### Mahosot Microbiology Review

**Issue No 6**

**July 2009**

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**The Bolaven Plateau from Attapeu**

We are very grateful to the Minister of Health, His Excellency Dr Ponmek Dalaloy, the Director of the Curative Department, Prof Sommone Phounsavath and the Director of Mahosot Hospital, Professor Chanpheng Thammavong for facilitating and supporting this project. With very many thanks to all the staff of Mahosot Hospital, those who checked this newsletter, CMPE, NCLE, UHS, MORU, WPRO, WHO-Vientiane, IFMT, Health Frontiers, Luang Nam Tha Hospital, SFE, Universities of Oxford & Marseille, Jichi Medical University, Hôpital Paul Brousse, LaRREC and the Mekong River Commission.

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This newsletter summarizes the results of the collaborative infectious disease & tropical medicine research carried out by the Wellcome Trust-Mahosot Hospital-Oxford Tropical Medicine Research Collaboration (or LOMWRU=Lao Oxford Mahosot Wellcome Research Unit for short). A Lao language version is available at the website below. Please let us know any suggestions for improvement or comments. Please distribute to all who are interested. A pdf file is available from the e-mail addresses below. In the next issue we hope to include full details of the CNS Infection study, problems in the treatment of typhus in pregnancy, new interesting information on infantile beriberi, the frequency of antibiotic activity in the urine of Lao patients, the geographical distribution of past scrub typhus and murine typhus infections in Vientiane, an evaluation of the tourniquet test in adults with suspected dengue, the clinical features of melioidosis in Laos and the causes of encephalitis. This newsletter is available from [http://www.tropmedres.ac/study-sites/asia/laos/links-and-publications.html](http://www.tropmedres.ac/study-sites/asia/laos/links-and-publications.html).

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In 1954 a new bacterium, *Rickettsia sennetsu*, was described in patients on the Japanese island of Kyushu. This was the first documented cause of a disease resembling infectious mononucleosis. Patients had fever, weakness, anorexia, generalised lymphadenopathy, hepatosplenomegaly and peripheral blood mononucleosis with atypical lymphocytes. The incubation period was ~14 days and no fatalities were reported. The causative agent was isolated from patients’ peripheral blood, lymph nodes and bone marrow and subsequently renamed *Ehrlichia sennetsu* and, most recently, *Neorickettsia sennetsu*. *Neorickettsia* are obligate intracellular bacteria, but unlike *Rickettsia*, grow in host-membrane lined cytoplasmic vacuoles. *Sennetsu* is Japanese for infectious mononucleosis. The other described *Neorickettsia* species parasitize mammals such as dogs and horses. The Japanese researchers suspected that patients contracted sennetsu by eating raw fish. In support of this hypothesis they found that of 96 people fed raw grey mullet (*Mugil cephalus*), 5% developed a sennetsu-like illness and *N. sennetsu* was cultured from the blood of 4 of those who became ill. Further investigation identified a closely related *Neorickettsia*, the ‘SF agent’, in *Stellantchasmus falcatus* fluke metacercaria in grey mullett, but ‘SF’ is not known to cause human disease. It remained uncertain whether sennetsu was contracted from fish as *N. sennetsu* was not found in Japanese fish. Since the 1980s sennetsu has apparently disappeared as a public health problem in Kyushu – perhaps because the consumption of raw grey mullet has declined with changes in food habits. In Laos raw fish and fermented fish paste are commonly eaten we speculated that *N. sennetsu* could be a local cause of undifferentiated fever and investigated this with the University of Marseille, France. We looked for evidence of past exposure to sennetsu in Lao people by looking for anti-sennetsu IgG antibodies in Lao blood donors and patients with fever, hepatitis or jaundice (n=1,132). They had a high prevalence (17%) of such antibodies compared to 4% and 0% from febrile patients (n=848) in Thailand and Malaysia, respectively. This suggests that many Lao have been exposed to sennetsu - however whether these people developed disease is not known.

