Educational Workshop

EW10: Clinical cases of infections caused by intracellular bacteria

Arranged with the ESCMID Study Group for Coxiella, Anaplasma, Rickettsia and Bartonella (ESCAR)

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G. Greub (Lausanne, CH) – no handout available
M. Wegdam-Blans (Veldhoven, NL) – no handout available
Raoult - Rickettsial infection

- May 2012, in France
- Young girl (7 years)
- Tick bike after walking in wood
- The tick attached in the scalp
- A black lesion at site of tick bite
- A lymphadenopathy painful in the neck

Clinical cases of infections caused by intracellular bacteria

Rickettsial infection

27/04/13

BERLIN

Cervical lymphadenopathy (left panel, arrow), inoculation on the scalp (middle panel), and residual alopecia 4 weeks later (right panel).
Raoult - 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

WHAT IS IT?

- TIBOLA
  Spotless rickettsiosis caused by Rickettsia slovaca and associated with Dermacentor ticks.
  Raoult D, Lakos A, Fenollar F, Beytout J, Brusqui P, Fournier PE.

- DEBONEL
  [DEBONEL] (Dermacentor-borne necrosis-erythematolymphadenopathy). A new tick-borne disease?
  Oteo JA, Barr 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
Raoult - Rickettsial infection

**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, Ravery C, Rolain JM, Brouqui P, Davoust B, Raoult D.
Raoult - Rickettsial infection

**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**

- 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

The geographic distribution of these rickettsiae likely parallels that of Dermacentor ticks

R. slovaca in ticks
R. raoultii in ticks
R. slovaca infection in human
R. raoultii infection in human
Raoult - Rickettsial infection

Rickettsia « la rioja »

Genetic characterisation of ompA, ompB and gltA genes from Candidatus Rickettsia rioja.

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.
Francisella tularensis
Eschar and neck lymphadenopathy caused by Francisella tularensis after a tick bite: a case report
Edouard S, Goren K, Turi T, Angiakko E, Soobabashri C, Raoult D.

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 tularemia.

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.
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.
Raoult - Rickettsial infection

**SYMPTOMATOLOGIE**

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

**EPIDEMIOLOGIE**

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

**TRAITEMENT**

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

**Culture tique**

- Mis en culture le 05/12/2012 - positive le 18/12/2012 :
  - Rickettsia slovaca

**Date prélèvement s marlab Barto ARN 16S 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 ng 1283791 neg neg 21 écouvillon 1283792 neg neg neg 32/36 32/36 neg neg neg neg neg neg neg

**Date Smarlab Barto C. B Rick F. T Diplo 05/12/2012 1213183 neg neg neg neg neg WB: négative

**SKIN BIOPSY IS THE KEY OF DISCOVERY OF NEW RICKETTSIAL DISEASES**
Raoult - Rickettsial infection

**SWABING**


**DIAGNOSTIC: PCR**

Tested gene | Citrate synthase | 16srRNA
--- | --- | ---
**Ants** | 40 | 0 | 0 | 0
**Lice** | 44 | 16 (36%) | 5 (11%) | 0

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 (%)</th>
<th>Aucun traitement (%)</th>
</tr>
</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>
<td></td>
</tr>
<tr>
<td>Raoult et al. 2002 (14)</td>
<td>1</td>
<td>10 (71,5)</td>
<td>Anxithromycline : 0</td>
<td>1 (7,1)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>4</td>
<td></td>
<td>3 (21,4)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oião et al. 2004 (6)</td>
<td>2</td>
<td>21 (95,5)</td>
<td>Josamycine : 1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td></td>
<td>4 (4,5)</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</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td></td>
<td>7 (7)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ibarra et al. 2006 (7)</td>
<td>5</td>
<td>43 (79,6)</td>
<td>Josamycine : 1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td></td>
<td>1 (1,9)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Anxithromycline : 10 (18,5)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Raoult - Rickettsial infection

