

Mahosot Microbiology Review

Issue No. 7

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Inside this issue:

| | |
|--|-------|
| Medical literature, Actinomycosis | 2 |
| Infantile beriberi | 3 |
| <i>Burkholderia pseudomallei</i> , soil & water | 4 |
| Pneumococci, Japanese encephalitis virus | 5 |
| Typhus exposure in Vientiane | 6 |
| Urinary antibiotics ! Beware the wild banana ! | 7 |
| ESBL, Myiasis | 8 |
| Diagnosis of typhoid, Has artemisinin resistance arrived in Laos ? | 9 |
| Typhus, fluoroquinolones and treatment | 10 |
| Treatment of typhus and meningitis | 11-12 |
| Treatment of melioidosis & typhoid | 13-14 |
| Treatment of septicaemia | 15 |
| Lao Medical Literature, The Tail End | 16-28 |
| Tests at the Microbiology Laboratory | 29 |



The extended Microbiology Laboratory at Mahosot Hospital looking SE

We are very grateful to the Minister of Health, His Excellency Prof. Dr. Eksavang Vongvichit, the Director of the Curative Department, Assoc. Prof. Dr. Chanphomma Vongsamphanh and the Director of Mahosot Hospital, Assoc. Prof. Dr. Bounthaphany Bounexouai 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, IFMT, IRD, MORU, Fondation Merieux, Institut Pasteur, WPRO, WHO-Vientiane, Health Frontiers, Luang Nam Tha, Salavan & Phonsavan Hospitals, SFE, HPA-UK, Natural History Museum-London, LSHTM, MRC Human Nutrition, Royal Brompton Hospital-London and the Universities of Oxford, Aix-Marseille and Paris Oest.

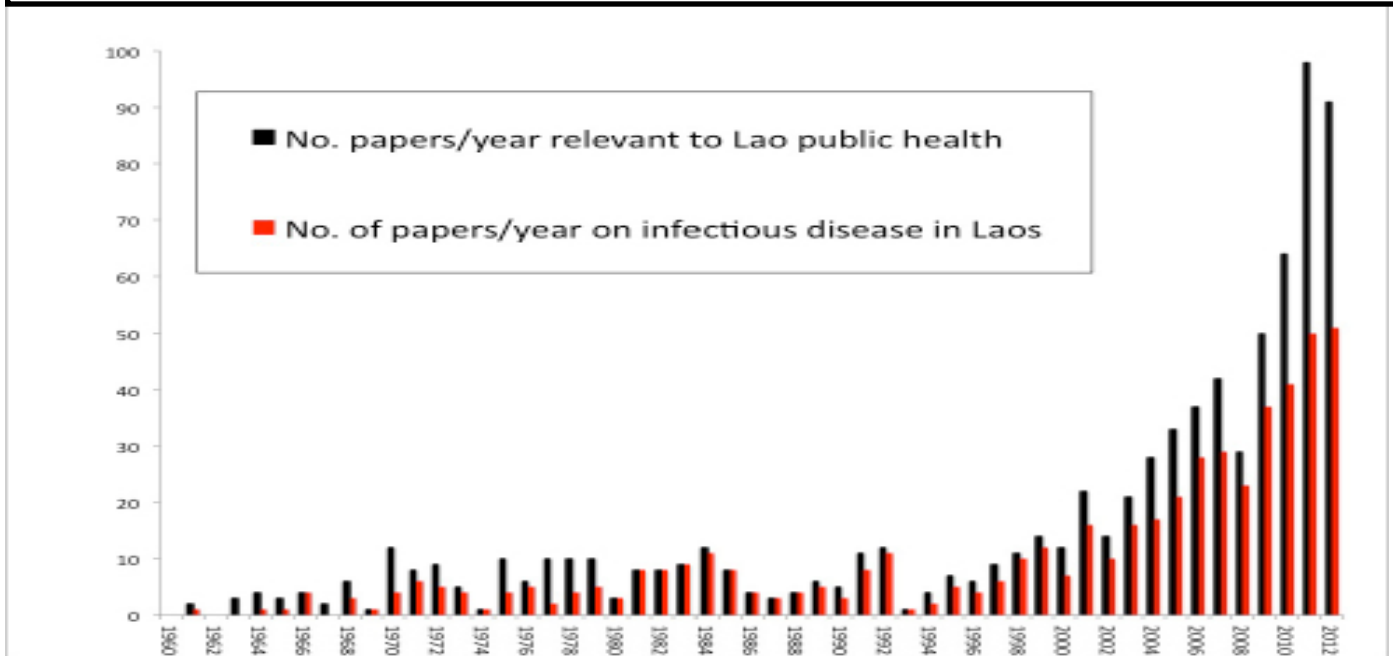
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This newsletter summarizes the results of the collaborative infectious disease & tropical medicine research carried out by the Lao-Oxford-Mahosot Hospital-Wellcome Trust Research Unit. A Lao language version is available at the website below. Please let us know any suggestions for improvement or comment and distribute to all who are interested. A pdf file is available from the e-mail addresses above. Due to delays in publication there are many new Lao relevant papers included (pages 16-28) so that we can catch up with these lists. We hope to publish MMR-8 in February 2014. This newsletter is available from <http://www.tropmedres.ac/study-sites/asia/laos/links-and-publications.html>.

