Whipple’s disease and *Tropheryma whipplei*
Whipple’s disease and *Tropheryma whipplei*

1907  First description of Whipple’s disease (Metabolic trouble)

1991  Identification of the agent of Whipple’s disease
      A rare bacterium causing a rare chronic infection

2000  First establishment of a strain of *T. whipplei*
      *Full genome sequence*
      *Molecular tools*
      *Genotyping*
      *Antibiotic susceptibilities*

2017  *T. whipplei*: A common bacterium

Prevalence of *T. whipplei* carriage

- **Saliva and stools specimens:**
  - Age
  - Exposure
  - Geographical area

<table>
<thead>
<tr>
<th>Population</th>
<th>Young children in France</th>
<th>French general adult population</th>
<th>Underground sewer workers</th>
<th>Adults in rural Senegal</th>
<th>Relatives of <em>T. whipplei</em> patients or carriers (France)</th>
<th>Children in rural Senegal in rural Laos</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prevalence faeces</td>
<td>≈1%</td>
<td>≈4%</td>
<td>≈12%</td>
<td>17.4%</td>
<td>38%</td>
<td>48%</td>
</tr>
<tr>
<td>Prevalence saliva</td>
<td>NA</td>
<td>0.2%</td>
<td>≈3%</td>
<td>1.7%</td>
<td>10%</td>
<td>9.5%</td>
</tr>
</tbody>
</table>

- **Duodenal biopsy:**
  - 0.25%
Classic Whipple’s disease

- A **disseminated disease** characterized by the **presence** of **typical histologic lesions** observed in **small-bowel** biopsies using PAS-staining

- Typical patient: 50 years-old Caucasian man

- Clinical manifestations: protean and non specific:
  As nearly all organs could be involved

Most often: arthralgia, weight-loss and/or chronic diarrhea

  Cardio-vascular manifestations
  Neurologic manifestations
  Ophthalmologic manifestations
  Mediastinal and/or mesenteric adenopathy
  Skin involvement and other…
Classic Whipple’s Disease

Review of the literature 2007:

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

<table>
<thead>
<tr>
<th>Feature</th>
<th>Patients with Whipple’s Disease</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male sex</td>
<td>770/886 (87)</td>
</tr>
<tr>
<td>Arthralgia or arthritis</td>
<td>244/335 (73)</td>
</tr>
<tr>
<td>Diarrhea</td>
<td>272/335 (81)</td>
</tr>
<tr>
<td>Weight loss</td>
<td>223/240 (93)</td>
</tr>
<tr>
<td>Fever</td>
<td>128/335 (38)</td>
</tr>
<tr>
<td>Adenopathy</td>
<td>174/335 (52)</td>
</tr>
<tr>
<td>Melanoderma</td>
<td>99/240 (41)</td>
</tr>
<tr>
<td>Neurologic signs†</td>
<td>33/99 (33)</td>
</tr>
<tr>
<td>Ocular signs†</td>
<td>6/99 (6)</td>
</tr>
<tr>
<td>Pleural effusion</td>
<td>26/190 (14)</td>
</tr>
</tbody>
</table>

Data 2010:
Main symptom: Arthralgia (88/113, 78%)
Misdiagnosis:
Inflammatory rheumatoid disease (56/113, 50%)
Improvement with a short course of antibiotics

Prodromal stage:
Average time = 6 years

Steady-state stage:
More rapid clinical progression with immunosuppressive therapy
Emergence of cases of localized \textit{T. whipplei} endocarditis

Total of published cases of localized \textit{T. whipplei} endocarditis (excluding our cases)

Our published and unpublished cases

Goldenberger \textit{et al.}
First detection

Gubler \textit{et al.}
First series reported

Miguelena \textit{et al.}
Gabus \textit{et al.}
Escher \textit{et al.}
Besnard \textit{et al.}
Mallmann \textit{et al.}

Ansemant \textit{et al.}
Daien \textit{et al.}
Lagier \textit{et al.}
Endocarditis due to *T. whipplei*


- Typical patient: 60 year-old Caucasian man

- Presence of arthralgia (75%)

- Apyrexia (fever observed for 31.6%)

- Previous valvular disease not systematically observed (30%)

- « Cardiologic » presentation:
  - Cardiac insufficiency (69.5%)
  - Acute ischemic stroke (27%)
  - Peripheral arterial emboli (13.5%)

