Clinical cases of infections caused by intracellular bacteria
Rickettsial infection

- May 2012, in France
- Young girl (7 years)
- Tick bite after walking in wood
- The tick attached in the scalp
- A black lesion at site of tick bite
- A lymphadenopathy painful in the neck
Cervical lymphadenopathy (left panel, arrow), inoculation on the scalp (middle panel), and residual alopecia 4 weeks later (right panel).
May 2012, in France

Young girl (7 years)

Tick bite after walking in wood

The tick attached in the scalp

A black lesion at site of tick bite

A lymphadenopathy painful in the neck
WHAT IS IT?

• **TIBOLA**

  Spotless rickettsiosis caused by *Rickettsia slovaca* and associated with *Dermacentor* ticks.
  Raoult D, Lakos A, Fenollar F, Beytout J, Brouqui P, Fournier PE.
  Clin Infect Dis. 2002 May 15;34(10):1331-6

• **DEBONEL**

  [DEBONEL (Dermacentor-borne-necrosis-erythematolymphadenopathy). A new tick-borne disease?].
  Oteo JA, Ibarra V.

• **SENLAT**

  Scalp eschar and neck lymphadenopathy caused by *Bartonella henselae* after Tick Bite.
- The tick: biting in winter/spring in the scalp

- The microorganisms

- How to manage
DERMACENTOR TICKS IN EUROPE

Ambush strategy:

- waits, falls
- bites head
- children
- cold season
- Females
DERMACENTOR SEEKING HAIR
Dermacentor reticulatus, the ornate dog tick
D. marginatus, the ornate sheep tick
- The tick
- The microorganisms
- The disease
- How to manage
CAUSATIVE AGENTS

1- *R. slovaca*

A new tick-transmitted disease due to *Rickettsia slovaca.*
Raoult D, Berbis P, Roux V, Xu W, Maurin M.

2- *R. raoultii*

*Rickettsia slovaca* and *R. raoultii* in tick-borne Rickettsioses.
Parola P, Roverey C, Rolain JM, Brouqui P, Davoust B, Raoult D.
**R. slovaca** infection (compared to MSF)

- Based on 17 PCR confirmed cases
- More tick bite found
- Lesion in the hair
- Less fever
- More cervical lymph node
- Sequels: residual asthenia, localised alopecia
- Low death rate
- Young age
- Females > males
- Low serological titres

Rickettsia slovaca infection
**R. raoultii**

- Cases very closely related to TIBOLA
- Clinical cases identical to that caused by *R. Slovaca*
- Present in *Dermacentor*, ¼ of that of *R. slovaca*
- Less pathogenic
- Present in all Eurasia

Abstract
Tick-borne lymphadenopathy (TIBOLA), also called Dermacentor-borne necrosis erythema and lymphadenopathy (DEBONEL), is defined as the association of a tick bite, an inoculation eschar on the scalp, and cervical adenopathies. We identified the etiologic agent for 65% of 86 patients with TIBOLA/DEBONEL as either Rickettsia slovaca (49/86, 57%) or R. raoultii (7/86, 8%).
Characteristics of TIBOLA/DEBONEL patients with certain or probable *Rickettsia slovaca* infection compared with patients with certain or probable *R. raoultii* infection*

