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Every child has the right to a healthy and loving life. By opening your heart, sharing your skill or providing support, you can make a profound difference in the life of a child and the future of a nation.

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Angkor Hospital for Children, in Siem Reap, Cambodia, is a pediatric teaching hospital funded by the NGO FRIENDS WITHOUT A BORDER.



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TROPICAL MEDICINE RESEARCH PROGRAMME

Angkor hospital for Children at Siem Reap



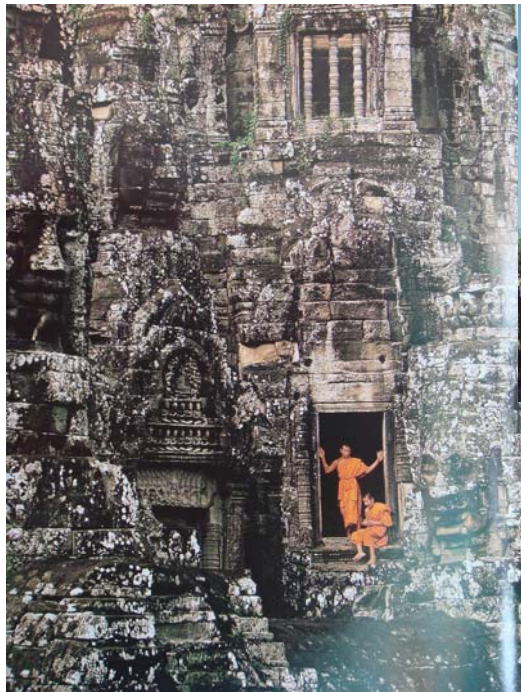
The cost-saving, effective diagnoses of melioidosis in Cambodia

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Mahidol University*



Angkor Wat



Bayon

Ta Prohm



- 80% of the 14 million people in Cambodia live in rural areas
- 65% live with inadequate drinking water supplies and 92% live with inadequate sanitation facilities
- 34% of the populations lives on less that US\$1.00 per day
- Nearly 50% of all Cambodian children are malnourished
- One in seven will die before their 5th birthday due to preventable causes





Angkor Hospital for Children (AHC) serves as the paediatric department for Siem Reap's Provincial Hospital since 1999.

Currently the outpatient department sees 400-450 children each day and maintains 50 inpatient beds.



MICRO LAB

October 2005



20USD

Price in Siem Reap 2005



Home made blood culture bottle
0.8 USD



Number of blood culture tested per year



Year	Blood culture
2005	352
2006	911
2007	1251
2008	1449



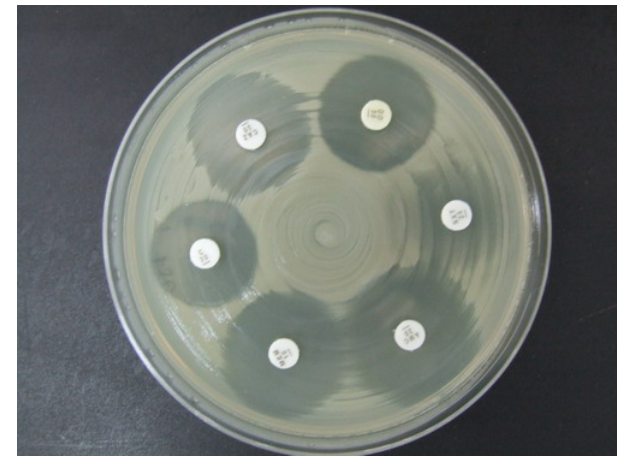
MICRO LAB



Coagulase test



Susceptibility test



Home made biochemistry test

(Gram negative bacilli)



