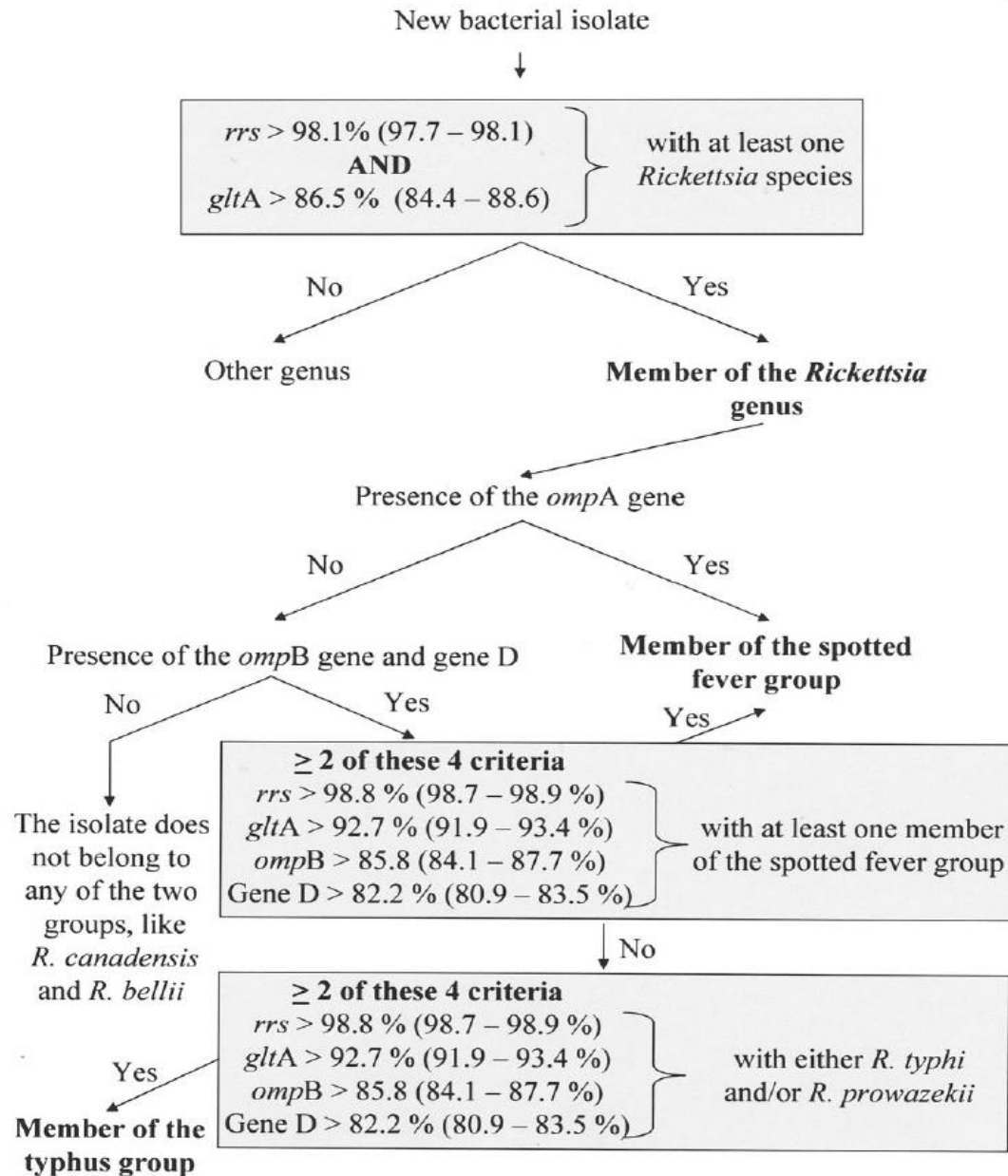
**Figure 7:** Organisation phylogénétique des espèces de *Rickettsia* basée sur la comparaison des séquences des gènes 16S rDNA, *gltA*, *ompA*, *ompB* et gène D (par la méthode des maximums de parcimonie) (Fournier *et al.*, 2003).