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Medical literature relevant to Laos

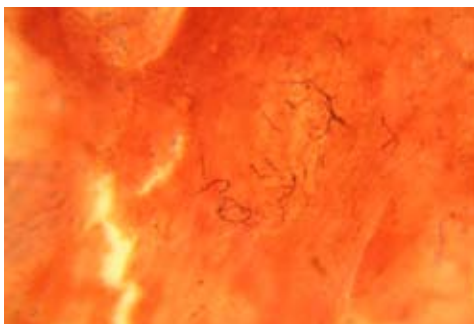
A dramatic increase in the volume of published scientific literature relevant to public health in Laos continues



Actinomycosis

Mycetomas, commonly known as Madura foot, are chronic, localized, slowly progressing infections of the cutaneous and subcutaneous tissues caused either by true fungi (eumycetoma) or by aerobic actinomycetes (actinomycetoma). They are acquired by traumatic implantation, especially in rural tropical agriculture communities. Although well recognized elsewhere in Asia, it has not been reported from Laos before (see Rattanavong *et al.* 2012).

A 30 year-old school teacher and rice farmer from Xieng Khouang was admitted to Mahosot Hospital with a massive growth on her left foot, without a history of trauma. The swelling had progressed slowly but painlessly over 5 years with multiple draining sinuses. Ten days before admission the foot had increased considerably in size, preventing her from walking. Gram stain and bacterial culture of tissue biopsies revealed a branching filamentous Gram-positive bacterium that was subsequently identified as *Actinomadura madurae* by 16S-rRNA sequencing. Foot X-ray demonstrated extensive osteolysis of the left metatarsal bones. She was treated with long-term co-trimoxazole and multiple 3-week cycles of amikacin with a good therapeutic response. As ~78% of Lao people work in agriculture and there are, as elsewhere in the rural tropics, few microbiology laboratories, it is likely that the condition is under-diagnosed.



Left - Gram stain of smear from left foot tissue biopsy revealed branching, filamentous Gram positive bacteria.

Right - Left foot of patient before treatment



Infantile beriberi

Infantile beriberi, or clinical thiamin (vitamin B₁) deficiency in infants, is a forgotten disease in Asia. In the late 19th century with the advent of mechanical rice milling, which removed rice husk containing thiamin, beriberi became a major public health problem in Asia, responsible for considerable mortality. Children aged ~ 2-3 months present in cardiac failure but usually rapidly improve if given thiamin injections. It remains relatively common in Vientiane, with 50-90 infants admitted with a clinical diagnosis of beriberi/year at Mahosot Hospital, probably resulting from prolonged intra- and post-partum food avoidance behaviours. A case control study [Soukaloun *et al.* 2003] suggested that, compared with control mothers, mothers of infants with beriberi had significantly less diet diversity, soaked glutinous rice significantly longer or were more likely to pour off excess water from non-glutinous rice, had fewer years of schooling, were more likely to report that income was inadequate for basic needs, were more likely to perform hard physical labour and to be married to farmers.

There has been very little recent research on the best diagnostic techniques to confirm the diagnosis. A case controlled study of 47 infants with beriberi and age-matched afebrile and febrile controls in Vientiane (see Soukaloun *et al.* 2011) addressed this. Contrary to the situation in adults, basal erythrocyte transketolase activity (ETK) was a better biochemical marker of infantile beriberi than the activation coefficient. Plasma troponin T may be a useful indicator of infantile beriberi in babies at risk. This holds promise as there are now rapid diagnostic tests for troponin.

Clinical disease may be the tip of an iceberg with subclinical thiamin deficiency also contributing to illness. To investigate this 778 sick infants were recruited during one year at Mahosot Hospital, without clinical evidence of beriberi, and ETK assays performed - 13.9 % of infants had basal ETK levels suggesting biochemical thiamin deficiency (see Khounnorath *et al.* 2011). Mortality was 5.5% but, among infants >2 months old, mortality was higher in those with low basal ETK (3/48, 6.3%) than in those with normal basal ETK (P=0.048, relative risk=9.06 (95%CI 0.97 - 85.1)). However, caution is required as the mortality difference is based on only 4 deaths among 197 infants aged >2 months with basal ETK data with borderline relative risk and significance. Clinically unapparent thiamin deficiency may contribute to mortality and a low clinical threshold for providing thiamin to sick infants is needed. Only a clinical trial will answer which groups of infants should receive supplementation. For sick infants thiamin should be given parenterally to increase tissue levels rapidly. There is evidence that gastrointestinal absorption of thiamin is saturated at doses of >5 mg, suggesting that oral doses above this give limited, if any, benefit.



'Hua wan' from Pakse - the root inside the bamboo container is used to make tea for the mother to drink to ward off harm when she does not properly observe post-partum food avoidance behaviour.