Commercial test kit for Gram negative bacilli



API 20E



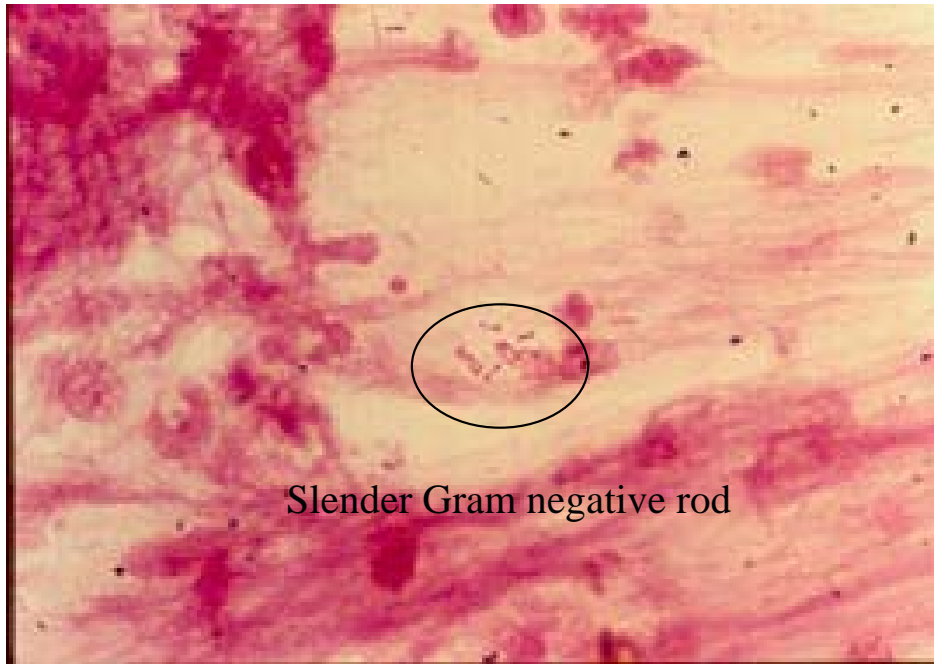
API 20NE

Isolation from blood

(Jan 2006 to Sep 2009)

Organisms (n=277)	Blood culture
<i>Salmonella typhi</i>	21.3
<i>Staphylococcus aureus</i> (MRSA 19%)	11.2
<i>Streptococcus pneumoniae</i> *	10.8
<i>Haemophilus influenzae</i> *	6.6
<i>Klebsiella pneumoniae</i>	5.9
<i>Escherichia coli</i>	5.9
<i>Acinetobacter spp.</i>	5.2
<i>Burkholderia pseudomallei</i>	3.8
<i>Pseudomonas aeruginosa</i>	3.5
<i>Neisseria meningitidis</i> *	1.7
Others	21.3

* An organism that difficult to isolates

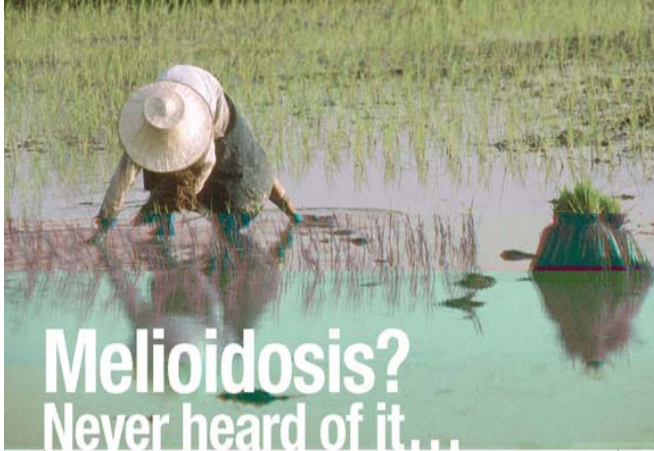


- *Burkholderia pseudomallei* is a soil saprophyte and the cause of melioidosis.
- Infection is acquired through inoculation, inhalation, or ingestion.
- Most reported cases occur in Thailand and northern Australia, but the diagnostic is not available across much of rural Asia.



Why melioidosis is important?

- Melioidosis is a leading cause of bacterial sepsis in northeast Thailand with mortality rate of 80%.
- The clinical presentation is highly variable and ranges from acute fulminant sepsis with widespread bacterial dissemination to a mild localised infection.
- Childhood infection accounts for around 10% of cases overall in this setting, with acute suppurative parotitis accounting for one third of pediatric cases.
- No reports in the literature of indigenous melioidosis or environmental isolation of *B. pseudomallei* in adjacent Cambodia.



Melioidosis? Never heard of it...

Deadly tropical infections that kill within 48 hours don't usually go unnoticed. But one killer has been largely ignored for decades. Now, thanks to worries about bioterror, it is being taken more seriously. Peter Aldhous reports.