## Gènes cibles

- **gltA, ompB, ompA, 16S rRNA et 17kD**

*Roux V 1997, Roux V2000, Fournier PE 1998, Tzianabos T 1989.*

- Identification d'espèces après séquençage  
permettra de faire un diagnostic d'espèces.



**Figure 4:** les recommandations de la taxonomie poly-phasique pour la classification des rickettsies (Fournier *et al.*, 2003)

## PCR conventionnelles

Sfax 2008-2012

**Tableau 4** : PCR conventionnelles chez des patients ayant une rickettsiose

	<i>ompA</i>	<i>ompB</i>	<i>gltA</i>	17KDa
<b>BCU (n=44)</b>				
Positivité	5 (11,3%)	5 (11,3%)	5 (11,3%)	7 (16%)
Séquençage	<i>R. conorii</i> subsp <i>israelensis</i> (3)	<i>R. conorii</i> subs <i>conorii</i> (2) <i>R. conorii</i> subsp <i>israelensis</i> (3)	-	SFG
<b>Sang-EDTA (n=101)</b>				
Positivité	1 (1%)	1 (1%)	1 (1%)	1 (1%)
Séquençage	<i>R. conorii</i> subsp <i>israelensis</i>	<i>R. conorii</i> subsp <i>israelensis</i>	-	-

## PCR en temps réel (qPCR)

- ✿ Sensibilité
- ✿ Spécificité (TaqMan)
- ✿ Rapidité
- ✿ Utilisation de sonde spécifique d'espèce++

## Assessment of Real-Time PCR Assay for Detection of *Rickettsia* spp. and *Rickettsia rickettsii* in Banked Clinical Samples

Cecilia Y. Kato,<sup>a</sup> Ida H. Chung,<sup>a</sup> Lauren K. Robinson,<sup>a</sup> Amy L. Austin,<sup>a,b</sup> Gregory A. Dasch,<sup>a</sup> Robert F. Massung<sup>a</sup>

Centers for Disease Control and Prevention, Rickettsial Zoonoses Branch,<sup>a</sup> and Laboratory Preparedness and Response Branch,<sup>b</sup> Atlanta, Georgia, USA

Two novel real-time PCR assays were developed for the detection of *Rickettsia* spp. One assay detects all tested *Rickettsia* spp.; the other is specific for *Rickettsia rickettsii*. Evaluation using DNA from human blood and tissue samples showed both assays to be more sensitive than nested PCR assays currently in use at the CDC.

January 2013 Volume 51 Number 1

- Sensibilité > Nested PCR
- Diagnostic rapide de la RMSF (*R. rickettsii*)



SHORT COMMUNICATION

## **Widespread use of real-time PCR for rickettsial diagnosis**

Aurélie Renvoisé, Jean-Marc Rolain, Cristina Socolovschi & Didier Raoult

Unité de Recherche en Maladies Infectieuses et Tropicales Emergentes CNRS-IRD UMR6236-198, Faculté de Médecine, Université de la Méditerranée, Marseille, France

FEMS Immunol Med Microbiol **64** (2012) 126–129

- 2 qPCR: SFG et TG
- 643 échantillons testés: 45 positifs
- 31/ 45: BCU ou ECU



## RESEARCH ARTICLE

# Comparison of Two Quantitative Real Time PCR Assays for *Rickettsia* Detection in Patients from Tunisia

Abir Znazen<sup>1\*</sup>, Hanen Sellami<sup>1</sup>, Emna Elleuch<sup>2</sup>, Zouhour Hattab<sup>3</sup>, Laroussi Ben Sassi<sup>4</sup>, Fatma Khrouf<sup>5</sup>, Hassen Dammak<sup>6</sup>, Amel Letaief<sup>3</sup>, Mounir Ben Jemaa<sup>2</sup>, Adnene Hammami<sup>1</sup>

February 23, 2015

- 2 duplex de qPCR:
  - SFG (RC0338) et TG (Rp278)
  - Toute rickettsies (16s rDNA) puis Rtt (TG)

Table 1. Positivity of qPCR according to serologic status among patients with confirmed diagnosis.