**Sennetsu in a Lao fish**

In collaboration with LaRREC and the Mekong River Commission, PCR for *N. sennetsu* was performed on a large variety of Lao fish from the Mekong River valley. Intestines from one individual each of *Channa gachua* and *Trichopsis vittata* were positive by PCR targeting the 16S rRNA gene. The percentage homologies with *N. sennetsu* of the two sequences obtained from these fish were 92.2 and 95.8%, respectively. This suggests that other species of *Neorickettsia*, but not *N. sennetsu* were present. One fresh gill tissue sample from *Anabas testudineus* (the climbing perch ‘pa kheng’) had a 16S rRNA sequence with 99.1% homology with *N. sennetsu*, confirmed by the two specific real-time PCRs for gltA and Omp85 genes. Sequences from these PCR products were 100% identical to that of *N. sennetsu*. *A. testudineus* can breath both in air and water and they are said to be able to climb trees! They are important food fish and occur throughout the Mekong River Basin, Sri Lanka to China, Indonesia, and the Philippines. Therefore, there is good evidence that sennetsu is in Lao fish, but it is uncertain whether it is in the flesh or in trematodes in the fish.
A Lao patient with sennetsu

A 17 year old unemployed Lao man, from a rice farming family in Vientiane Province, presented at Mahosot Hospital in April 2003 with fever, headache and weakness for 2 weeks. On admission, he was pale, jaundiced and febrile, with hepatosplenomegaly and palpable inguinal and cervical lymph nodes. He was anemic (hematocrit 19%) with a normal peripheral white cell count (6.7 x 10^9/L) except relative and absolute lymphocytosis (neutrophils 38%, lymphocytes 60%, monocytes 2%; local upper reference range for lymphocytes <45% and <3.6 x 10^9/L, respectively) and a normal platelet count (180,000/mm^3) and a negative malaria blood smear. Atypical lymphocytes were not searched for. He had an elevated ESR (75mm/1st hour) but negative hemocultures after 7 days of incubation. Serum direct bilirubin 20.5 µmol/L (reference range <4.3 µmol/L) and total bilirubin 32.0 µmol/L (<14.5 µmol/L), AST 86 IU/L (<37 IU/L) and ALT 43 IU/L (<40 IU/L) were raised. Abdominal ultrasound demonstrated homogenous splenomegaly. In 2000 he had been admitted to hospital with splenomegaly but did not receive blood transfusions. He had not visited any other provinces in Laos or been abroad. On the 2nd day he was empirically treated with ofloxacin (200 mg twice a day) for 7 days for suspected typhoid, was afebrile on the 3rd day and was discharged home on the 5th day.

When reviewed in December 2006 he was well but had splenomegaly without palpable hepatomegaly or lymphadenopathy. His red cells were not G6PD deficient by the methaemoglobin reduction test, but haemoglobin cellulose acetate electrophoresis demonstrated the presence of haemoglobin H. Although sennetsu causes splenomegaly and may cause jaundice, these signs may have been a consequence of Hb H disease rather than neorickettsiosis. He remembered eating boiled snakehead fish (Channa species), including their intestines, in the weeks before becoming ill. He ate raw homemade ‘padek’ or raw fermented fish paste 2-3 times a week for many years. The fish used to make the padek, from the Nam Ngum lake, were ‘pa khao’, ‘pa kadert’, ‘pa kheng’, ‘pa khor’, ‘pa xieum’ and ‘pa khagneng’ which are probably Puntius sp., Trichogaster trichopterus, Anabas testudineus, Channa striata, Ompok bimaculatus and Mystus multiradiatus, respectively. PCR targeting the 16S rRNA gene of Anaplasmataceae from the admission buffy coat was positive and gave a sequence with 100% homology with N. sennetsu, confirmed by the two specific real-time PCR with 100% homology. None of the remaining available 90 buffy coats from Lao patients were PCR positive for N. sennetsu. Therefore, there is evidence for sennetsu in at least one Lao patient. However, whether sennetsu is an important cause of disease in Laos remains to be determined. If it is, sennetsu may be important elsewhere in the region, given the frequent consumption of raw fish in east Asia.

In cell culture N. sennetsu are resistant to penicillin, erythromycin, co-trimoxazole, and surprisingly chloramphenicol, but sensitive to doxycycline and ciprofloxacin. It therefore can probably be treated with relatively inexpensive and readily available antibiotics.