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>TIBOLA/DEBONEL patients, n = 86</th>
<th>No. <em>R. slovaca</em> infections (%)</th>
<th>No. <em>R. raoultii</em> infections (%)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female sex</td>
<td></td>
<td>33/49 (67)</td>
<td>7/7 (100)</td>
<td>0.04</td>
</tr>
<tr>
<td>Mean age, y</td>
<td></td>
<td>32</td>
<td>32</td>
<td>0.90</td>
</tr>
<tr>
<td>Age ≤12 y</td>
<td></td>
<td>20/49 (41)</td>
<td>3/7 (43)</td>
<td>0.46</td>
</tr>
<tr>
<td>Hiking or recreational activities such as a walk in the forest</td>
<td>21/28 (75)</td>
<td>4/5 (80)</td>
<td>0.44</td>
<td></td>
</tr>
<tr>
<td>Fever§</td>
<td></td>
<td>21/39 (54)</td>
<td>4/5 (80)</td>
<td>0.27</td>
</tr>
<tr>
<td>Painful eschar</td>
<td></td>
<td>14/22 (64)</td>
<td>3/3 (100)</td>
<td>0.30</td>
</tr>
<tr>
<td>Painful adenopathies</td>
<td>18/26 (69)</td>
<td>5/5 (100)</td>
<td>0.20</td>
<td></td>
</tr>
<tr>
<td>Face edema</td>
<td></td>
<td>6/31 (19)</td>
<td>2/5 (40)</td>
<td>0.30</td>
</tr>
<tr>
<td>Rash</td>
<td></td>
<td>7/30 (23)</td>
<td>1/5 (20)</td>
<td>0.68</td>
</tr>
<tr>
<td>Headache</td>
<td></td>
<td>16/30 (53)</td>
<td>4/4 (100)</td>
<td>0.10</td>
</tr>
<tr>
<td>Alopecia</td>
<td></td>
<td>16/27 (59)</td>
<td>0/4</td>
<td>0.09</td>
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<tr>
<td>Asthenia</td>
<td></td>
<td>23/33 (70)</td>
<td>5/5 (100)</td>
<td>0.20</td>
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<tr>
<td>Prolonged asthenia</td>
<td>10/29 (35)</td>
<td>2/4 (50)</td>
<td>0.46</td>
<td></td>
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<tr>
<td>Chronic asthenia#</td>
<td>4/28 (14)</td>
<td>1/4 (25)</td>
<td>0.51</td>
<td></td>
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</tbody>
</table>
The geographic distribution of these rickettsiae likely parallels that of *Dermacentor* ticks.
Genetic characterisation of ompA, ompB and gltA genes from Candidatus Rickettsia rioja.
Portillo A, Ibarra V, Santibáñez S, Pérez-Martínez L, Blanco JR, Oteo JA.
NOM : G  
Prénom : G  
Sexe : F  
Date de naissance :  

Hôpital/Service : Hopital Fréjus  
Médecin : Dr Pascal Del Giudice  
Tel/Email : 04. 94 40 22 00  

SYMPTOMATOLOGIE  

- Fièvre non-X  
- Escarre unique / localisation : cuir chevelu  
- Escarres multiples / localisation :  
- Adénopathies / localisation : cervicales + douleurs ++  

EPIDEMIOLOGIE  

- Voyage NON-X  
- Piqûre de tique / Identification morphologique:  
  Dermacentor marginatus (femelle gorgée)  
  Spectrométrie de masse: Dermacentor marginatus score 1.9  
- Piqûre unique / localisation : cuir chevelu  

TRAITEMENT : Antibiotique / molécule: DOXY 4 jj  

PHOTO: X OUI  
- 2ème sérum demandé  

Culture: X Mis en culture le 08/11/2012  

BioMol  

<table>
<thead>
<tr>
<th>Date</th>
<th>échan</th>
<th>smarlab</th>
<th>Cođe</th>
<th>Barto</th>
<th>Rick</th>
<th>R. slovac</th>
<th>R. rault</th>
</tr>
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<td>tique</td>
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<td>pos</td>
<td>neg</td>
<td>21/22</td>
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<td>neg</td>
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<td>ecouv</td>
<td>1281734</td>
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<td>neg</td>
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<td>neg</td>
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<tr>
<td>22/11/2012</td>
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<td></td>
<td>croute</td>
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<td>neg</td>
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Sérologie  