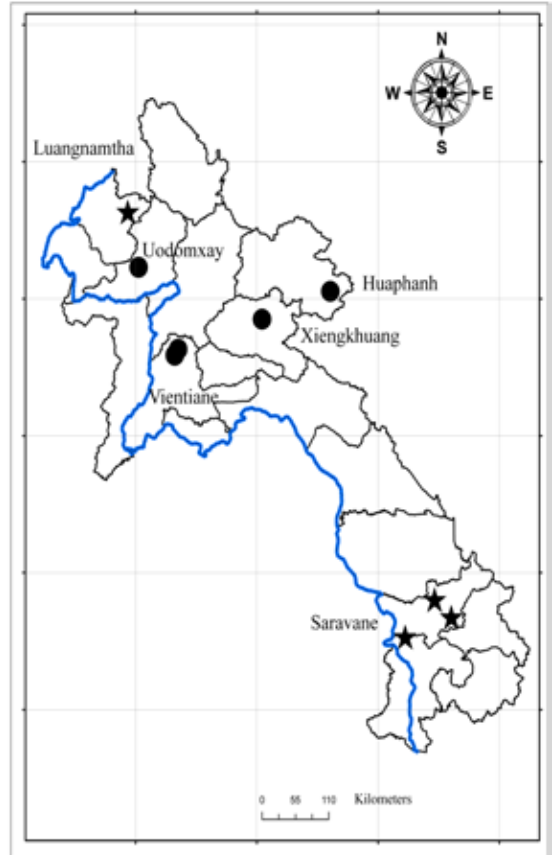
Burkholderia pseudomallei, water and soil

Melioidosis (caused by *Burkholderia pseudomallei*) is an important cause of sepsis in SE Asia and N Australia, especially in Thailand and Laos. We still do not understand the spatial distribution of the pathogen in soil and water and there has been relatively little work to understand this and what chemical, physical or biological aspects of the environment are important. In Laos, *B. pseudomallei* is a significant cause of sepsis around Vientiane capital and has been isolated in soil around the city in the Mekong River valley. We see patients who appear to have been infected elsewhere in the Mekong Valley but very few who we are sure have lived all their lives in the highlands. Two studies have tried to shed light on *B. pseudomallei* in the environment.

First, we explored whether *B. pseudomallei* occurs in Lao soil distant from the Mekong River, drawing three axes across north-west, northeast and southern Laos to create 9 sampling areas in 6 provinces (see Rattanavong *et al.* 2011). Within each sampling area a rice field was selected at random and holes, in a grid of 100 sampling points each 5m apart, dug. Soil was obtained from a depth of 30 cm and cultured for *B. pseudomallei*. Four of 9 sites (44%) were culture positive. The highest isolation frequency was in east Saravane, where 94% of soil samples were *B. pseudomallei* positive with a very high (the world record) geometric mean (range) concentration of ~464 (25-10,850) CFU/g soil. At Luang namtha, only one sample (1%) was culture positive. Therefore, *B. pseudomallei* occurs in Lao soils beyond the immediate vicinity of the Mekong River in southern Laos. Health workers in NE and NW Laos may be able to put melioidosis lower on their differential diagnosis than in southern Laos.

Second, we used Moore's swabs, made of gauze (see Vongphayloth *et al.* 2012), to sample paddy field, lake, river, borehole and storage tank water. Thirty-six and six percent of water samples collected around East and West Saravane, respectively, were swab culture positive. Low pH and high turbidity were independently associated with culture of *B. pseudomallei*. Most positive water samples were from the Sedone River, downstream of the East Saravane site. Could river *B. pseudomallei* culture provide an index of *B. pseudomallei* soil density in the watershed?

This work suffers from uncertainty as to whether *B. pseudomallei* can be present and viable in soil/water but is not culturable by current techniques. Comparisons of molecular assays with culture are needed. Culture is extremely labour intensive and in beeastern Lao the abundant UXO means that digging sites have to be checked first for ordnance.



Top - The distribution of *B. pseudomallei* at 9 sampling sites in Laos, 2009; star = culture positive and circle = negative. The blue line represents the course of the Mekong River.

Bottom - A Moore's gauze swab being lowered into a small well, retaining any *B. pseudomallei* present, in Salavane.



Pneumococci and vaccination policy

Invasive pneumococcal disease (IPD) is a major cause of morbidity and mortality globally with an estimated 1.6 million people dying of pneumococcal disease each year. Of these 0.7–1 million are children under the age of 5 years living in the developing world. IPD has been described from Vientiane but there are no data on the local serotypes - important to guide planned Lao vaccination policies. A prospective hospital-based study of the circulating serotypes in Laos was conducted (see Moore *et al.* 2009). IPD was defined as patients culture positive for *S. pneumoniae* in blood and/or cerebrospinal fluid (CSF), and/or positive for *S. pneumoniae* in CSF using a real-time PCR assay targeting the *lytA* gene. A novel real-time PCR assay, developed in Laos, was used to determine serotypes.

Of 10,799 patients with haemocultures and 353 patients with CSF samples, 0.21% and 5.4%, respectively, were positive for *S. pneumoniae* (n=35). Two isolates associated with meningitis were penicillin-nonsusceptible, with MICs of 0.39 and 0.125 µg/mL. All *S. pneumoniae* tested were susceptible to ceftriaxone.

The most frequent serotype was 1, followed by serotypes 5, 6A/B/C, 14 and 23F. Serotype represented in the PCV-7 vaccine infected 39% of patients, with 73% and 76% coverage for the PCV-10 and PCV-13 vaccine, respectively. Although the sample size is small, these data suggest that the PCV-7 vaccine may have relatively low efficacy in Laos and that PCV-13 would be optimal.