Mention melioidosis in most circles—even those with a passing interest in tropical medicine—and you'll be met with blank stares. The infection is often misdiagnosed because the bacterium that causes it, the soil-dwelling *Burkholderia pseudomallei*, triggers multiple symptoms that mimic those of other diseases. In parts

new antibiotics, to see if they can reduce this toll. But for Thailand's overstretched health-care system, such drugs are prohibitively expensive. No pharmaceutical company has volunteered to donate its products, so Chaowagat's plans seem stalled. "If we use our own money, we have a problem," she says.

first became sick 26 years later. Vanaporn Wuthikannun, who works on *B. pseudomallei* in the Wellcome Trust unit at Mahidol University in Bangkok, has cultured the bacterium from a sample kept in distilled water for a decade. "It's very tough," she observes. For the most part, *B. pseudomallei* is thought to get its nutrition from rotting



Once ignored as an obscure disease, melioidosis and the frighteningly versatile bacterium that causes it are drawing attention as a bioterror threat

Racing to Defuse a Bacterial Time Bomb

UBON RATCHATHANI, THAILAND—A Thai man with lank black hair and grizzled stubble lolls on a cot parked in a hallway outside a crowded ward. The 61-year-old farmer answers tersely as a senior physician, Wipada Chaowagat, quizzes him. When the man was admitted on 9 May with sepsis and an abscess in his chest wall, Wipada fingered an old nemesis: melioidosis. But although nearly nine out of 10 melioidosis patients in Thailand with septic shock die, somehow the farmer beat the odds. After spending 2 months in Sappasithpraxong Hospital here in northeastern Thailand, the taciturn man with watery eyes is almost well enough to go home.

Wipada can't explain how the farmer, who suffers from kidney disease, managed to fend off a bacterium, *Burkholderia pseudomallei*, that in its fiercest incarnation kills most of its victims. Indeed, there is no shortage of scientific puzzles surrounding melioidosis. Over the 2 decades that Wipada has studied the once-obscure malady, more and more experts have become intrigued by the ability of *B. pseudomallei* to alter its form and survive in environments as disparate as soil, distilled water, and the human body.

"There's something incredibly interesting and important going on with *pseudomallei*, and nobody knows what that is," says Colin Manoi, a geneticist at the University of Washington, Seattle.

Melioidosis is largely confined to Southeast Asia and northern Australia and, fortunately for the rest of the world, researchers don't anticipate the shape-shifting bug breaking out of its ecological cage anytime soon. But its characteristics make it an insidious threat as a bioweapon. The bacteria can hide in the body for decades. Once the time bomb detonates, a constellation of symptoms allows melioidosis to masquerade as other ailments. Although many patients are rushed to the hospital with acute disease, others have symptoms more akin to tuberculosis or cancer, says Sharon Peacock of the Mahidol-Oxford Tropical Medicine Research Unit (MORU) in Bangkok. Misdiagnosis can prove fatal: *B. pseudomallei* is impervious to all but a few antibiotics.

"It's not as scary as anthrax or smallpox," says Peacock, who has spent 20 years on the trail of melioidosis. "But it still has a significant terror factor. Once soil is contaminated, *B. pseudomallei* is very hard to get rid of."

Scientists know they are up against a worthy foe. "Viruses are very smart. Bacteria are normally not so smart," *pseudomallei* acts like a virus" in its deviousness, says Suresh Chakravarti, director of the Melioidosis Research Center at Thailand's Khon Kaen University. Hoping to strengthen their defenses, researchers launched a drug trial this month at Sappasithpraxong. And a pilot experiment is under way in Ubon Ratchathani to take the battle to *B. pseudomallei*'s home turf: Thailand's ubiquitous rice paddies.



Certain aspects, especially in northeastern Thailand, are at high risk of contracting melioidosis.

The Great Mimicker

Melioidosis was first described from opium addicts in Burma in 1911, and in 1947, two cases involving POWs held in Siam were reported in the *British Medical Journal*. It wasn't until the Vietnam War, however, when U.S. soldiers came home with the disease, that melioidosis attracted significant attention in the West. The first Thai case was reported in 1955. "No one had ever seen this before," says tropical medicine specialist Sompong Panayagapita, former president of Vichaiyut Hospital in Bangkok. In the 1960s,

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B. pseudomallei was isolated from 30% of soil samples ranged from 1-5,000 (median 90 CFU/g, IQR 20-250CFU/g of soil)

***Burkholderia pseudomallei* Antibodies in Children, Cambodia**

Vanaporn Wuthiekanun,* Ngoun Pheaktra,†
Hor Putchhat,† Lina Sin,† Bun Sen,†
Varun Kumar,† Sayan Langla,*
Sharon J. Peacock,*‡ and Nicholas P. Day*‡