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>N°</th>
<th>PCR Rickettsia positive sur écouvillon cutané de l’escarre (%)</th>
<th>PCR Rickettsia positive sur biopsie cutanée (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wang JM et al. 2009 (36)</td>
<td>4</td>
<td>6/7 (85,7)</td>
<td>NP</td>
</tr>
<tr>
<td>Bechah Y et al. 2011 (33)</td>
<td>9</td>
<td>8/9 (88,9)</td>
<td>5/5 (100)</td>
</tr>
<tr>
<td>Mouffok N et al. 2011 (35)</td>
<td>39</td>
<td>26/41 (63,4)</td>
<td>4/4 (100)</td>
</tr>
<tr>
<td>Renvoisé A et al. 2012 (37)</td>
<td>45</td>
<td>8/42 (19)</td>
<td>31/150 (20,6)</td>
</tr>
</tbody>
</table>
Znazen - Bartonella infection

Bartonella infection

Abir Znazen, Adnene Hammami.
Laboratory of Microbiology, Habib Bourguiba University Hospital Sfax, Tunisia.

1875: outbreak of Oroya Fever
1885: Daniel Carrion (Peruvian medical student) inoculated himself with infected material
Alberto Barton: discovery of the agent of Carrion’s Disease (Bartonella bacilliformis)

Historical overview
Znazen - Bartonella infection

Outlines

- Taxonomy and genus description
- Epidemiology and transmission
- Clinical manifestations
- Case presentation:
  - Bacillary angiomatosis
  - Infective endocarditis

Taxonomy and genus description

- Gram negative rods
- Facultative intracellular
- Slow growth on blood agar medium
- Inert in most biochemical tests
  - Limitation of Standard biochemical methods for identification
- Molecular methods:
  - Position of *Bartonella* species
Znazen - Bartonella infection
Znazen - Bartonella infection

- Rapidly growing number of *Bartonella* species and genotypes
- Multilocus typing and other molecular techniques
- Full genome analyses of *Bartonella* strains appear to be a more promising approach for developing a natural typing system.

Natural cycle => Hemotropic life form:
- Persistent intra-erythrocyte infection
- Specific mammalian host
- Transmission via arthropod vector

Ubiquity

- Various animals: reservoir of zoonotic infections (pets++)
  - *Cats*: *B. henselae*, *B. clarridgeiae* and *B. koehlerae*
    - Well adapted host: asymptomatic bacteremia
    - Seroprevalence: 14 to 50%
    - Cat fleas: many species++, role in transmission
  - *Dogs*: *B. vinsonii Subsp. berkoffii* (endomyocarditis), *B. henselae*, *B. clarridgeiae* (endocarditis), *B. wasonhensis*, *B. elizabethae*, *B. quintana* and *B. bovis*
  - *Cattle*: *B. bovis* (Europe, North America and Asia)
  - *Rats*: *B. elizabethae*
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Table 1: Bartonella species and their vectors and hosts

<table>
<thead>
<tr>
<th>Bartonella species</th>
<th>Vectors</th>
<th>Hosts</th>
</tr>
</thead>
<tbody>
<tr>
<td>B. vinsonii subsp. berkhofii</td>
<td>Tick, dog</td>
<td>Human</td>
</tr>
<tr>
<td>B. vinsonii subsp. vinsonii</td>
<td>Vole</td>
<td>Vole, ear mite, Human</td>
</tr>
<tr>
<td>B. vinsonii subsp. arupensis</td>
<td>Tick</td>
<td>Mouse, vole</td>
</tr>
<tr>
<td>B. quintana</td>
<td>Body louse</td>
<td>Human</td>
</tr>
<tr>
<td>B. taylorii</td>
<td>Cat, mouse, vole</td>
<td>Mouse</td>
</tr>
<tr>
<td>B. henselae</td>
<td>Cat, tick</td>
<td>Cat, human</td>
</tr>
<tr>
<td>B. koehlerae</td>
<td>Cat, mouse, vole</td>
<td>Cat</td>
</tr>
<tr>
<td>B. alsatica</td>
<td>Cat, mouse, vole</td>
<td>Mouse</td>
</tr>
<tr>
<td>B. grahamii</td>
<td>Mouse, vole, mouse</td>
<td>Mouse</td>
</tr>
<tr>
<td>B. elizabethae</td>
<td>Mouse, vole</td>
<td>Mouse, vole</td>
</tr>
<tr>
<td>B. tribocorum</td>
<td>Rat</td>
<td>Cat, mouse, vole</td>
</tr>
<tr>
<td>B. birtlesii</td>
<td>Mouse</td>
<td>Mouse</td>
</tr>
<tr>
<td>B. doshiae</td>
<td>Vole</td>
<td>Vole</td>
</tr>
<tr>
<td>B. rochalimae</td>
<td>Dog, fox</td>
<td>Dog, fox, human</td>
</tr>
<tr>
<td>B. clarridgeiae</td>
<td>Cat, mouse, vole</td>
<td>Cat, mouse, vole</td>
</tr>
<tr>
<td>B. bovis</td>
<td>Cattle</td>
<td>Cattle</td>
</tr>
<tr>
<td>B. capreoli</td>
<td>Roe deer, deer</td>
<td>Roe deer, deer</td>
</tr>
<tr>
<td>B. chomelii</td>
<td>Cattle, deer</td>
<td>Cattle</td>
</tr>
<tr>
<td>B. schoenbuchensis</td>
<td>Roe deer</td>
<td>Roe deer</td>
</tr>
<tr>
<td>B. bacilliformis</td>
<td>Sand flies</td>
<td>Human</td>
</tr>
</tbody>
</table>