Japanese encephalitis virus and vaccination policy

Although there has been no information on the causes of encephalitis in Laos, the Japanese encephalitis virus (JEV) is likely to be important as it occurs in all adjacent countries. Diagnosis of JEV is difficult because it is clinically indistinguishable from other causes of acute encephalitis and there are serological cross-reactions with dengue and other flavivirus antibodies. Detection of the virus in cerebrospinal fluid (CSF) is rarely successful so we use ELISAs to detect anti-JEV IgM in CSF confirming the importance of JEV in Laos (see Moore *et al.* 2012). CSF from 515 patients admitted at Mahosot Hospital (2003-2008) with suspected central nervous system infections were tested; 234 (45%) with acute encephalitis, 256 (50%) with meningitis and 157 (31%) with both syndromes.

The median (IQR; range) age of patients was 24 (8-38; 0.05-85) years and 32% were <15 years. CSF from 14.5% of patients with encephalitis and 10.1% from those with encephalitis and meningitis were positive for anti-JEV IgM. Of JEV IgM positive patients, 42% had convulsions, 63% had a reduced Glasgow Coma Score, the median (range) CSF white cell count was 125 (0-653)/µL with a median percentage of lymphocytes of 37 (0-90)%. Patients came from northern Laos and there is also evidence from sera of JEV throughout the country. As JEV is an important preventable disease, the expanded use of ELISA assays would help define the burden of disease. These data suggest that JEV vaccination should be considered.

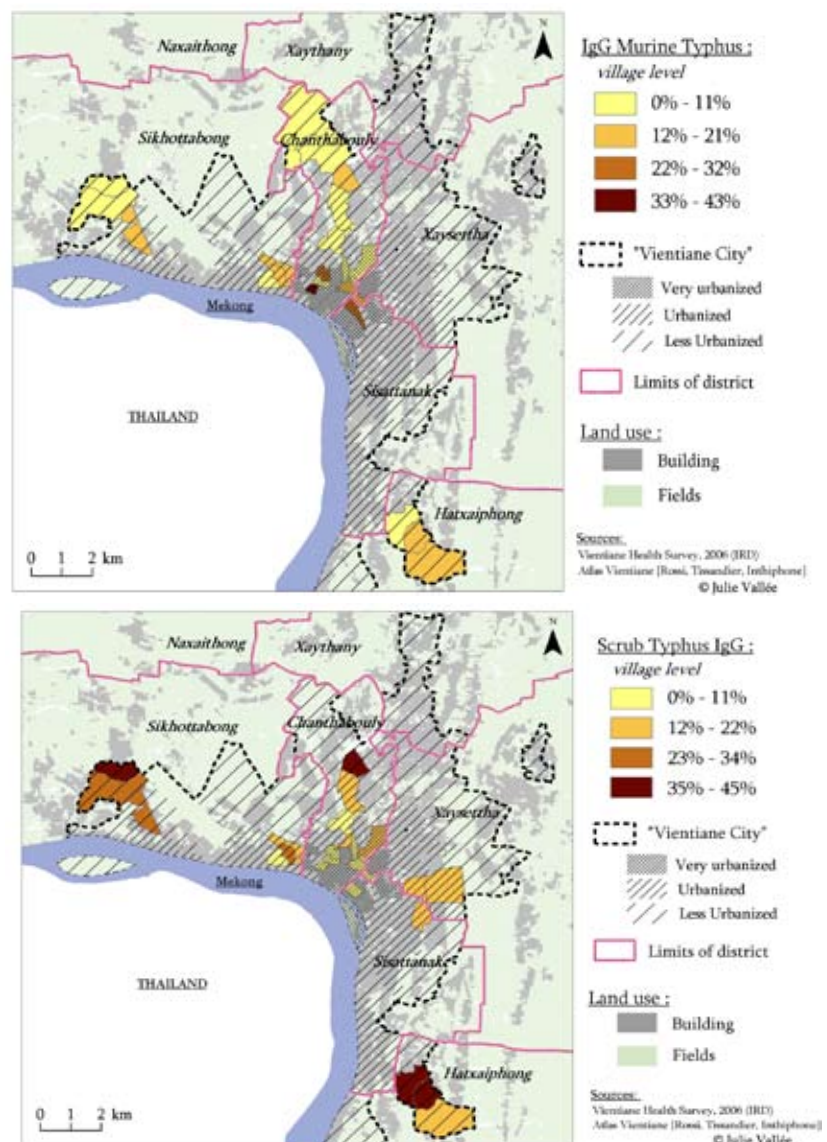
Typhus exposure and risks in Vientiane

Both scrub typhus and murine typhus are common causes of fever in Vientiane. Comparative risk factors for infection are unclear. As part of a large project examining urbanisation in Vientiane, we therefore determined the frequency of IgG seropositivity against *Orientia tsutsugamushi* (scrub typhus) and *Rickettsia typhi* (murine typhus), as indices of prior exposure to these pathogens, in randomly selected adults in urban and peri-urban Vientiane City (n=2,002, adults ≥ 35 years)(see Vallée *et al.* (2010). We performed ELISAs on eluates from filter paper blood spots.