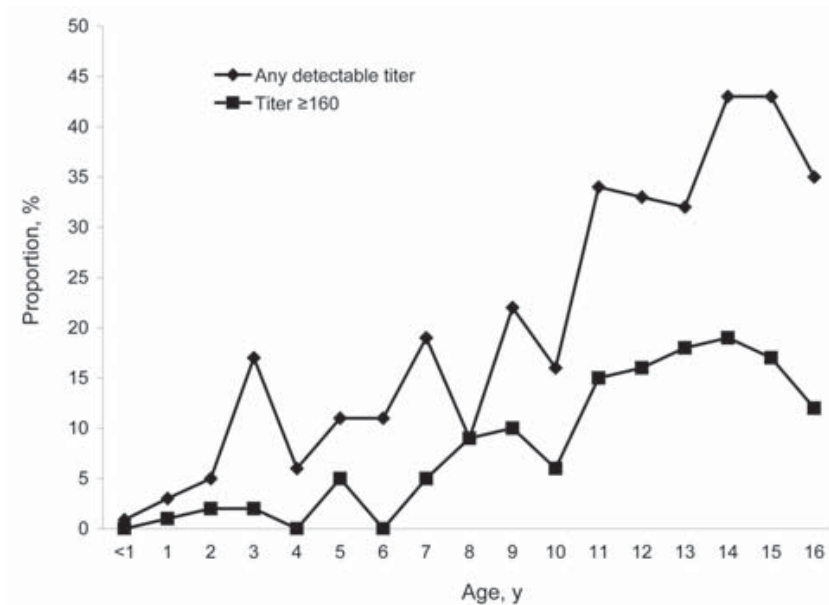
Antibodies to *Burkholderia pseudomallei* were detected in 16% of children in Siem Reap, Cambodia. This organism was isolated from 30% of rice paddies in the surrounding vicinity. Despite the lack of reported indigenous cases, melioidosis is likely to occur in Cambodia.

and to determine whether this from their environment.

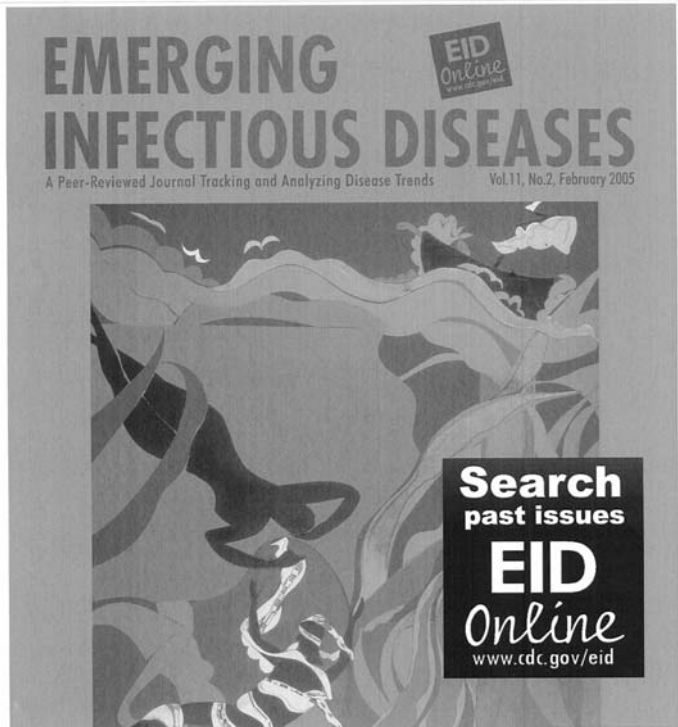
The Study

A prospective, cross-sectional study was conducted at Angkor Hospital for Children, Siem Reap, Cambodia, from 2005 through April 2006. Unselected consecutive serum samples were collected from children between birth and 16 years of age from the biochemistry and hematology laboratory of Angkor Hospital for Children, Siem Reap. Blood samples were collected from outpatients and inpatients. These blood tests were ordered by the primary physician for other reasons, and the sample used represented surplus material. Samples were centrifuged at 3,000 rpm for 10 min and the serum stored at -30°C. Target sample numbers were 40-60 per year group. An anonymous database was created to record sex, age, and indirect hemagglutination assay (IHA) titer. The presence and titer of antibodies to *B.*

Indirect hemagglutination assay titer for 968 children



Antibodies to *Burkholderia pseudomallei* were detected in 16% of Children in Siem Reap