Climbing perch, ‘pa kheng’ or Anabas testudineus in a Vientiane market
Malaria has historically been a major public health problem in Laos - the majority is *Plasmodium falciparum* malaria with perhaps ≤10% being *P. vivax*. Research began in the 1960s during the construction of the Nam Ngum dam. Falciparum malaria resistance to first line therapy (chloroquine) and second line therapy (sulphadoxine-pyrimethamine or SP) spread throughout adjoining countries and by 2000 their national policies had changed to artemisinin-combination therapy (ACT). However, although anecdotal reports suggested that chloroquine was still efficacious there had been no research to check this. The first project of the Collaboration was therefore to examine the efficacy of chloroquine and SP in Lao patients, in conjunction with the Centre for Malariology, Parasitology & Entomology (CMPE). Other groups, from France, Germany & Japan also worked with CMPE on similar projects in diverse parts of Laos. The key technique for determining antimalarial sensitivity is to perform clinical trials of the drug in question at the correct doses in consenting patients with uncomplicated falciparum malaria. The time to disappearance of the parasites in the patients’ blood is measured and patients are followed up for some weeks to check whether they develop new infections or whether the original infection recurs. These can usually be distinguished by a PCR technique to examine whether the parasites have the same genotype or are different. The length of follow up depends on the half-life of the antimalarial medicine in the patients blood and in the past this has often been too short. The first study was conducted in Muang Fueng, Vientiane Province, in 2000. As malaria declined in Vientiane Province the study site moved to Phalanxay District in Savannakhet Province in 2001. In 2006 the study site changed to Xepon District, also in Savanakhet Province.
In the first small trial (n=29) at Mueng Fueng with 42 day follow up, 78% of patients failed chloroquine therapy and 38% failed SP - they had recrudescence infections with *P. falciparum*. Of great concern the majority (64%) of chloroquine treatment failures were high grade early failures (RII & RIII) (Mayxay *et al.* 2003a). The team then moved to Phalanxay where they conducted a similar but larger trial (n=100) again with 42 day follow up. 36% patients failed chloroquine and 18% failed SP, with 43% of the chloroquine failures being high grade. Failure rates among children were 4.9 x higher than failure rates in adults, presumably because of anti-malarial immunity acquired during childhood protects adults. A higher proportion of patients treated with SP than chloroquine had gametocytes in their blood - if these gametocytes were viable this suggests that malaria transmission would be higher after SP therapy (Mayxay *et al.* 2003b).

These trials also illustrate the importance of long follow up. In the Phalanxay study if the conventional 14 day follow up had been used, 94% of low grade treatment failures would have been missed and the efficacy of both medicines overestimated. Trials in Vang Vieng, Sekong and Attapeu gave similar results.

The evidence from these studies was used by the Lao Government to decide to change first line treatment of falciparum malaria to artemisinin derivative combination therapy (ACT) in 2005.
With clear evidence that chloroquine and SP had failed as treatments for falciparum malaria in Laos as they had elsewhere, more information was needed on which ACT would be most appropriate. The team therefore conducted a clinical trial in Phalanxay (n=330) comparing chloroquine plus SP, artesunate plus mefloquine and artemether coformulated with lumefantrine (Mayxay et al. 2004). Again, patients were followed for 42 days. The cure rates were 93% for chloroquine+SP, 97% for artemether-lumefantrine and 100% for artesunate+mefloquine. The cure rate for artesunate+mefloquine was significantly greater than for chloroquine+SP. The mean parasite clearance time was significantly longer in patients treated with chloroquine+SP (2.9 days) in comparison to artesunate+mefloquine (2.1 days) and artemether-lumefantrine (2.1 days). Mean fever clearance times were also significantly longer in the chloroquine+SP group than in the two ACT groups. The percentage of patients with gametocytes detected at any time point after treatment was much higher in the chloroquine+SP group (26%) than in either of the ACT groups (~5%). The proportion of patients with ≥1 recorded potential side effect was higher in the group receiving artesunate+mefloquine (52%) than in those receiving artemether-lumefantrine (27%) or chloroquine+SP (44%). These results suggested that either artesunate+mefloquine or artemether-lumefantrine would be efficacious therapies for uncomplicated falciparum malaria in Laos, but artesunate+mefloquine was associated with more frequent side effects. A similar trial in Luang Nam Tha also showed high cure rates with these 2 ACTs. In 2005 CMPE and the Government of Laos recommended changing the national policy to artemether-lumefantrine.

Could dihydroartemisinin-piperaquine be an appropriate therapy for Laos in the future?