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<th>Smarlab</th>
<th>Barto</th>
<th>C. B</th>
<th>Rick</th>
<th>F. T</th>
<th>Borrelia</th>
<th>Diplo</th>
<th>A.phago</th>
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<tbody>
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<td>08/11/2012</td>
<td>1212062</td>
<td>neg</td>
<td>neg</td>
<td>neg</td>
<td>neg</td>
<td>neg</td>
<td>neg</td>
<td>neg</td>
</tr>
<tr>
<td>22/11/2012</td>
<td>1212618</td>
<td>neg</td>
<td>neg</td>
<td>neg</td>
<td>neg</td>
<td>neg</td>
<td>neg</td>
<td>neg</td>
</tr>
</tbody>
</table>

gltA gène: Rickettsia sp. DmS1 (591/592) AY129300  
ompA gène: Candidatus Rickettsia rioja (452/453) EF028201, Rickettsia sp. Jl-02 (544/551) AY 093696
Scalp eschar and neck lymphadenopathy caused by Bartonella henselae after Tick Bite.

Rickettsia slovaca and Rickettsia raoultii have been associated with a syndrome characterized by scalp eschar and neck lymphadenopathy following tick bites. However, in many cases, the causative agent remains undetermined. We report 3 cases of this syndrome caused by Bartonella henselae, and we propose the term "SENLAT" to collectively describe this clinical entity.
**BARTONELLA HENSELAE**

**DIAGNOSTIC**

Adenopathie cervicale bilatérale
Escarre cutanée : ulceration inflammatoire du scalp

→ Tableau évoquant plutôt un TIBOLA

→ Evolution à une semaine : l'évolution : persistance des ADP parotidiennes bilatérales (centimétrique à droite, 2 cm à gauche), fistulisation à la peau de l'ADP parotidienne gauche, avec tarissement spontané de l'écoulement puriforme en une dizaine de jours. Persistance d'une asthénie.

**FACTEURS DE RISQUE**

Contact avec animaux car vit en zone semi-urbaine
Travaille dans 1 centre équestre : pense s'être fait piqué par une tique ? Possède des chats

**TRAITEMENT**

15 J de DOXY

<table>
<thead>
<tr>
<th>Date</th>
<th>Smarlab</th>
<th>prélèvement</th>
<th>Rickettsia sp.</th>
<th>Bartonella sp.</th>
<th>Bartonella henselae</th>
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<tbody>
<tr>
<td>09/04/2009</td>
<td>2961732</td>
<td>BCU</td>
<td>neg</td>
<td>30,84/33,89</td>
<td>30,45/33,62</td>
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</table>

**2. NOM : H**
Prénom : Y
Sexe : M

Hôpital NANCY, Dr. Waton

**DIAGNOSTIC**

Adenopathie cervicale bilatérale
Escarre cutanée du cuir chevelu

→ PCR positive sur BCU à *Bartonella henselae.*
INTRODUCTION:
In 25 to 35% of cases, the aetiological agent of scalp eschar and neck lymphadenopathy after a tick bite remains undetermined. To date, Rickettsia slovaca, Rickettsia raoultii and more recently Bartonella henselae have been associated with this syndrome.

CASE PRESENTATION:
A four-year-old Caucasian boy was admitted to hospital with fever, vomiting and abdominal pain. On physical examination, an inflammatory and suppurating eschar was seen on the scalp, with multiple enlarged cervical lymph nodes on both sides. Although no tick was found in this scalp lesion, a diagnosis of tick-borne lymphadenopathy was suggested, and explored by serology testing and polymerase chain reaction of a biopsy from the eschar. Francisella tularensis DNA was found in the skin biopsy and the serology showed titres consistent with tularaemia.