Scrub typhus seropositivity (bottom map) was significantly higher among adults living in the periphery (28.4%) than in the central zone (13.1%) of Vientiane. In contrast, seroprevalence of murine typhus IgG antibodies (top map) was significantly higher in the central zone (30.8%) as compared to the periphery (14.4%). In multivariate analysis, adults with a longer residence in Vientiane were at significant greater risk of past infection with murine typhus and at lower risk for scrub typhus.

Those with no education, living on low incomes, living on plots of land with poor sanitary condition, living in large households and farmers were at higher risk of scrub typhus and those living in neighborhoods with high building density and close to markets were at greater risk for murine typhus and at lower risk of scrub typhus past infection.

The association of murine typhus seropositivity with homes close to markets suggest that enhanced market rubbish disposal and rodent control may help reduce the incidence of the disease. Scrub typhus is not conventionally thought of a disease of cities. However, it infects people in palm plantations, primary forest, beaches and in city gardens and the term 'scrub' is misleading. It is possible that Vientiane city dwellers are infected during residence in rural areas, visits to the countryside - for example to collect bamboo shoots or to fish-or in gardens and parks within the city.



Antibiotic activity in urine !

Antibiotic resistance is a major global public health problem, affecting treatment decisions, patient outcome, health care expenditure and public perceptions of health care. Widespread unregulated provision of antibiotics, dispensing of insufficient doses, reduced adherence to complete dose regimens and the poor quality of the drug supply are thought to contribute to the spread of antibiotic resistance.

We estimated the proportion of Lao in- and out-patients who had taken antibiotics before medical consultation by detecting antibiotic activity in their urine added to lawns of reference organisms (*Bacillus stearothermophilus*, *Escherichia coli* and *Streptococcus pyogenes*). Urine containing antibiotics kills the bacteria. In the retrospective (N=2,058) and prospective studies (N=1,153), 49.7% and 36.2%, respectively, of Vientiane patients had urinary antibiotic activity detected. The highest frequency of estimated antibiotic pre-treatment was found in patients recruited with suspected central nervous system infections and community-acquired septicaemia (both 56.8%). In Vientiane, children had a higher frequency of estimated antibiotic pre-treatment than adults (60.0% v 46.5%; P<0.001). Antibiotic use based on patients histories was significantly less frequent than when estimated from urinary antibiotic activity. Although Laos appears to have lower levels of antibiotic resistance in comparison to adjacent countries, ESBL positive *E. coli* (see page 8) and *Klebsiella pneumoniae* clinical isolates are common in Vientiane. It is likely that as the economy improves a greater volume and diversity of antibiotics will be consumed. The high frequency of antibiotic use in the community, as revealed by urinary antibiotic activity, may engender worsening drug resistance. This suggests that enhanced antibiotic stewardship and pharmacy regulation are required.

Beware the wild banana !

Six patients with bowel obstruction after eating wild bananas (BOWB) were recently described from Luang Namtha (see Selsak *et al.* 2011). Six required enterotomy for phytobezoars. On asking 227 other patients/relatives, 46% had eaten wild banana seeds. 45% knew of complications, including constipation (38%), appendicitis/abdominal pain/vomiting (3% each) and bloated stomach/death (1% each). Middle/highland Lao ethnicity was associated with wild banana consumption and male sex with consumption and unawareness. Of 44 doctors at all surgically-equipped hospitals in Laos, 33 knew of BOWB, describing patients as usually young, adult middleland Lao males. Country-wide, 46 patients with BOWB are known to have been operated on in 2009 (incidence ~0.8/100,000). All consumed WB seeds. BOWB is widespread in Laos, especially among men consuming WB seeds on an empty stomach in the forest. Alarming modes of local treatment were described, including the insertion of sticks and the piping of gasoline into the rectum. More engagement and education are needed to warn people of



WB phytobezoars from bowel of Lao patient.
Max length 5 cm

Extended-spectrum beta-lactamase (ESBL) bacteria

Since 2004 we have seen increasing numbers of patients infected with Extended-spectrum beta-lactamase (ESBL) producing *Escherichia coli* and *Klebsiella pneumoniae* at Mahosot Hospital. These can be extremely difficult to treat as they are resistant to cephalosporins and often also to gentamicin and fluoroquinolones. The molecular epidemiology of ESBLs from Laos was investigated in 54 *E. coli* isolates; the majority of isolates (76%) were from bloodstream and urinary tract infections, with a further 20% from pus samples.

CTX-M genotypes were found in all ESBL-*E. coli* clinical isolates from 2004-2009, with variants similar to those seen in neighbouring China and Thailand (CTX-M-14,-15 and -55-like, CTX-M-27). There was common concomitant ciprofloxacin/gentamicin resistance (68%). Global ESBL-*E. coli* lineages (ST-131, ST-405) were common, but ST-648, previously identified amongst birds, was the second most common sequence type, possibly related to the high frequency of backyard poultry farming in Laos (see Stoesser *et al.* 2011). These data suggest that it will be very important to enhance antibiotic stewardship measures in Laos, especially for cephalosporins, and investigate affordable alternative antibiotics for therapy.

Chromoblastomycosis and Myiasis

Chromoblastomycosis is a worldwide chronic infection of the skin and subcutaneous tissue, most commonly found in tropical and subtropical areas. It is mainly caused by the fungal genera *Fonsecaea*, *Phialophora* and *Cladophialophora* that are saprophytes in soil and plants. The lower limbs are most commonly infected and the nodular and/or verrucous plaques can develop centripetal satellite lesions. The most frequent complication is bacterial secondary infection, but malignancies have also been recorded.