However, one potential problem with artemether-lumefantrine is that some research has suggested that fat is required for the good absorption of lumefantrine in the intestine. Lao people often prefer not to take fatty food when they have a fever and low fat intake was documented in the above trial. The good efficacy of artemether-lumefantrine, despite low fat intake, may be explained by greater parasite susceptibility and perhaps higher levels of background immunity. However, if artemisinin resistance appears in Laos or immunity declines, as it will with lower incidence, the efficacy of artemether-lumefantrine may decline. Another ACT, dihydroartemisinin-piperaquine (Artekin), which was also developed in China is a potentially less expensive coformulated ACT with less dietary interaction. The team conducted a clinical trial of Artekin versus artesunate–mefloquine in uncomplicated falciparum malaria at Phalanxay (Mayxay et al. 2006) showing excellent 42-day cure rates for the two groups (99% and 100%, respectively) (n=220). Artekin may represent an alternative ACT for the future in Laos.
A 53-year-old Tai Dam farmer was admitted in 2008, at the Provincial Hospital of Luang Namtha, with 2 days of fever, chills, severe headaches, nausea and vomiting, and a cough productive of white sputum. On admission he had crepitations audible at the left lung base. With suspected community-acquired pneumonia he was started on oral amoxicillin. Malaria smear and HRP-2 rapid test for *Plasmodium falciparum* malaria were negative. The patient developed upper abdominal and lower back pain and increasing dyspnoea and was pale and jaundiced with a respiratory rate of 42/min, temperature 39°C, blood pressure 90/60 mmHg, heart rate irregular 120/min, bibasal inspiratory crepitations, tender hepatomegaly with axillary and inguinal lymphadenopathy. As community-acquired septicaemia or leptospirosis were suspected, therapy was changed to ceftriaxone. However, he deteriorated, became apnoeic and could not be resuscitated. Blood cultures, sent to Vientiane, grew *Chromobacterium violaceum*. The organism was sensitive to gentamicin, chloramphenicol, ofloxacin, co-trimoxazole but resistant to ampicillin, ceftriaxone, and ceftazidime by disc diffusion testing. *Chromobacterium violaceum* is a Gram negative facultative anaerobic bacillus, found in soil and stagnant water. It usually has a violet pigmented appearance on agar. Since its discovery as a human pathogen in 1927 in Thailand and Vietnam, it is important to bear in mind as empirical therapy for septicaemia (eg ampicillin, ceftriaxone and specific treatment for melioidosis (ceftazidime), which it resembles clinically, will not be effective.

Malaysia only ~150 human cases have been reported worldwide, mostly from tropical and subtropical areas. It has been described in Thailand and Vietnam. The current best treatment for septicaemic melioidosis is a minimum of 10 days intravenous ceftazidime.

### Dosage adjustment for ceftazidime in patients with renal impairment

The current best treatment for septicaemic melioidosis is a minimum of 10 days intravenous ceftazidime. As patients with melioidosis often have impaired renal function we give here a suggested dose regime for different ranges of serum creatinine for adults of about 50 kg.

<table>
<thead>
<tr>
<th>Serum creatinine μmol/L</th>
<th>Ceftazidime Dose</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Less than 176</td>
<td>Less than 2</td>
<td>2g every 8 hours</td>
</tr>
<tr>
<td>176 - 352</td>
<td>2-4</td>
<td>1g every 8 hours</td>
</tr>
<tr>
<td>353 - 528</td>
<td>4-6</td>
<td>1g every 12 hours</td>
</tr>
<tr>
<td>Greater than 528</td>
<td>&gt;6</td>
<td>1g every 24 hours</td>
</tr>
</tbody>
</table>

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Noma

Noma, or cancrum oris, is a necrotizing ulcerative stomatitis that destroys the mouth and face. It usually starts in early childhood and is associated with severe poverty, malnutrition and infections. Acute noma occurs predominantly in malnourished children aged 1-4 years living in the remotest and poorest parts of the world. It is most frequently described from sub-Saharan Africa but is under reported. There have been very few reports from Asia. There has been very little research and the pathophysiology is not understood. Patients with acute noma present with bad breath, fever, malnutrition, and gingival ulceration. If recognized early, the patient can be treated successfully with antibiotics, oral hygiene and nutritional measures. Untreated, the ulcer rapidly progresses and, within days, soft tissue, bone, and teeth are lost, leaving a hole in the face. It has a 80-90% untreated mortality rate. Most children die, without a diagnosis or reporting. The survivors are left severely disfigured, functionally impaired, and unable to chew and speak. Their appearance, difficulties with speech and eating result in isolation and psychological scarring. Reconstructive surgery is very difficult, expensive and rarely available to noma survivors.

Since 2002 a series of 12 patients with noma from six of the seventeen Lao provinces have been identified (Srour et al. 2008). Subsequent enquiries recorded a total of 28 noma survivors in Lao. Patients with noma were not actively searched for and it is likely that many more would be discovered if looked for in remote areas.