CONCLUSION:
This is, to the best of our knowledge, the first reported case of scalp eschar and neck lymphadenopathy after tick bite infection caused by F. tularensis.
SENLAT tularémie

infection à *bartonella henselae*

infection à *Rickettsia slovaca*
- The tick

- The microorganisms

- How to manage
  - Ticks
  - Serology
  - Skin samples
  - Treatment
A method for rapid species identification of ticks may help clinicians predict the disease outcomes of patients with tick bites and may inform the decision as to whether to administer postexposure prophylactic antibiotic treatment. We aimed to establish a matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS) spectrum database based on the analysis of the legs of six tick vectors: Amblyomma variegatum, Rhipicephalus sanguineus, Hyalomma marginatum rufipes, Ixodes ricinus, Dermacentor marginatus, and Dermacentor reticulatus. A blind test was performed on a trial set of ticks to identify specimens of each species. Subsequently, we used MALDI-TOF MS to identify ticks obtained from the wild or removed from patients. The latter tick samples were also identified by 12S ribosomal DNA (rDNA) sequencing and were tested for bacterial infections. Ticks obtained from the wild or removed from patients (R. sanguineus, I. ricinus, and D. marginatus) were accurately identified using MALDI-TOF MS, with the exception of those ticks for which no spectra were available in the database. Furthermore, one damaged specimen was correctly identified as I. ricinus, a vector of Lyme disease, using MALDI-TOF MS only. Six of the 14 ticks removed from patients were found to be infected by pathogens that included Rickettsia, Anaplasma, and Borrelia spp. MALDI-TOF MS appears to be an effective tool for the rapid identification of tick vectors that requires no previous expertise in tick identification. The benefits for clinicians include the more targeted surveillance of patients for symptoms of potentially transmitted diseases and the ability to make more informed decisions as to whether to administer postexposure prophylactic treatment.
Date of symptoms:
- Fever
- Eschar only/localisation: Eschar multiples/localisation:
- Adenopathy/localisation:
- Rash:
  - Maculo-papulus
  - Purpuric
  - Vesiculus

Other manifestation: erythematous lesions

Travel place/date:

Morphological identification: *Ixodes ricinus*

Mass spectrometry: *I. ricinus* score 1.5

Single bite/localisation:
multiple bite/localisation:

Animals contact:

-> Identify risk factor

<table>
<thead>
<tr>
<th>Date</th>
<th>smarlab</th>
<th>Bartonella</th>
<th>C. burnetii</th>
<th>Rickettsies</th>
<th>Coxiella-like</th>
<th>Borrelia</th>
<th>A. phagoc</th>
</tr>
</thead>
<tbody>
<tr>
<td>08/10/2012</td>
<td>1279089</td>
<td>neg</td>
<td>neg</td>
<td>neg</td>
<td>neg</td>
<td>neg</td>
<td>ec</td>
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</tbody>
</table>
NOM : L  
Prénom : P  
Sexe : F  
Date de naissance:  

Hôpital/Service: Hôpital Nord/MIT  
Médecin : Dr Aubry  
Tel/Email : 68933  

SYMPTOMATOLOGIE
☐ Date de début des symptômes : 01/12/2012  
☐ Fièvre NON  
X Escarre unique / localisation : cuire chevelu  
X Adénopathies / localisation : cervicale + lymphangite

EPIDEMIOLOGIE
☐ Voyage lieu / date :  
X Piqûre de tique / Identification : Dermacentor marginatus  
Masse spectre : 1.837 Dermacentor marginatus  
X Piqûre unique / localisation : cuire chevelu

TRAITEMENT : Antibiotique / molécule: Doxy 200mg/j débuté le 01/12/2012 tout de suite après détection de tique

Date prélèvement smarlab Barto ARN C. B. Rick R. slovaca R. raoultii F. T Borrelia 16S Ehr spiro C. like Diplo Actine
05/12/2012 tique 1283793 neg neg 18/21 17/20 neg neg 35
sang 1283791 neg neg neg neg neg neg 21
écouvillon 1283792 neg neg neg 32/36 32/36 neg neg neg neg neg neg neg neg  

WB: négative

Culture tique Mis en culture le 05/12/2012- positive le 18/12/2012: Rickettsia slovaca
WESTERN BLOT ADSORPTIONS CROISEES