A 72 year-old farmer was admitted to Luang Namtha Provincial Hospital, northern Laos, with a growth on the left lower leg which began 1 week after a forefoot leech bite 10 years previously. He presented with a cauliflower-like mass and plaque-like lesions on his lower leg/foot and cellulitis with a purulent tender swelling of his left heel.

He was thought initially to have leprosy or skin cancer, but skin scrapings from the left lower leg lesions revealed typical brownish, round, thick-walled, multiseptate sclerotic cells in a wet film, confirmed with the 10% potassium hydroxide technique.

During wound dressing, 22 maggots were discovered in the heel wound and identified as third instar larvae of the Old World screwworm fly, *Chrysomya bezziana*. Presumably flies laid eggs in the open wound.

PCR of a biopsy of a left lower leg nodule demonstrated *Fonsecaea pedrosoi*, *F. monophora*, or *F. nubica*. He was successfully treated with long term terbinafin plus itraconazole pulse-therapy and local debridement (see Slesak *et al.*



Left - Lower leg of the patient at presentation with typical lesions on his foot that spread centripetally up to his knee.

Below - Contaminating screwworm fly *Chrysomya bezziana* maggots from patient's heel. They are ~1 cm long



Simple but accurate diagnosis of typhoid

Although the bacterium causing typhoid, *Salmonella enterica* serovar Typhi, was first described 132 years ago, most typhoid patients globally do not have access to reliable laboratory diagnosis. There remains an urgent need for inexpensive, rapid, portable, and simple techniques for diagnosing typhoid in locations away from sophisticated hospital settings, where the burden of diseases is the greatest. Typhoid diagnosis could be enhanced by making the identification of *S. Typhi* in blood cultures simpler, less expensive and faster. We investigated whether the combination of blood culture bacterial growth with an *S. Typhi* antigen rapid diagnostic test (RDT) on the blood culture fluid could be an accurate and inexpensive tool for the accelerated diagnosis of patients with acute typhoid in Laos (see Castonguay-Vanier *et al.* 2013). These RDTs were developed for detecting *S. Typhi* in stools. We adapted them for detecting *S. Typhi* in blood culture fluid when the fluid becomes turbid.

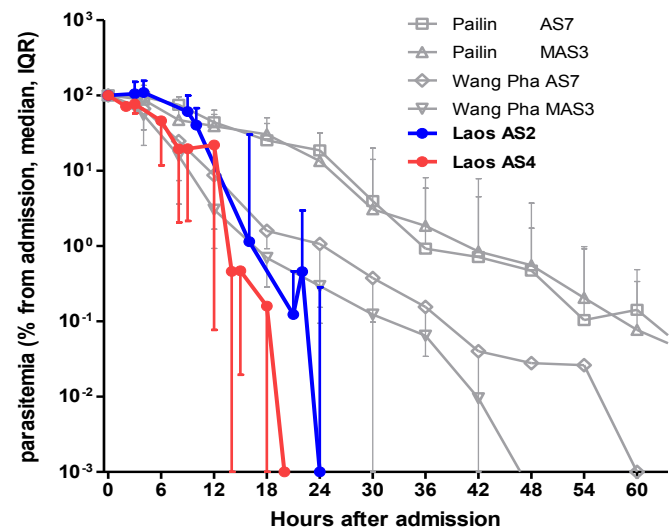
Using reference strains of Gram negative bacteria, the RDT was only positive for Group D *Salmonella enterica*, including *S. Typhi*. In a prospective study of 6,456 blood culture bottles from 3,028 patients over 15 months, 392 blood culture bottles (6.1%) from 221 (7.3%) patients had Gram negative rods (GNR) seen in the blood culture fluid. The sensitivity, negative predictive value, specificity and positive predictive value were 96.7%, 99.5%, 97.9% and 87.9%, respectively, for patients with proven *S. Typhi* bacteremia and 91.2%, 98.4%, 98.9% and 93.9% for patients with Group D *Salmonella*. The RDT accelerated diagnosis by a median of one day and were less expensive and labour intensive than conventional reference assays.

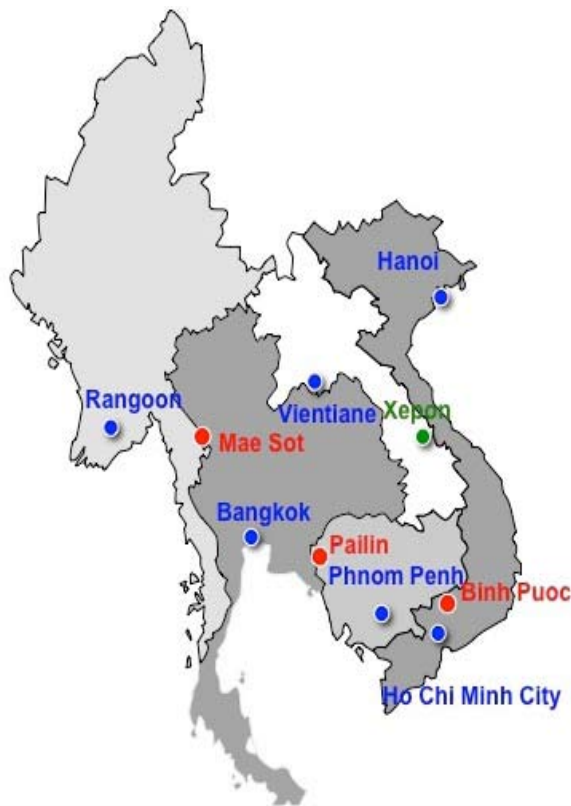
Although, this does not address the problem that blood cultures only detect *S. Typhi* in ~40-80 % of patients with typhoid, it suggests that the combination of blood cultures with RDTs are promising as accurate tools for accelerated, inexpensive diagnosis. We hope that they will be trialed in other locations and be useful for diagnosis in peripheral diagnostic labs and in investigation of suspected typhoid outbreaks.