	Total	Serology (+) on first serum (N = 66)	Serology (-) on first serum (N = 16)	p
Skin biopsies	44	29	15	
PanRick	24(54.5%)	12 (41.4%)	12(80%)	<b>0.03</b>
Rtt	3(6.8%)	2(6.9%)	1(6.7%)	0.7
RC00338	19(43.2%)	10(34.5%)	9(60%)	0.1
Rp278	2(4.5%)	1 (3.4%)	1(6.7%)	0.5
Whole blood samples	79	63	16	
PanRick	5(6.3%)	2(3.1%)	3(18.7%)	<b>0.05</b>
Rtt	2(2.5%)	2(3.1%)	0	0.7
RC00338	3(3.8%)	0	3(18.7%)	<b>0.007</b>
Rp278	1(1.2%)	1(1.6%)	0	0.8

doi:10.1371/journal.pntd.0003487.t001

# Comparison of Real-Time Quantitative PCR and Culture for the Diagnosis of Emerging Rickettsioses

Emmanouil Angelakis, Hervé Richet, Jean-Marc Rolain, Bernard La Scola, Didier Raoult\*

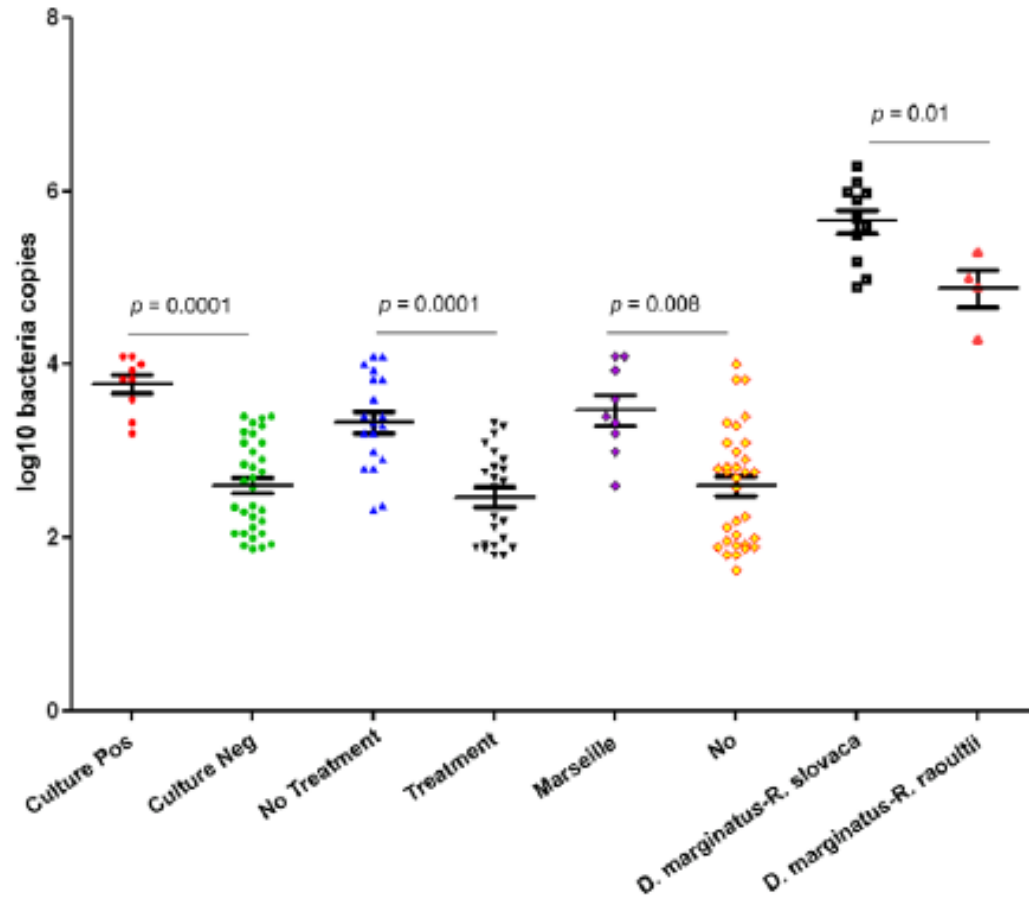
URMITE UMR 6236, CNRS-IRD, Faculté de Médecine et de Pharmacie, Marseille, France