Homelessness, alcoholism, poverty, poor conditions of hygiene, body louse.
Znazen - Bartonella infection

B. tamiae
Other Bartonella species

B. vinsonii subsp. Berkhoffii
B. elizabethae
B. claridgeiae
B. waahoensis
B. koehlerae

B. vinsonii subsp. Berkhoffii
B. claridgeiae
B. waahoensis
B. koehlerae

B. korthiae
B. tamiae

Table 3: clinical presentation associated with Bartonella sp.

<table>
<thead>
<tr>
<th>species</th>
<th>Human disease</th>
</tr>
</thead>
<tbody>
<tr>
<td>B. elizabethae</td>
<td>Endocarditis, neuroretinitis</td>
</tr>
<tr>
<td>B. claridgeiae</td>
<td>CSD, sepsis, endocarditis</td>
</tr>
<tr>
<td>B. koehlerae</td>
<td>Endocarditis, CSD</td>
</tr>
<tr>
<td>B. vinsonii subsp. Berkhoffii</td>
<td>Endocarditis, arthralgia / myalgia / headache / fatigue</td>
</tr>
<tr>
<td>B. waahoensis</td>
<td>Fever and myocarditis</td>
</tr>
<tr>
<td>B. tamiae</td>
<td>Fever</td>
</tr>
<tr>
<td>B. grahamii</td>
<td>Neuroretinitis</td>
</tr>
<tr>
<td>B. doshae</td>
<td>CSD</td>
</tr>
<tr>
<td>B. alsatica</td>
<td>Endocarditis, lymphadenitis</td>
</tr>
</tbody>
</table>
Znazen - Bartonella infection

B. henselae pathogenesis

- Cat scratch disease
- Infective endocarditis
- Peliosis
- Hepatitis
- Chronic bacteraemia
- Bacillary angiomatosis

Ctenocephalides felis

B. quintana pathogenesis

- Trench fever
- Chronic bacteraemia
- Bacillary angiomatosis
- Infective endocarditis

Clinical case 1
Znazen - Bartonella infection

Clinical case 1
- 50 year old women
- Immunocompetent
- Djbeniana (rural area)
- Low socioeconomic level
- Multiple angiomatosis lesions persisting more than 6 months.

Figure: angiomatosis lesions localized on the left hand

Diagnosis
- Histological lesions: +++
- Hematoxylin eosin staining
- Warthin starry staining

Microbiology:
- ITS-PCR: Bartonella quintana
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Bacillary angiomatosis

**Treatment**
- Erythromycin: 3g/day during 3 months
- Outcome: favorable

Clinical case 2

<table>
<thead>
<tr>
<th>Patient's characteristics</th>
<th>Clinical features</th>
</tr>
</thead>
<tbody>
<tr>
<td>61 year old men</td>
<td>Continuous fever</td>
</tr>
<tr>
<td>Immunocompetent</td>
<td>Dyspnea</td>
</tr>
<tr>
<td>Sfax (Awabel locality)</td>
<td>Abdominal pain</td>
</tr>
<tr>
<td>Low socioeconomic status</td>
<td></td>
</tr>
<tr>
<td>(hygiene)</td>
<td></td>
</tr>
<tr>
<td>No history of any</td>
<td></td>
</tr>
<tr>
<td>cardiac disease</td>
<td></td>
</tr>
</tbody>
</table>
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At admission:
- Fever: 39°
- BP: 100/40mmHg, HR: 84bpm, RR: 20C/mn
- Systolic and diastolic murmur in the mitral and aortic areas
- Chest X ray: infiltrates
- Abdominal echography: hepatomegaly