Has artemisinin resistance arrived in Laos ?

There is now, sadly, evidence for *Plasmodium falciparum* artemisinin resistance in Cambodia, Vietnam and the Thai/Burma border. We conducted a randomized clinical trial to assess parasite clearance times (PCT) and the efficacy of 4mg/kg and 2mg/kg oral artesunate for 3 days followed by artemether-lumefantrine in patients with uncomplicated falciparum malaria at Xepon, Savannakhet Province (see Mayxay *et al.* 2012). The mean (range) PCT was 23.2 (12-46) h. Ten patients (23%) remained parasitemic on day 1 after treatment but no patient had patent asexual parasitemia on day 2 or 3. The 42-day PCR-corrected cure rate was 100%. In conclusion, no evidence of *P. falciparum* in vivo resistance to artesunate was found in southern Laos. However, this will need to be checked regularly.

Red & blue lines are Lao parasite clearance at 2 (AS2) and 4 (AS4) mg/kg artesunate whilst grey lines are for Pailin (Thai/Cambodia border) and Wang Pha (Thai/Burma border)





The map, left, shows mainland SE Asia with capitals (blue) and sites with evidence for prolonged artemisinin treatment parasite clearance (red) and the Lao study site of Xepon that still has fast parasite clearance (green).

Typhus, fluoroquinolones and treatment

Scrub typhus (*Orientia tsutsugamushi*) is a public health concern for a population of over a billion humans in Asia and Australia with an estimated incidence of one million cases/year. Although doxycycline remains the standard therapy, fluoroquinolones have been used successfully in a few patients. However, there is also clinical evidence that these compounds are ineffective in the treatment of scrub typhus. In order to clarify this, we determined the *in vitro* susceptibility of *O. tsutsugamushi* strain Kato to ciprofloxacin and sequenced the quinolone-resistance-determining-region (QRDR) of the *gyrA* gene, the target of fluoroquinolones, from 18 Lao patients (see Tantibhedhyangkul *et al.* 2009). *O. tsutsugamushi* were resistant to ciprofloxacin and ofloxacin *in vitro* (MIC = 8 μ g/ml) and all sequences obtained, including those from the two available genomes of *O. tsutsugamushi* (strains Boryong and Ikeda), had a Ser83Leu mutation in their QRDR domain that is known to be associated with fluoroquinolone resistance. These results suggest that fluoroquinolones should not be used in the treatment of scrub typhus. We do not know how effective fluoroquinolones are for murine typhus. Similarly, it is unclear how effective azithromycin is for treatment of either scrub typhus or murine typhus in Laos (more data in next MMR).

There are no consensus guidelines for the definition of severe scrub typhus or murine typhus. In theory doxycycline and chloramphenicol should be efficacious but the optimum treatment is unclear. For those with reduced Glasgow Coma Score or unable to swallow, intravenous therapy (although we do not have intravenous doxycycline in Laos) or nasogastric tube administration of doxycycline and chloramphenicol would be required (see Page 11).

Treatment Guidelines

Please check paediatric guidelines for neonates and children and consider pregnancy testing

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. We take no responsibility for their use.

Treatment of typhus

Oral **Doxycycline** 4mg/kg stat followed by 2mg/kg every 12 hours for 7 days

[Usual dose in adults 200mg stat followed by 100mg every 12 hours for 7 days]

OR

Oral **Chloramphenicol** 50–75mg/kg/day for 7 days

[Usual dose in adults 500mg every 6 hours for 7 days]

The risks to children <8 years of 1 week 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.

Do pregnancy test before giving doxycycline to women of childbearing age. Suggest to warn patients of gullet pain

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

* *Listeria monocytogenes* appears to be rare 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

Guidelines : Treatment of Meningitis

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

| | | | | |
|-----|----------|-----------|-----------|-----------|
| Age | 0-7 days | 8-28 days | <15 years | >15 years |
|-----|----------|-----------|-----------|-----------|

| | | | | |
|---------------|--------------|--------------|--------------|----------|
| Daily dose IV | 150mg/kg/day | 200mg/kg/day | 300mg/kg/day | 12 g/day |
|---------------|--------------|--------------|--------------|----------|

| | | | | |
|---------------|-----------|-------------|-----------|----------|
| Dose Interval | every 8 h | every 6-8 h | every 6 h | every 4h |
|---------------|-----------|-------------|-----------|----------|