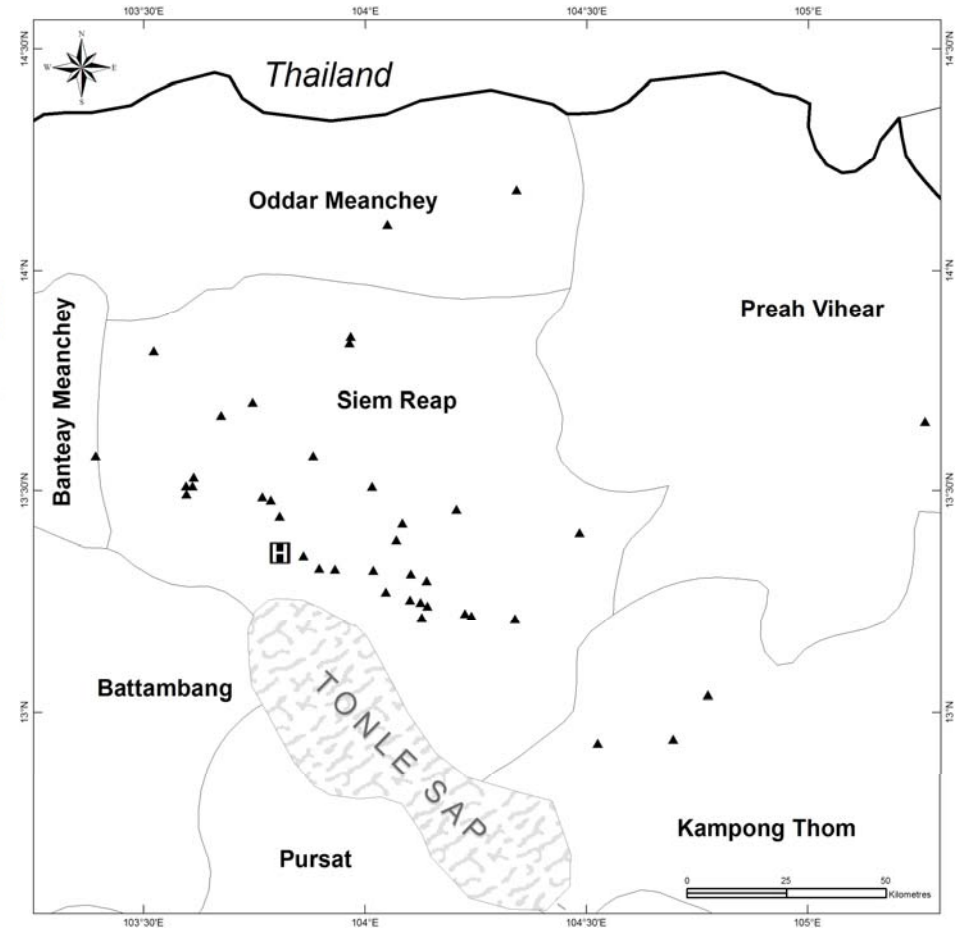
METHOD

- A laboratory-based study was conducted between October 2005 and December 2008 to identify children with one or more clinical samples positive for *B. pseudomallei*
- Demography and clinical information were collected
- The causative isolates were genotyped using multilocus sequence typing (MLST)
- Neighbour-joining trees were re-constructed

RESULTS

39 cases of culture-proven melioidosis were identified between Oct 2005 and Dec 2008

(2005 (3 months), n=2; 2006, n=9; 2007, n=13; 2008, n=14)

