What causes non-malarial, haemoculture negative fever?

<table>
<thead>
<tr>
<th>Disease</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Scrub typhus</td>
<td>16%</td>
</tr>
<tr>
<td>Leptospirosis</td>
<td>10%</td>
</tr>
<tr>
<td>Dengue</td>
<td>10%</td>
</tr>
<tr>
<td>Murine typhus</td>
<td>10%</td>
</tr>
<tr>
<td>Spotted fever</td>
<td>3%</td>
</tr>
<tr>
<td>JEV</td>
<td>3%</td>
</tr>
<tr>
<td>'Doxycycline responsive illnesses'</td>
<td>39%</td>
</tr>
</tbody>
</table>

Clinical associations

Very few clinical or laboratory features were useful in distinguishing these diseases

**Lymphadenopathy**

Murine typhus (3%) v scrub typhus (46%)

\[ \text{OR} (95\%\text{CI}) = 26 (3 - 213) \]

**Peripheral white count <8.5 10^9/L**

Dengue (62%) v scrub typhus (16%)

\[ \text{OR} (95\%\text{CI}) = 17 (5 - 66) \]

Severe disease

- Patients with scrub typhus had higher frequency of abnormal chest examination (27%) than patients with murine typhus (3%) \(p=0.002\)
- Respiratory rate was faster for scrub typhus patients than for those with murine typhus \(p=0.0012\)

- Raised transaminases > x 3 upper limit of reference range in ~ 1/3 of typhus patients

- Meningism, severe headache, dyspnoea, renal impairment:
  - 17 (27%) of patients with scrub typhus
  - 9 (23%) of patients with murine typhus
Rickettsial culture
Propagation of rickettsia in L929 and Vero cell cultures in BSL3 Laboratory
Infection of cultured cells with buffy coat. Many uncertainties:
- Antibiotics?
- Optimum blood volume?
- Optimum incubation temperature?
Determination of rickettsia infection in cell cultures by IFA and Real-Time PCR assays
16s PCR & RFLP genotyping of O.tsutsugamushi

Rickettsial Culture
Propagation of rickettsia in L929 and Vero cell cultures in BSL3 Laboratory
Began September 2008
From Mahosot
- 40 patients O. tsutsugamushi
- 11 patients R. typhi
From rural Laos since September 2009
- 18 patients O. tsutsugamushi
For MLST and antibiotic susceptibility testing in 2010

Treatment
For both diseases we use in adults:
- 200mg doxycycline stat followed by 7 days 100mg every 12 hours
Murine typhus - No clinical trials - despite being a global disease!
Therefore for both diseases random, unblinded clinical trials:
- Doxycycline 7 days versus 3 days versus azithromycin 3 days
Murine typhus trial recruitment finished

Kyushu, Japan 1953
Syndrome of fever, generalised lymphadenopathy, hepatosplenomegaly, peripheral blood mononucleosis, lymphopaenia with atypical lymphocytes
Rickettsia sennetsu, Ehrlichia sennetsu and most recently Neorickettsia sennetsu. The first cause of ‘glandular fever’ identified

Laos
- Fish - very important part of diet
- Multiple rivers, ponds and fish farms
- Consumption of raw or partially cooked fish common, as suggested by high prevalence of Opisthorchis infection
- ‘Pa dek’ – fermented fish paste
- THEREFORE - if sennetsu is a fish/trematode borne zoonosis - we might expect it in Laos

Laos- seroprevalence
- Percentage of patients with anti-sennetsu IgG>1/100
  - Blood donors – Vientiane (n=194) 17%
  - Undifferentiated fever - Vientiane (319) 14%
  - Undifferentiated fever - Savannakhet (229) 15%
  - Febrile hepatitis/jaundice – Vientiane (390) 20%
  - Undifferentiated fever - Malaysia (40) 0%
  - Undifferentiated fever - Thailand (808) 3%
Anabas testudineus

Climbing perch

Occurs throughout the Mekong River Basin, Sri Lanka to China, Indonesia, and the Philippine Islands. It is a small (~10 cm long) omnivore and an important food fish in SE Asia and usually cooked as a whole fish.

PCR for N. sennetsu positive on gill tissue

Trichopsis vittata

A small (<7 cm in length) carnivore found in the Mekong and also in Thailand, Viet Nam and Indonesia. Also distributed to many non-endemic countries in the aquarium trade.

Neorickettsia - 95.8% homology with N. sennetsu

Undercooked/raw fish consumption

Some 45 million people are estimated to have Opisthorchis/Clonorchis infections - suggesting that they have eaten raw/undercooked fish and therefore at risk of sennetsu?

We are very grateful to the Minister of Excellence Dr. Ponmek Dalaloy, and the Director of the Curative Department, Ministry of Health, Professor Sommone Phounsavath and the Directors of Mahosot Hospital for support for this study.

We thank all the staff of laboratories and wards Mahosot Hospital and our collaborators, especially:

University of Marseille (Didier Raoult, Jean Marc Rolain & Philippe Parola)
US Navy (Al Richards)
Mekong River Commission/LARREC
Institut de Recherche pour le Développement (Julie Valle)