- Aortic valve involvement (61%)

- Cardiac vegetation (82%)
Encephalitis due to *T. whipplei*


- 30 cases reported in the literature (15 these last 5 years)

-Can mimic symptoms of almost any other neurologic condition:

  - Cognitive changes may even extend to dementia
  - Psychiatric symptoms such as depression and personality changes
  - Supranuclear ophthalmoplegia
  - Myoclonus
  - Hypothalamic involvement
  - Movement abnormalities of the eye muscles:
    - Oculomasticatory or oculofaciockeletal myorhythmia
Acute infections: Pneumonia (2007)

- In the bronchoalveolar lavage fluid (BAL) of a child with pneumonia (USA)
  - Harris J, De Grote MA, Sagal SD, //, Pace NR. PNAS 2007;104:20529-33..

- In 6 (3%) of 210 BAL from patients with pneumonia in intensive care units (Marseille, France)

  Broad range 16S rRNA PCR detected only Tw in the BAL of a patient with pneumonia

  Molecular detection of Tw in association with other bacteria from the saliva in the BAL of other patients with aspiration pneumonia

- Isolation of T. whipplei in pure culture from the BAL of a patient with interstitial acute pneumonia

  From January 2013 to December 2014, from all the 1438 BALs (Marseille, France):

  88 BALs (6.1%) positive for T. whipplei

  Tw commonly associated with aspiration pneumonia (18/88 patients)

  Tw detected as a unique pathogen in 9 patients with pneumonia

Acute infections: Gastro-enteritis (2010)

- In 36 (15%) of stools of 241 children from 2-4 year-old hospitalized in Marseille (France)

- High bacterial loads (>10^4/g)

- Absence of *T. whipplei* in stools after patient recovery

- No case in the controls

- Positive serology in patients significantly higher than in controls

- 33% co-infection with identified pathogens


-Murine model with previously inflammed colonic tissues

Other studies about the role of *T. whipplei* as an agent of gastroenteritis

**Rural Ghana**

Faeces from children of 0-12 months:

35 diarrheic children (23.3%) positive for *T. whipplei*

43 children without diarrhea (11.4%) of positive for *T. whipplei*


**Marseille, France**

From December 2009 to January 2013:

- 3796 rectal swabs from children of less than 6-year-old from the Emergency Department

555 diarrheic children 22 (4%) positive for *T. whipplei*

3241 children without diarrhea (1.7%) positive for *T. whipplei* (p=0.001)

*T. whipplei* was more commonly observed in Autumn

Acute infections: Bacteremia (2010)

- In 13 (6.4%) of 204 blood specimens from patients with fever and without malaria in Dielmo and Ndiop (rural Senegal)
- Cough, significantly more frequent in febrile patients with *T. whipplei* bacteremia than those without
- An agent of unexplained fever, including respiratory infection?


New study about *T. whipplei* bacteremia in Senegal including 2024 febrile people

-Febrile patients: 36/786 (4.6%)
-Age-matched controls without fever: 1/385 (0.25%), p<10⁻³
Excellent specificity and sensitivity

Production of rabbit polyclonal antibodies specifically directed against *T. whippelii*

Proposition of a new diagnostic tool:

Immunohistochemistry

- Periodic acid-Schiff staining (PAS)


Duodenal biopsy

- Immunohistochemistry


Immunohistochemistry

Lymph node biopsy

Blood monocytes

Aqueous humor

Brain biopsy

Cardiac valve
Patient 1. A 40-year-old woman that mainly presented sleep disturbances, abnormal eye movements, light dementia, arthralgia, and dysphonia without digestive signs.

Maxillary sinus biopsy (IHC x 200)

Laryngeal biopsy (IHC x 200)
Patient 2. A 67-year-old man that presented fever, myalgia, and arthralgia without digestive signs.

Muscle biopsy (IHCx200)
Patient 3. A 77-year-old dead man that initially presented mesenteric lymphadenopathy, hepatomegaly, and splenomegaly with the presence of granulomas, which led to the diagnosis of sarcoidosis. No arthralgia and no digestive signs were observed prior to the start of corticoid therapy, the alteration of the generalized status of the patient and his death.