Biological features:
- CRP: 144mg/l
- ESR: 70 mm
- WCC: 17120/mm³, hb: 8.2g/dl, Pl: 65000/mm³
- Liver enzymes: 33/19 IU/l
- 3 sets of blood cultures: negative
Znazen - Bartonella infection

ETO (March 15 2013):
- Aortic valve: mobile vegetation (20mm)
- Mitral valve: many vegetations with calcification + perforation
- Perivalvular abscess

Diagnosis: infective endocarditis

Which items are in favor of Bartonella endocarditis?
- Geographic origin
- Poorness
- Defective hygiene
- Destructive valve lesions
The clinical and pathological characteristics of Bartonella endocarditis:

- Previous history of a valvular disease
- More often affecting the aortic valve
- Highly vegetative lesions with calcification

Raoult D, JAMA Int Med, 2003

Which microbiological investigations should be performed?

- Serology:
  - Coxiella burnetii
  - Bartonella
  - Brucella
- Secondly: Chlamydia, Legionella, Mycoplasma...

- PCR on whole blood:
  - Standard PCR/ RT-PCR
  - Genes: ITS, rnpb, fur, pap, Groel...
Znazen - Bartonella infection

Serology results

<table>
<thead>
<tr>
<th></th>
<th>Chlamydia pneumoniae</th>
<th>Bartonella quintana/henselae</th>
<th>Coxiella burnetii</th>
<th>Phase I/II</th>
</tr>
</thead>
<tbody>
<tr>
<td>IgT</td>
<td>6400/3200</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>IgG</td>
<td>2048</td>
<td>-</td>
<td>Neg</td>
<td></td>
</tr>
<tr>
<td>IgM</td>
<td>Neg</td>
<td>-</td>
<td>Neg</td>
<td></td>
</tr>
<tr>
<td>IgA</td>
<td>Neg</td>
<td>-</td>
<td>Neg</td>
<td></td>
</tr>
</tbody>
</table>

- What is your diagnosis?
  
  Bartonella endocarditis
  
  Cross reactions between Chlamydia and Bartonella +++

- Previously: almost all cases of Chlamydia endocarditis were in fact Bartonella endocarditis.
  
  (Maurin M, JCM, 1997)

- In our series of BCNE: 13 patients with Bartonella IE showed positive serology to three chlamydial species
  
  (Znazen A, AJTMH, 2005)

- Micro immunofluorescence assay:
  
  - Gold standard
  
  - Cross reactions between species

- Western blott with cross adsorption:
  
  - To confirm species
  
  - Antigen consuming
Znazen - Bartonella infection

IgG titer > 1:800:
- PPV of 0.81 in patients with chronic infection
- PPV of 0.955 in patients with infective endocarditis

Clinical case (continues):

Patient was operated on March 22 2013

Prosthetic Aortic and mitral valves

For removed cardiac valves, which investigations should be indicated?

- Culture
- PCR
- Histology
- Immunohistochemistry
Znazen - Bartonella infection

Antimicrobial drug susceptibility of *Bartonella*:
- Penicillin
- Cephalosporin
- Aminoglycosides
- Chloramphenicol
- Doxycycline, tetracycline
- Macrolides
- Rifampin
- Fluoroquinolones
- Cotrimoxazole

Raoult D, *JAMA Internal Medicine*, 2003
Rolin JM, *AAC*, 2004

Treatment
- Aminoglycosides: 14 days
- Recommended antibiotherapy:
  - Doxycycline: 100mg×2/day for 6 weeks
  - Gentamicin: 3mg/kg/day for 2 weeks

Take home message
- *Bartonella* species: several host reservoirs
  - *B. quintana*: detected in fleas!
- Wide clinical spectrum
- Diagnosis: Serology / PCR++
- MLST + complete genome sequences:
  - Natural history: reservoir-transmission-IE
- *Bartonella* Network:
  - Better description of epidemiology and pathogenicity
Znazen - Bartonella infection

Special thanks to:

- Cardiology department, Hedi Chaker university hospital
- Infectious diseases department, Hedi Chaker university hospital
- Laboratory of Microbiology, Habib Bourguiba university hospital