March 2012

**Table 3.** Results for the 26 patients with suspected rickettsiosis who had an acute and a convalescent-phase serum sample.

	Positive Serology		<i>Rickettsia</i> -positive PCR	Culture positive	Total positive
	Acute sample	Convalescent-phase sample			
Patients with skin biopsies	2 (7%)	17 (65%)	14 (53%)	5 (19%)	17 (65%)

doi:10.1371/journal.pntd.0001540.t003



**Figure 1. Comparison between the cycles and log<sub>10</sub> values of the number of hypothetical protein copies/ml for *R. africae*.**  
doi:10.1371/journal.pntd.0001540.g001

## Écouvillonnage de l'escarre



FIG 16 Use of swabs of skin eschar in the diagnosis of a case of *Rickettsia sibirica mongolitimonae* by qPCR.

Tableau 5: positivité des PCR dans l'écouvillonnage de l'escarre et la biopsie cutanée

Etude	Écouvillon	Biopsie cutanée
Wang 2009	4/4 (100%) <i>(escarre+pustules)</i>	-
Bechah 2011*	8/9(88,9%)	5/5 (100%)
Mouffok 2011	26/41 (63,4%)	4/4 (100%)
Tunisie 2012-2104	10/13 (77%)	42/53 (79,2%)

\* : étude expérimentale

# Autres méthodes diagnostiques: entomologie



## Detection of *Rickettsia* in *Rhipicephalus sanguineus* Ticks and *Ctenocephalides felis* Fleas from Southeastern Tunisia by Reverse Line Blot Assay

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Institut Pasteur de Tunis, Université Tunis El Manar, Tunis, Tunisia<sup>a</sup>; Hôpital Habib Bourguiba<sup>b</sup> and Hôpital Hédi Chaker,<sup>c</sup> Université de Sfax, Sfax, Tunisia

Ticks ( $n = 663$ ) and fleas ( $n = 470$ ) collected from domestic animals from southeastern Tunisia were screened for *Rickettsia* infection using reverse line blot assay. Evidence of spotted fever group *Rickettsia* was obtained. We detected *Rickettsia felis* in fleas, *Rickettsia massiliae* Bar 29 and the *Rickettsia conorii* Israeli spotted fever strain in ticks, and *Rickettsia conorii* subsp. *conorii* and *Rickettsia* spp. in both arthropods. The sensitivity of the adopted technique allowed the identification of a new association between fleas and *R. conorii* subsp. *conorii* species. The presence of these vector-borne *Rickettsia* infections should be considered when diagnosing this disease in humans in Tunisia.