The age at onset was 3 years in 25%, 4 in 8%, 5 in 8%, 6 in 33%, 7 in 17% and 8 in 8%. The median (range) year of onset was 1989 (1981-2003). This could mean that the incidence of noma has declined with increased development, but this may not be the case as children with the sequelae of noma are often hidden and the non-systematic identification of patients is unlikely to be representative of the actual age distribution. More detailed research is needed on the incidence of this disease in rural Asia. An expressive name in Lao for noma ‘disease of mouth rotting’ has been suggested. Noma is an ominous stigma of severe poverty and the description of this disease emphasizes the importance of poverty reduction and nutritional improvement in Lao development.

Lao PDR

Provinces with noma recorded. Orange presents those recorded by Srour et al. (2007) and yellow subsequent patients.

A Lao child with acute noma. His parents carried him 12 hours to the nearest hospital.

Photo courtesy of Bryan Watt.
### Leptospiral Culture

Leptospirosis is a very difficult disease to diagnose clinically and using laboratory techniques. In Laos it is often a challenge to distinguish leptospirosis from scrub typhus, murine typhus, dengue and septicaemia clinically. Very few Lao patients with leptospirosis at Mahosot Hospital have Weil’s disease (leptospirosis with jaundice and renal failure) - most have undifferentiated fever.

Diagnostic techniques are also difficult. As the antibody response is slow, antibody tests have low sensitivity in the first week of illness, which is when patients present and need treatment! The diagnosis can be made serologically using comparison of admission and convalescent sera using Microscopic Agglutination Tests (MAT) but this requires a ‘zoo’ of live leptospires of different types and the result is not available in time to help the individual patient.

So far PCR tests have not been very successful and culture of leptospires is, like for rickettsia, a long process. It needs Ellinghausen, McCullough, Johnson, and Harris (EMJH) medium and dark-field microscopy.

We have been trying to grow leptospires from the blood clot left over from taking serum from patients. In the last 2 years we have grown leptospires from 35 patients and we saw a peak of isolation after the floods in Vientiane of August 2008. When we grow leptospires we try to find the patient to check that they have had appropriate therapy (eg a penicillin, a tetracycline or ceftriaxone). We will compare the results from culture with these from serology and PCR and type the leptospires (using Multilocus Sequence Typing) with MORU.

Photograph by dark field microscopy of leptosiral culture. They are motile and spiral about 10-20 micrometers long.

### Hepatitis A - more evidence

In the last newsletter we reported the results of Lao Hepatitis & Jaundice study. We pointed out that anti-HAV IgM was positive in 36% of Lao patients but that 35% of those with anti-HAV IgM detected were \( \geq 40 \) years. This was surprising as HAV is normally acquired when younger. The detection of anti-HAV IgM is conventionally considered the gold standard for the diagnosis of acute HAV and is thought to usually persist for 3-6 months after disease onset. This result was investigated further by the National Reference Centre for HAV, Hôpital Paul Brousse, Villejuif, France. IgG anti-HAV avidity assays were performed on sera from 51 HAV IgM positive patients; 42 (82%) patients had IgG anti-HAV avidity \( > 70\% \), suggesting polyclonal activation and not acute HAV. Of 10 patients with low IgG avidity 8 were HAV PCR positive, whilst none of those with avidity index \( > 70\% \) (42 patients) were HAV PCR positive. It is therefore likely that the high HAV IgM frequency resulted from nonspecific polyclonal activation of memory cells and NOT acute HAV. This problem has only been demonstrated before in France but has important implications for HAV diagnosis everywhere. It is likely that false positive HAV IgM results are more common than is realised.
**Rickettsial Culture**

Since September last year we have been trying to culture rickettsia from all patients with positive rapid test results for scrub typhus and murine typhus. As these organisms are Category 3 pathogens the culture on L929 and Vero cells is carried out in the Biosafety Level 3 laboratory in the Infectious Disease Centre. When a positive culture is suspected the organism is identified using immunofluorescence and PCR assays. We try to find the patient to check that they have had appropriate therapy. To date we have grown *Orientia tsutsugamushi* (the cause of scrub typhus) from 30 patients and *Rickettsia typhi* (the cause of murine typhus) from 10 patients. It takes 2-4 weeks for a culture to become positive so this test is not helpful for deciding treatment for individual patients. However, this work will allow us to determine the antibiotic sensitivity of the organisms and examine their genetic diversity. Doxycycline resistant scrub typhus has been reported from northern Thailand and it will be important to check the susceptibility of Lao *O. tsutsugamushi*. There is only serological evidence for Spotted Fever Group (SFG) of *Rickettsia* in Laos - we hope to obtain better evidence of what species are clinical problems in Laos. Confusingly most SFG in Laos seem to cause a ‘spotless’ disease!