Specimens from November 2004

Spleen biopsy (IHC x 200)  
Liver biopsy (IHC x 400)
Patient 4. A 73-year-old man that presented with pericarditis during a relapse of classic WD after treatment with trimethoprim-sulfamethoxazole.

Pericardial biopsy (IHC x 100)
Patient 5. A 57-year-old man was admitted to our hospital for acute heart failure in July 2010. The patient’s history revealed previous hospitalization for polyarthralgia in 2005 without diagnosis. He had been admitted a month before to a secondary hospital because of a worsening general condition, including a 38-kilogram weight loss and fever.

Endomyocardial biopsy (IHC x100)
Molecular biology

First molecular tools:
- PCR targeting 16S rRNA
  - False positive (*A. odontolyticus, C. gingivalis*) ++

- Quantitative real time PCR targeting the intertransgenic spacer

In 2003, 2 full genome sequences from 2 different *T. whipplei* strains

Development of quantitative real time PCR targeting repeated sequences
1. **Cell-culture**
3 shell vials for each sample
HEL cells +/- Antimicrobials

2. **Culture axenic medium**
Genome analysis:
Lack of numerous ways allowing the synthesis of amino acids
Cell-free culture medium that provided all the missing amino acids

3. **Culture of T. whipplei from faeces**
Axenic medium + Glutaraldehyde (2%)

4. **Culture of T. whipplei from saliva and BAL**
Axenic medium
Samples successively filtered until 0.45-µm pore filters

---

**T. whipplei culture**

- Observation every month for approximately 6 months
- IF using specific antibodies
- Specific quantitative PCR
- Flask of 25 cm² for the strain’s establishment

**IF + PCR**

### Summary of established strains of *T. whipplei* in our center since 2000

<table>
<thead>
<tr>
<th>Primo-cultures</th>
<th>Established strains</th>
</tr>
</thead>
<tbody>
<tr>
<td>17 cardiac valves</td>
<td>7</td>
</tr>
<tr>
<td>14 cerebro-spinal fluids</td>
<td>14</td>
</tr>
<tr>
<td>13 duodenal biopsies</td>
<td>3</td>
</tr>
<tr>
<td>12 synovial fluids</td>
<td>12</td>
</tr>
<tr>
<td>5 blood specimens</td>
<td>4</td>
</tr>
<tr>
<td>4 saliva samples</td>
<td>4</td>
</tr>
<tr>
<td>3 lymph nodes</td>
<td>2</td>
</tr>
<tr>
<td>1 skeletal muscular biopsy</td>
<td>1</td>
</tr>
<tr>
<td>1 stools specimen</td>
<td>1</td>
</tr>
<tr>
<td>1 skin biopsy</td>
<td>1</td>
</tr>
<tr>
<td>1 broncho-alveolar fluid</td>
<td>1</td>
</tr>
<tr>
<td>1 synovial biopsy</td>
<td>0</td>
</tr>
<tr>
<td>1 aqueous humor</td>
<td>0</td>
</tr>
</tbody>
</table>

Serological diagnosis


3 reaction patterns of 60 patients with classic WD

110 kDa

<table>
<thead>
<tr>
<th>Pattern</th>
<th>Frequency</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weaker reaction after deglycolysation</td>
<td>33 (55%)</td>
<td></td>
</tr>
<tr>
<td>No response</td>
<td>23 (38%)</td>
<td></td>
</tr>
<tr>
<td>Equal response before and after deglycosylation</td>
<td>4 (7%)</td>
<td></td>
</tr>
</tbody>
</table>

3 reaction patterns of 26 *T. whipplei* carriers

110 kDa

<table>
<thead>
<tr>
<th>Pattern</th>
<th>Frequency</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weaker reaction after deglycolysation</td>
<td>3 (11.5%)</td>
<td></td>
</tr>
<tr>
<td>No response</td>
<td>2 (8%)</td>
<td></td>
</tr>
<tr>
<td>Equal response before and after deglycosylation</td>
<td>21 (81%)</td>
<td></td>
</tr>
</tbody>
</table>

- Not performed in routine
Diagnosis of Classic Whipple’s disease


**Suspicion of classic Whipple disease**

- **Saliva / Stool PCR screening**
  - Positive / Positive (PPV: 95.2%)
    - High suspicion
  - Positive / Negative
    - Possible contamination
  - Negative / Positive
    - Bacterial load in stool $\geq 10^4$ cfu/g
      - High suspicion (PPV: 100%)
  - Negative / Negative (NPV: 99.2%)
    - Unlikely classic Whipple disease