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

Chloramphenicol

| | | | | |
|-----|----------|-----------|-----------|-----------|
| Age | 0-7 days | 8-28 days | <15 years | >15 years |
|-----|----------|-----------|-----------|-----------|

| | | | | |
|---------------|-------------|-------------|-----------------|----------|
| Daily dose IV | 25mg/kg/day | 50mg/kg/day | 75-100mg/kg/day | 4-6g/day |
|---------------|-------------|-------------|-----------------|----------|

| | | | | |
|---------------|-----|--------|----|----|
| Dose Interval | 24h | 12-24h | 6h | 6h |
|---------------|-----|--------|----|----|

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

AND

Ampicillin

| | | | | |
|-----|----------|-----------|-----------|-----------|
| Age | 0-7 days | 8-28 days | <15 years | >15 years |
|-----|----------|-----------|-----------|-----------|

| | | | | |
|---------------|--------------|--------------|--------------|----------|
| Daily dose IV | 150mg/kg/day | 200mg/kg/day | 300mg/kg/day | 12 g/day |
|---------------|--------------|--------------|--------------|----------|

| | | | | |
|---------------|-----------|-------------|-----------|----------|
| Dose Interval | every 8 h | every 6-8 h | every 6 h | every 4h |
|---------------|-----------|-------------|-----------|----------|

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.

If you suspect TB meningitis please send as much CSF as you can

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.

Please ensure haemoculture, and other relevant samples such as urine, throat swab, are taken before commencing antibiotics.

Guidelines : Treatment of Severe Melioidosis

Dosage adjustment for ceftazidime in patients with renal impairment

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.

| Serum creatinine | | Ceftazidime Dose |
|-------------------------|--------------|-------------------------|
| µmol/L | mg/dL | |
| Less than 176 | Less than 2 | 2g every 8 hours |
| 176 - 352 | 2-4 | 1g every 8 hours |
| 353 -528 | 4-6 | 1g every 12 hours |
| Greater than 528 | >6 | 1g every 24 hours |

SEVERE MELIOIDOSIS - 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 in 2 divided doses for 12-20 weeks - dose adjusted for body weight. See top of Page 14.

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]

Guidelines : Oral Treatment of Melioidosis

Co-trimoxazole dose based on body weight (see Dance 2011)

| Body weight/kg | Dose (TMP/SMX) mg every 12 hours |
|----------------|----------------------------------|
| < 40 | 160/800 mg |
| 40-60 | 240/1200 mg |
| > 60 | 320/1600 mg |

LOCALISED MELIOIDOSIS – ORAL TREATMENT

For patients with melioidosis at ONE site WITHOUT septicaemia

Co-trimoxazole in 2 divided doses for 12-20 weeks - dose adjusted for body weight. See above.

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]

Treatment of typhoid

Uncomplicated typhoid Ofloxacin 15mg/kg/day po in 2 divided doses for 3 days

Severe typhoid

eg. hypotension, abdominal pain, intestinal hemorrhage/perforation, encephalopathy, seizures, pneumonia
Ask for surgical review plus ceftriaxone 60mg/kg/day iv/im in single dose/day for 10-14 days

Consider dexamethasone 3mg/kg infusion iv over 30 mins followed by 1mg/kg over 30 mins every 6 hours for eight additional doses.

The risks to children <8 years of 3 days ofloxacin are likely to be less than the risks of not giving ofloxacin.

NB Fever clearance is usually shorter with fluoroquinolones than iv ceftriaxone and fever may disappear ~ one day after fluoroquinolones are stopped

Empirical treatment of Septicaemia

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]

[beware 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]

If intra-abdominal sepsis suspected, suggest adding metronidazole (500 mg every 8 hours)

Please try to take all microbiology samples and multiple blood cultures before starting antibiotics

SUGGESTIONS FOR SPECIAL SITUATIONS

If you suspect endocarditis:

Suggest taking six separate blood culture sets and inform cardiologist

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 [Copies available from the Microbiology Laboratory]

2009 - since last newsletter

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THE TAIL END

An 18-year-old Lao student and rice farmer from Saravan Province presented at Mahosot Hospital with widespread abscess formation and significant scarring of her left buttock of three years duration (see Wootton *et al.* 2012). Initially starting as a small painless pustule, without antecedent trauma or injection, the lesion had grown and been treated with incision and drainage and a week's course of ampicillin. Although blood cultures were negative, pus from the buttock grew *Burkholderia pseudomallei*. Tests for diabetes and thalassaemia were negative. Chest XR was normal but she had splenic abscesses visible on abdominal ultrasound. A fistulogram showed no connection with internal organs but she had a large collection in her buttock that was drained. She was treated with iv ceftazidime for three weeks and then oral doxycycline and co-trimoxazole for 16 weeks with significant improvement.



wellcome trust



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}

please only send if patient has UTI symptoms, except in pregnancy

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 - stool - for suspected gastrointestinal tract bleeding. ^{1 day}

CSF - for suspected CNS infection or other intracranial pathology - please call Lab +

- 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. *S. suis* 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}

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

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 do 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}

Hand, Foot and Mouth Disease - throat swab, vesicle fluid, stool and serum. Please phone Lab.

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, phone number, ward, Hospital Number and requesting doctors name (not just the signature !).