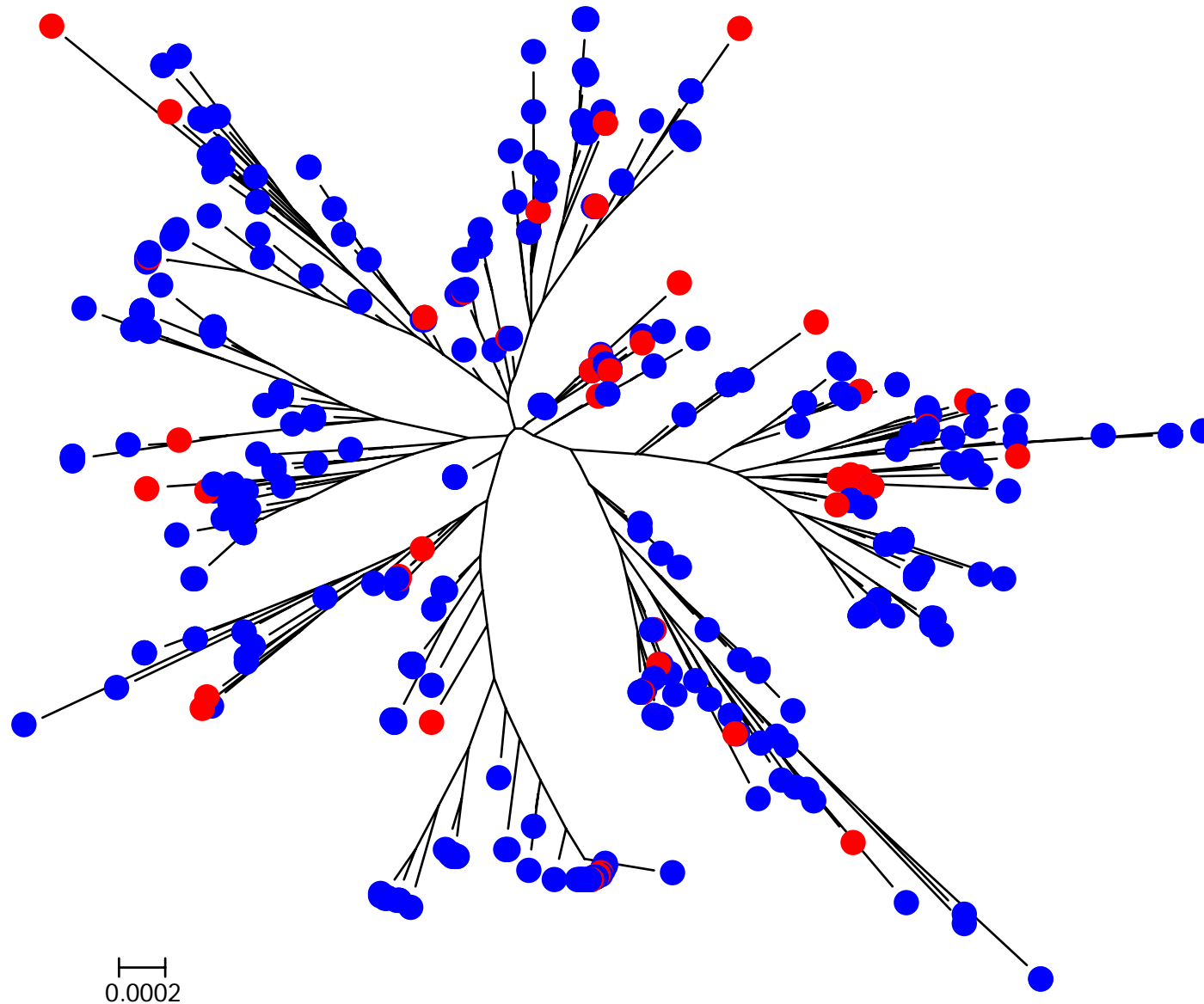


RESULTS: Summary data for 39 children with melioidosis



Variable	no.	
Male gender	15	(38%)
Age (yrs), median (range, IQR [†])	7.8	(1.6 to 16.2, 4.1-12.4)
Underlying disease present	4	(10%)
Source of <i>B. pseudomallei</i> isolate		
Blood [†]	9	(23%)
Pus	29	(74%)
Respiratory secretions	1	(3%)
Severity of infection [†]		
Localized	27	(69%)
Disseminated	12	(31%)
Type/site of infection		
Acute suppurative parotitis	15	(38%)
Superficial soft tissue abscess	7	(18%)
Blood culture positive, no focus identified	6	(15%)
Lymph node abscess	4	(10%)
Pneumonia	3	(8%)
Meningitis	1	(3%)
Other	3	(8%)
Died during admission	8	(21%)
Death attributable to melioidosis	7	(18%)
Time to death for attributable deaths (days), median (range)	2	(Day of admission to day 5)

Neighbour-joining tree using concatenated sequences of all seven loci for Cambodian invasive and soil isolates (n=53), together with data downloaded from the MLST website for all Thai *B. pseudomallei* isolates that have been reported in previous publications (n=462). Red circles denote Cambodian isolates and blue circles denote Thai isolates.



DISCUSSION

- This is the first description of pediatric melioidosis in Cambodia.
- Many of the isolates responsible were defined as novel STs, but Cambodian isolates from soil and invasive disease were highly related to a collection of isolates in nearby Thailand.
- 39 cases are likely to represent the tip of the iceberg since diagnosis relies on microbial culture, which is rarely available in this setting.

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Dr. William Housworth **Dr. Varun Kumar**
 Director of AHC Senior paediatrician



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