**Confirmation:** Histologic lesions observed in small-bowel biopsies using PAS-staining and/or specific immunochemistry (or qPCR)
Diagnosis of localized *T. whipplei* infection


**Suspicion of localized infection**

- **Infective endocarditis**
  - Blood+
  - Valvular biopsy ++
  - qPCR

- **Encephalitis/neurologic disorders**
  - CSF +
  - Brain biopsy ++
  - qPCR

- **Uveitis**
  - Aqueous humor +
  - qPCR

- **Adenopathy**
  - Lymph nodes biopsy +
  - qPCR

- **Arthritis**
  - Articular fluid ++
  - qPCR
High genetic variety of *T. whipplei*

In this tree: 61 genotypes from 198 specimens

- **Carriers**
- **T. whipplei** chronic infections (classic and localized)

**Cases of gastroenteritis**

Genotype*: Same genotype detected between the family members of a same family

Sene: Senegal
Looking for *T. whipplei* source and reservoir in Senegal


**105 water samples**
- 92 canaris
- 6 wells
- 7 water open

**317 Ixodid ticks**

**118 stools of “domestic” animals**
- Chickens, donkeys, goats, cattle, ducks, domestic pigeons, sheep, dogs

**207 dust samples**

**Humans:**
Currently the main identified reservoir and source of *T. whipplei* in these populations

**76 mosquitoes**
- *A. gambiae*

**239 fleas**

**317 Ixodid ticks**
Exhaustive comparison between households

Exhaustive in-site questionnaire

1. Number of inhabitants of the household (age/sex)
2. Description of the household
3. Food
4. Water
5. Sanitary
6. Hand washing
7. Waste

<table>
<thead>
<tr>
<th>Households</th>
<th>Stool carriage</th>
<th>Saliva carriage</th>
<th>Bacteremia</th>
<th>Toilets</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
<td>Yes (Closed)</td>
</tr>
<tr>
<td>11</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
<td>Yes (Open)</td>
</tr>
<tr>
<td>19</td>
<td>22.2%</td>
<td>0%</td>
<td>18.75%</td>
<td>No</td>
</tr>
<tr>
<td>39</td>
<td>83.3%</td>
<td>0%</td>
<td>19%</td>
<td>No</td>
</tr>
<tr>
<td>29</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
<td>Yes (Closed)</td>
</tr>
<tr>
<td>14</td>
<td>50%</td>
<td>0%</td>
<td>7.69%</td>
<td>Yes (Open)</td>
</tr>
<tr>
<td>16</td>
<td>44.4%</td>
<td>0%</td>
<td>5.35%</td>
<td>No</td>
</tr>
<tr>
<td>22</td>
<td>42.85%</td>
<td>8.33%</td>
<td>2.56%</td>
<td>No</td>
</tr>
</tbody>
</table>

- Limited access to toilets and exposure to human faeces facilitated the feco-oral transmission of *T. whipplei*. 
Proposition of a way of a transmission of *T. whipplei*

<table>
<thead>
<tr>
<th>Population</th>
<th>Faeces</th>
<th>Saliva</th>
<th>Seroprevalence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Europe general population</td>
<td>50.00%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>French homeless people*</td>
<td>10.00%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Europe underground sewer worker*</td>
<td>20.00%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rural Senegalese population</td>
<td>40.00%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>French relatives of Tw patients and carriers</td>
<td>50.00%</td>
<td>10.00%</td>
<td></td>
</tr>
</tbody>
</table>

In Senegal
- No presence in:
  - Water samples
  - Arthropod vector
  - Almost no presence (<1%) in:
    - Domestic animals
    - Dusts

In France
- Intrafamilial circulation

High prevalence in humans
Almost none environmental presence

**Human-to-human transmission**
- Depending of the hygiene condition
- Oro-oral (Good)
- Feco-oral (Poor)

**Circulation of same clones in families**
Proposition of a natural history of *T. whipplei*

**EXPOSURE TO Tropheyma whipplei**
Feco-oral or oro-oral interhuman transmission

**PRIMO-INFECTION**
% symptomatic?

**ACUTE INFECTIONS**
- Diarrhea
- Bacteremia
- Pneumonia
- Others?

**CARRIAGE**
- Prevalence depending of age, exposure and geographic area

**CURE**

**MOST OF THE CASES**

**SPECIFIC HOST**
Immuno-genetic factors?

**SUB-ACUTE OR CHRONIC INFECTIONS**
- Classic Whipple’s disease
- Localized infections
  - Endocarditis
  - Encephalitis
  - Uveitis
  - Adenitis
  - Pneumonia
  - Osteo-articular infection
  - Others?