**Treatment of meningitis**

On the following page we give provisional guidelines for the treatment of meningitis. We would like to emphasise:

* if bacterial meningitis is suspected please give empirical antibiotic therapy before LP or immediately after LP, depending on the clinical situation.

* if *R. typhi* or *O. tsutsugamushi* are suspected, suggest to add (not replace) doxycycline to an antibiotic that will counter conventional bacterial meningitis.

* ceftriaxone should also be active against leptospiral meningitis

* we have not found *Listeria monocytogenes* yet, but that does not necessarily mean that it is not a cause of meningitis in Vientiane. This organism is resistant to third-generation cephalosporins.

* we have found *S. pneumoniae* resistant to penicillin - therefore we do not recommend penicillin for empirical therapy - suggest a 3rd generation cephalosporin such as ceftriaxone.

* There is evidence that steroids may be beneficial in *M. tuberculosis* meningitis but the evidence for benefit in those with *Haemophilus influenzae* b or *S. pneumoniae* meningitis in SE Asia remains unclear.
Antibiotic Recommendations

**Ceftriaxone** *(a third generation cephalosporin is the preferred empirical therapy)*

80-100 mg/kg/day IV divided into 2 daily doses

[Usual dose for 50 kg adult ~2 g IV every 12h]

**OR** in neonates

**Cefotaxime**

Aged 0-7 days 100–150 mg/kg/day IV (dose interval every 8–12h) or aged 8-28 days 150–200 mg/kg/day IV (dose interval every 6–8h)

**AND IF** *Listeria* is suspected (usually in infants < 1 month old)

**Ampicillin**

<table>
<thead>
<tr>
<th>Age</th>
<th>0-7 days</th>
<th>8-28 days</th>
<th>&lt;15 years</th>
<th>&gt;15 years</th>
</tr>
</thead>
<tbody>
<tr>
<td>Daily dose IV</td>
<td>150mg/kg/day</td>
<td>200mg/kg/day</td>
<td>300mg/kg/day</td>
<td>12 g/day</td>
</tr>
<tr>
<td>Dose Interval</td>
<td>every 8 h</td>
<td>every 6-8 h</td>
<td>every 6 h</td>
<td>every 4h</td>
</tr>
</tbody>
</table>

If ceftriaxone (or other 3rd generation cephalosporin) is not available

**Chloramphenicol**

<table>
<thead>
<tr>
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<th>0-7 days</th>
<th>8-28 days</th>
<th>&lt;15 years</th>
<th>&gt;15 years</th>
</tr>
</thead>
<tbody>
<tr>
<td>Daily dose IV</td>
<td>25mg/kg/day</td>
<td>50mg/kg/day</td>
<td>75-100mg/kg/day</td>
<td>4-6g/day</td>
</tr>
<tr>
<td>Dose Interval</td>
<td>24h</td>
<td>12-24h</td>
<td>6h</td>
<td>6h</td>
</tr>
</tbody>
</table>

[Usual dose for 50kg adult chloramphenicol 1.5g six hourly]

**AND**

**Ampicillin**

<table>
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<td>every 6 h</td>
<td>every 4h</td>
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</table>

If suspect rickettsial disease add in: oral **Doxycycline** 4mg/kg stat followed by 2mg/kg every 12 hours for 1 week. In adults this will normally be 200mg stat followed by 100mg every 12 hours for 1 week. The risks to children <8 years of doxycycline therapy are likely to be less than the risks of not giving doxycycline. In pregnant women, oral azithromycin for 7 days is probably the best option and may also be efficacious in children.

Please always send blood cultures to the laboratory when you do a lumbar puncture. We then have a greater chance of finding the causative organism.

Tests for IgM antibodies against pathogens may represent recent infections before the presenting illness!

Therefore, please always treat these results with some suspicion. Until we have better DNA or antigen detection tests for typhus and rickettsia please assume that patients may have ‘conventional’ bacterial meningitis and treat with a 3rd generation cephalosporin.

There is still no specific therapy for Japanese B viral encephalitis or dengue encephalitis.