ANTIGENES: RSLO  RRAO

1239665

Dilution serum: 1/50
Dilution Adsorption: 1/10
adsorption: NUIT

Serum du 05/11/12
RSLO NEG
RRAO NEG

NON ABSORBE

ABSORBE RSLO

ABSORBE RRAO

Conclusion: RSLO
SKIN BIOPSY IS THE KEY OF DISCOVERY OF NEW RICKETTSIAL DISEASES

## DIAGNOSTIC: PCR

<table>
<thead>
<tr>
<th>Tested</th>
<th><em>R. prowazekii</em></th>
<th><em>B. quintana</em></th>
<th><em>B. recurrentis</em></th>
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</thead>
<tbody>
<tr>
<td>Ants</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>40</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lice</td>
<td>16 (36%)</td>
<td>5 (11%)</td>
<td>0</td>
</tr>
<tr>
<td>44</td>
<td></td>
<td></td>
<td></td>
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</tbody>
</table>

### Tested gene
- Citrate synthase
- Citrate synthase
- ITS
- 16srRNA

- Negative controls are critical
- Two different genes for confirmation of atypical/unique case
- Avoid “open” nested PCR and positive controls
Antibiotics used in the literature in the case of SENLAT series (Scalp Eschar and Neck Lymphadenopathy after tick-bite).

<table>
<thead>
<tr>
<th>Auteurs</th>
<th>N</th>
<th>Doxycycline (%)</th>
<th>Macrolide (%)</th>
<th>Autre traitement a (%)</th>
<th>Aucun traitement (%)</th>
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</thead>
<tbody>
<tr>
<td>Lakos 2002 (4)</td>
<td>8</td>
<td>16 (18,6)</td>
<td>NP</td>
<td>45 (52,3)</td>
<td>14 (16,3)</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Raoult et al. 2002 (14)</td>
<td>1</td>
<td>10 (71,5)</td>
<td>Azythromycine : 3 (21,4)</td>
<td>0</td>
<td>1 (7,1)</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td></td>
<td></td>
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<tr>
<td>Oteo et al. 2004 (6)</td>
<td>2</td>
<td>21 (95,5)</td>
<td>Josamycine : 1 (4,5)</td>
<td>0</td>
<td>0</td>
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<tr>
<td></td>
<td>2</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gouriet et al. 2006 (10)</td>
<td>1</td>
<td>13 (92,9)</td>
<td>Josamycine : 1 (7)</td>
<td>0</td>
<td>0</td>
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<tr>
<td></td>
<td>4</td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Ibarra et al. 2006 (7)</td>
<td>5</td>
<td>43 (79,6)</td>
<td>Josamycine : 1 (1,9)</td>
<td>0</td>
<td>0</td>
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<tr>
<td></td>
<td>4</td>
<td></td>
<td>Azythromycine : 10 (18,5)</td>
<td>0</td>
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</tbody>
</table>
Performance for the Rickettsia spp. PCR. made from a skin swab the eschar or a skin biopsy of the lesion to the diagnosis of rickettsiosis.

<table>
<thead>
<tr>
<th>Auteurs</th>
<th>Na</th>
<th>PCR <em>Rickettsia</em> positive sur écouvillon cutané de l’escarre (%)</th>
<th>PCR <em>Rickettsia</em> positive sur biopsie cutanée (%)</th>
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<tbody>
<tr>
<td>Wang JM et al. 2009 (36)</td>
<td>4</td>
<td>6/7 (85.7)</td>
<td>NP</td>
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<td>Bechah Y et al. 2011 (33)</td>
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<td>8/9 (88.9)</td>
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<td>Mouffok N et al. 2011 (35)</td>
<td>39</td>
<td>26/41 (63.4)</td>
<td>4/4 (100)</td>
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<td>Renvoisé A et al. 2012 (37)</td>
<td>45</td>
<td>8/42 (19)</td>
<td>31/150 (20.6)</td>
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