### ❖ Taux d'infection:

✓ *Rh. sanguineus*: 37,4%

✓ *C. felis*: 8,3%

❖ *R. conorii* dans les puces



# Matrix-Assisted Laser Desorption Ionization–Time of Flight Mass Spectrometry for Rapid Identification of Tick Vectors

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RESEARCH ARTICLE

## Detection of *Rickettsia* spp in Ticks by MALDI-TOF MS

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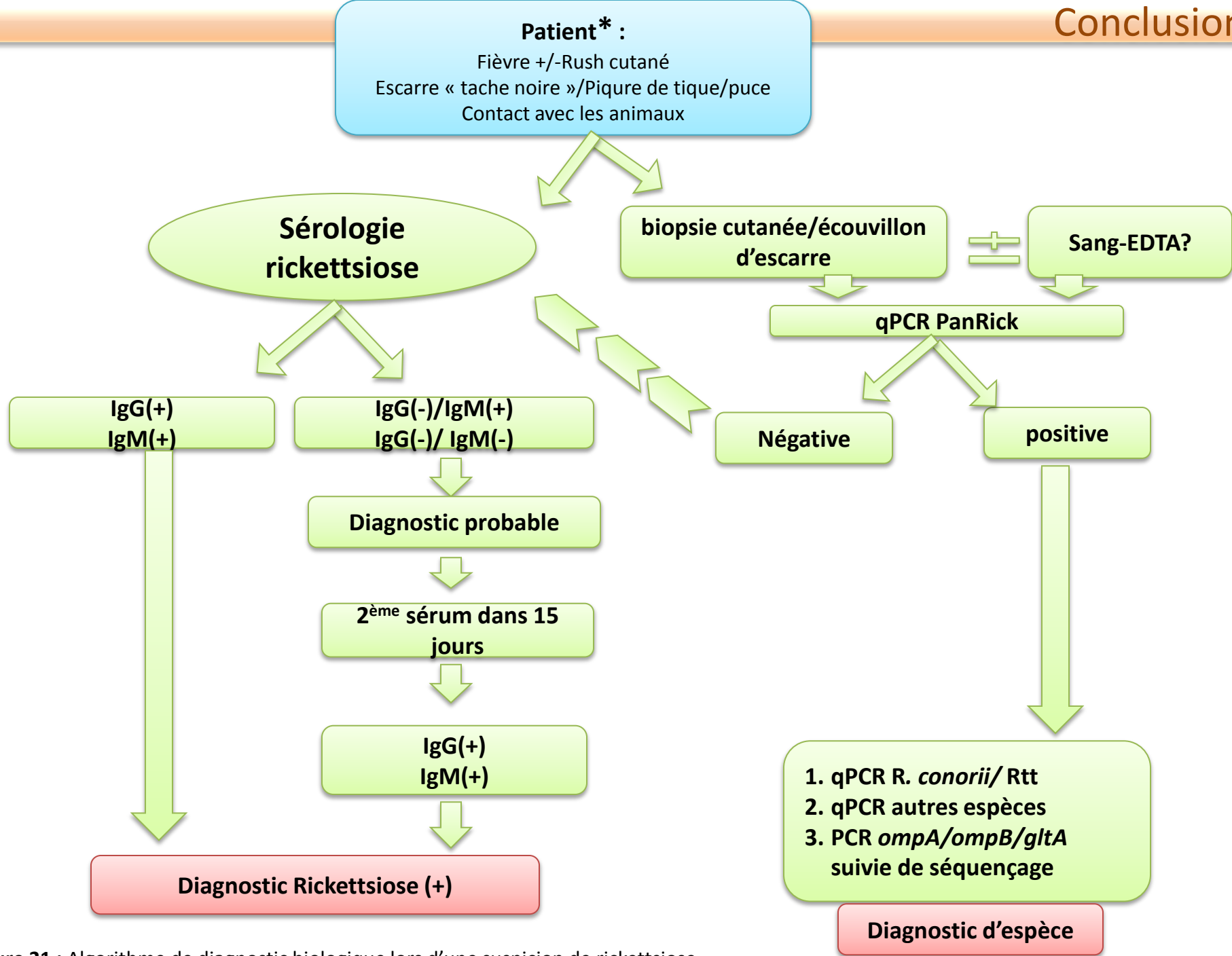


Figure 21 : Algorithme de diagnostic biologique lors d'une suspicion de rickettsiose

# Conclusions



# Conclusion

- Sérologie:
  - Diagnostic rétrospectif
  - De routine +++
- Biologie moléculaire
  - ◊ Diagnostic plus accessible
  - ◊ Prélèvements plus simples
  - ◊ Techniques moins coûteuses
- Nouvelles technologies

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June 13-16, 2015

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