The recommendations in these pages are derived from the latest drug resistance data from the pathogens isolated in Mahosot Hospital. Please note that these are only guidelines and the condition of the individual patient, renal and liver function, drug contraindications and allergies need to be considered before prescribing. Please check these doses before prescribing. For children please consult paediatric guidelines. These guidelines are provisional, adapted from Tunkel et al. (2004, CID 39, 1267-84), and will be revised when we have the analyzed the results from the CNS Infection study.

Guidelines : Treatment of Meningitis
Guidelines : Treatment of Severe Melioidosis

The recommendations in these pages are derived from the latest drug resistance data from the pathogens isolated in Mahosot Hospital. Please note that these are only guidelines and the condition of the individual patient, renal and liver function, drug contraindications and allergies need to be considered before prescribing. Please check before prescribing. For children please consult paediatric guidelines.

A recent paper has advised on consensus guidelines for the dosage of amoxicillin-clavulanate in oral eradication therapy of melioidosis (Cheng et al. 2008. Am J Trop Med Hyg 78:208-9). They recommend the doses below on the basis of pharmacokinetic data and clinical experience. They suggest that oral amoxicillin-clavulanate (coamoxiclav) should be used at a dose of 20/5 mg/kg three times a day. Where coamoxiclav is available in fixed 4:1 combinations, they suggest 1000/250mg three times a day for patients <60kg. Where coamoxiclav is only available as 2:1 combinations they suggest 500 mg/250 mg three times a day daily with additional amoxicillin (500 mg three times daily). For patients > 60 kg, they use a maximum dose of 1500/375 mg three times daily. They do not recommend twice daily regimens because of inadequate clavulanate.

SEVERE SELIOIDOSIS - PARENTERAL FOLLOWED BY ORAL THERAPY

Ceftazidime IV 120 mg/kg/day in 3 divided doses for minimum of 10 days
[Usual dose for 50 kg adult ~ 2 g iv every 8 hours]

OR

Amoxicillin-clavulanate IV 150 mg/kg/day in 6 divided doses for minimum of 10 days
[Usual dose for 50 kg adult ~ 1.2 g iv every 4 hours]

THEN oral eradication treatment of:

Co-trimoxazole 10/50 mg/kg/day in 2 divided doses for 12-20 weeks
[Usual dose for 50 kg adult ~ 2 x 960 mg tablets every 12 hours]

COMBINED WITH

Doxycycline 4 mg/kg/day in 1 daily dose for 12-20 weeks
[Usual dose for 50 kg adult ~ 2 x 100 mg capsules every 24 hours]

OR, AS LONE THERAPY

Amoxicillin-clavulanate 60/15 mg/kg/day in 3 divided doses for 20 weeks
[Usual dose for 50 kg adult 2 x 500/125 mg capsules every 8 hours]
A study examined 123 episodes of recurrent melioidosis in 116 patients in Ubon Ratchathani, NE Thailand. They used a combination of typing techniques to distinguish relapse and reinfection. They reported that 92 episodes (75%) of recurrent disease were attributable to relapse and 31 episodes (25%) were attributable to reinfection (Maharjan et al. 2005. J Clin Microbiol 43:6032–6034). In a study to look at risk factors, no risk factors for reinfection were identified. However, multivariate analyses identified choice and duration of oral antimicrobial therapy as the most important determinants of relapse, followed by positive blood culture result and multifocal distribution. Patients treated with an appropriate oral antibiotic regimen for 12-16 weeks had a 90% decreased risk of relapse, compared with patients who were treated for ≤8 weeks (Limmathurotsakul et al. 2006, Clin Infect Dis 43:979-86).

**LOCALISED MELIOIDOSIS – ORAL TREATMENT**

For patients with melioidosis at ONE site WITHOUT septicaemia

Co-trimoxazole  10/50 mg/kg/day in 2 divided doses for 8 –20 weeks  
[Usual dose for 50 kg adult ~ 2 x 960 mg tablets every 12 hours]

WITH

Doxycycline  4 mg/kg/day in 1 daily dose for 8 - 20 weeks  
[Usual dose for 50 kg adult ~ 2 x 100 mg capsules every 24 hours]

OR, AS LONE THERAPY

Amoxicillin-clavulanate 60/15 mg/kg/day in 3 divided doses for 20 weeks  
[Usual dose for 50 kg adult 2 x 500/125 mg capsules every 8 hours]  
Maximum dose 1500/375 mg every 8 hours
Empirical treatment of Septicaemia