**CHILDHOOD**

**ADULT**
Acquired resistance to trimethoprim/sulfamethoxazole

- Empirical treatment of classic Whipple’s disease:
  1-year of oral trimethoprim/sulfamethoxazole (STX): Spectacular efficacy but a lot of relapses

Literature: 34 patients with classic WD that have presented a relapse or failure with trimethoprim-sulfamethoxazole therapy

<table>
<thead>
<tr>
<th>Study</th>
<th>Year</th>
<th>Country</th>
<th>Number of patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cooper</td>
<td>1994</td>
<td>United-States of America</td>
<td>1</td>
</tr>
<tr>
<td>Feurle</td>
<td>1994</td>
<td>Germany</td>
<td>2</td>
</tr>
<tr>
<td>Frésard</td>
<td>1996</td>
<td>France</td>
<td>1</td>
</tr>
<tr>
<td>Schnider</td>
<td>1996</td>
<td>Austria</td>
<td>5</td>
</tr>
<tr>
<td>Vital-Durand</td>
<td>1997</td>
<td>France</td>
<td>1</td>
</tr>
<tr>
<td>Garas</td>
<td>2000</td>
<td>Australia</td>
<td>1</td>
</tr>
<tr>
<td>Levy</td>
<td>2000</td>
<td>France</td>
<td>1</td>
</tr>
<tr>
<td>Bakkali</td>
<td>2008</td>
<td>France</td>
<td>1</td>
</tr>
<tr>
<td>Fenollar</td>
<td>2009</td>
<td>France</td>
<td>4</td>
</tr>
<tr>
<td>Lagier</td>
<td>2010</td>
<td>France (9) and Switzerland (1)</td>
<td>10</td>
</tr>
<tr>
<td>Fenollar</td>
<td>2010</td>
<td>Canada and United-States of America</td>
<td>2</td>
</tr>
<tr>
<td>Pauletti</td>
<td>2010</td>
<td>Italia</td>
<td>1</td>
</tr>
<tr>
<td>Uryu</td>
<td>2012</td>
<td>Japan</td>
<td>1</td>
</tr>
<tr>
<td>Feurle</td>
<td>2012</td>
<td>Germany</td>
<td>1</td>
</tr>
<tr>
<td>Fenollar</td>
<td>2013</td>
<td>France</td>
<td>2</td>
</tr>
</tbody>
</table>
Resistance to trimethoprim/sulfamethoxazole


- Full genome sequencing of *T. whipplei* and antibiotic susceptibility testing:
  = Resistance of *T. whipplei* to trimethoprim

-Acquired resistance to sulfamethoxazole:
  - Among patients that experienced clinical failure during treatment with sulfamethoxazole
  - Apparition of mutations in the *folP* gene that encodes the target of sulfonamides
New therapeutic propositions

- Treatment of *T. whipplei* chronic infections:

  Doxycycline (100 mg X 2 per day) and Hydroxychloroquine (200 mg X 3 per day)
  For at least one year

  Spectacular efficacy, until now no clinically acquired resistance observed, but…

End of the treatment
in February 2007

Relapse in October 2008

Duodenal biopsies
Conclusions

1907:  **Whipple’s disease** = Metabolic disease

1991:  **Whipple’s disease** = Infectious disease caused by a rare bacterium

2017:  **T. whipplei** = Common bacterium and Whipple’s disease, the top of the iceberg of the infections caused by **T. whipplei**
   - Asymptomatic carriage
   - Chronic infections, such as Whipple’s disease
   - Acute infections

- Main hypothesis:

Many persons in any given population are exposed to **T. whipplei**

AND

Some of those with predisposing immuno-genetic factors
As yet undelineated
Subsequently develop the disease