Please check paediatric guidelines for neonates and children

**COMMUNITY—ACQUIRED SEPTICAEMIA**

Gentamicin IV 5 - 7 mg/kg once a day

[Usual dose for 50 kg adult ~ 240 – 360 mg IV once a day]
[behave renal impairment for doses subsequent to the first]

**WITH EITHER**

Ampicillin IV 25 - 100mg/kg every 6 hours
[Usual dose for 50kg adult = 1 g IV every 6 hours]

**OR ALTERNATIVE, MORE EXPENSIVE, REGIMEN WITH WIDER COVERAGE**

Ceftriaxone IV or IM 50-100mg/kg every 24 hours
[Usual dose for 50kg adult = 2 - 4 g every 24 hours]

**SUGGESTIONS FOR SPECIAL SITUATIONS**

**If you suspect melioidosis, while you wait culture results:**
Use co-amoxiclav or, if you very strongly suspect melioidosis, use ceftazidime

**If the patient is an infant:**
Add cloxacillin

**If you suspect bacterial meningitis plus septicaemia:**
Ceftriaxone is recommended to be given every 12 hours rather than every 24 hours

**If you suspect Pneumococcal meningitis plus septicaemia:**
Suggest using ceftriaxone every 12 hours, rather than a penicillin
## Recent Medical Papers From Laos

**2009 - to date**


### 2008


Seilmaier M, Guggemos W. (2008) [Severe febrile illness with renal impairment after travel to Southeast Asia.]. Internist (Berl) 49(11):1374-6


2007 (since listing in last newsletter)


Rodnick JE. (2007) Laos: how a new family medicine residency was developed (with the help of Canadians). Fam Med 39:515-6


**Microbiology Tests Available at the Microbiology Laboratory, Mahosot Hospital**

The superscript number is the number of days/weeks we will try to issue the final report by.

asap = as soon as possible

Blood culture - for suspected septicaemia [blood culture set for UI-2]. \(^8\) days

Pus - for suspected bacterial infection [sterile swab]. \(^3\) days

Urine - for suspected UTI [sterile white capped tube]. \(^3\) days

Throat swab - for suspected melioidosis [sterile swab]. \(^4\) days

Stool - for suspected bacterial/amoebic GI infection [sterile white capped tube]. \(^3\) days

Pleural fluid - for suspected bacterial or *Paragonimus* lung infection [sterile white capped tube]. \(^3\) days

Ascitic fluid - for suspected bacterial ascitis [sterile white capped tube]. \(^3\) days

Pericardial fluid - for suspected bacterial pericarditis [sterile white capped tube]. \(^3\) days

Broncho-alveolar lavage - for suspected lung bacterial infection [sterile white capped tube]. \(^3\) days

STI - vaginal/urethral discharge swab - direct microscopic wet mount and Gram stain exam. \(^1\) day

Weber test - for suspected gastrointestinal tract bleeding. \(^1\) day

CSF - for suspected CNS infection or other intracranial pathology +

- sterile white capped tube x 3 & fluoride tube for CSF
- please always do blood culture (UI-2) set if CNS Infection suspected
- CSF cell count, bacterial culture, Gram stain, Indian Ink stain. \(^1\) day/LJ culture -TB. \(^6\) weeks
- dengue/JEV serology. \(^7\) days
- scrub typhus & murine typhus rapid tests. \(^1\)-\(^2\) days
- bacterial PCR for *S. pneumoniae, H. influenzae* b and *N. meningitidis*. asap
- viral PCR for CMV, Enterovirus, HSV, VZ, JEV, dengue, mumps, measles, influenza, West Nile, tick-borne encephalitis and Nipah viruses. asap
- viral culture. \(^3\) weeks

Leptospiral culture - automatically performed on all white capped tubes in UI-2 set. \(^4\) weeks

Rickettsial serology - scrub typhus & murine typhus rapid tests same day (and subsequent IFA) for suspected typhus. Automatically performed on all who have LP

Rickettsial culture - we request this for all patients who are rapid test positive for scrub or murine typhus or if the patient is suspected as having Spotted Fever Group infection [EDTA pink capped tube]. \(^8\) weeks

Dengue/JEV IgM/IgG/NS1 ELISAs - we will do automatically for all patients with suspected CNS Infection and during the ‘dengue season’ will run samples every Wednesday. \(^7\) days

+ if patient needs a CT brain before LP but cannot afford cost please inform Microbiology Laboratory

We will phone the ward if we grow a clinically significant organism but cannot issue a full report yet

Please always label specimens and form with patient’s name, age, ward, Hospital Number and requesting doctor’s name (not just the signature !)