



***The ever-changing threat of
rickettsial diseases***

MORU 
Tropical Health Network

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APRC2 Sponsors

The Organising Committee would like to thank the following sponsors for their generous support:



InBios

We also thank Fuller laboratories, Fullerton, CA, USA and Jasmine Internet (Thailand), Co. Ltd. for their financial support.

Welcome

Dear APRC2 attendees,

It's a huge privilege for all of us in the MORU Network to welcome you to the 2nd Asia Pacific Rickettsia Conference! Over the past ten years there has been a real explosion in rickettsial research, driven principally in the Asia-Pacific region by the slow but increasing realisation that scrub typhus is a genuine and rather major public health problem. Over the past decade the number of publications on *Orientia* and scrub typhus has more than doubled, reflecting the hard work that many of you have put into uncovering the epidemiology, pathophysiology, clinical features and treatment of this neglected infection. Our conference is being held in the heartland of scrub typhus, and hopefully many of you will take the opportunity on Tuesday afternoon to see some of the remote rural areas where the disease has its biggest impact.

The Scientific Committee has put together a cornucopia of talks and poster sessions covering a wide range of topics on rickettsial diseases in the Asia Pacific and beyond. We hope you have a wonderful time in Chiang Rai, savouring the science, fostering new friendships and collaborations, experiencing the local culture and cuisine (don't miss the Khao Soi!), and enjoying the wonderful hospitality of our Thai hosts.

Best wishes

Nicholas Day

Director, MORU Tropical Health Network

Committee Members

Organising Committee

Stuart Blacksell	<i>Mahidol Oxford Research Unit, Bangkok, Thailand.</i>
Nicholas Day	<i>Mahidol Oxford Research Unit, Bangkok, Thailand.</i>
Matthew Robinson	<i>Lao-Oxford-Mahosot Hospital-Wellcome Research Unit, Vientiane, Lao PDR.</i>
Tri Wangrangsimakul	<i>Chiang Rai Clinical Research Unit, Chiang Rai, Thailand.</i>

Scientific Committee

Mohammad Yazid Abdad	<i>National Centre for Infectious Diseases, Singapore.</i>
Shuji Ando	<i>National Institute of Infectious Diseases, Tokyo, Japan.</i>
Elizabeth Batty	<i>Mahidol Oxford Research Unit, Bangkok, Thailand.</i>
Stuart Blacksell	<i>Mahidol Oxford Research Unit, Bangkok, Thailand.</i>
Kittipong Chaisiri	<i>Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand.</i>
Nicholas Day	<i>Mahidol Oxford Research Unit, Bangkok, Thailand.</i>
Stephen Graves	<i>Australian Rickettsial Reference Laboratory, Geelong, Australia.</i>
Cecilia Kato	<i>Centers for Disease Control and Prevention, Rickettsial Zoonoses Branch, Atlanta, GA, USA.</i>
Serge Morand	<i>CNRS ISEM-CIRAD ASTRE, Kasetsart University, Bangkok, Thailand.</i>
Paul Newton	<i>Lao-Oxford-Mahosot Hospital-Wellcome Research Unit, Vientiane, Lao PDR.</i>
Allen Richards	<i>Department of Preventive Medicine and Biostatistics, Uniformed Services University of the Health Sciences, Bethesda, MD, USA.</i>
Matthew Robinson	<i>Lao-Oxford-Mahosot Hospital-Wellcome Research Unit, Vientiane, Lao PDR.</i>
Jeanne Salje	<i>Mahidol Oxford Research Unit, Bangkok, Thailand.</i>
John Stenos	<i>Australian Rickettsial Reference Laboratory, Geelong, Australia.</i>
Khamsing Vongphayloth	<i>Institut Pasteur du Laos, Vientiane, Lao PDR.</i>
Tri Wangrangsimakul	<i>Chiang Rai Clinical Research Unit, Chiang Rai, Thailand.</i>

General Information

Meeting Venue

APRC2 is being held at *The Riverie by Katathani* in the city of Chiang Rai, Thailand.

Address: 1129 Kraisorasit Rd, Vieng District,
Muang, Chiang Rai, 57000 Thailand
Telephone: +66 53 607 999

Registration & Help Desk

On Sunday 3rd November, the conference Registration Desk will be near the hotel reception area. Delegates will receive their ID badge (which will allow access to conference rooms, meals and activities) and conference bag. From Monday 4th November onwards, the Registration/Help Desk will be located at the *Doi Tung Foyer* (outside the main meeting hall).

Assistance during the conference

If you have any questions or require assistance at any time during the meeting please speak to a member of the organising committee or conference staff. For hotel or accommodation related enquires please speak to a member of the hotel front desk.

Speakers

If you are giving an oral presentation, please visit the speakers' corner (*Doi Come*) or help desk well in advance of your presentation time to ensure your slides are uploaded and compatible. Slides should be in the widescreen format at 16:9 ratio in PowerPoint or similar in Keynote.

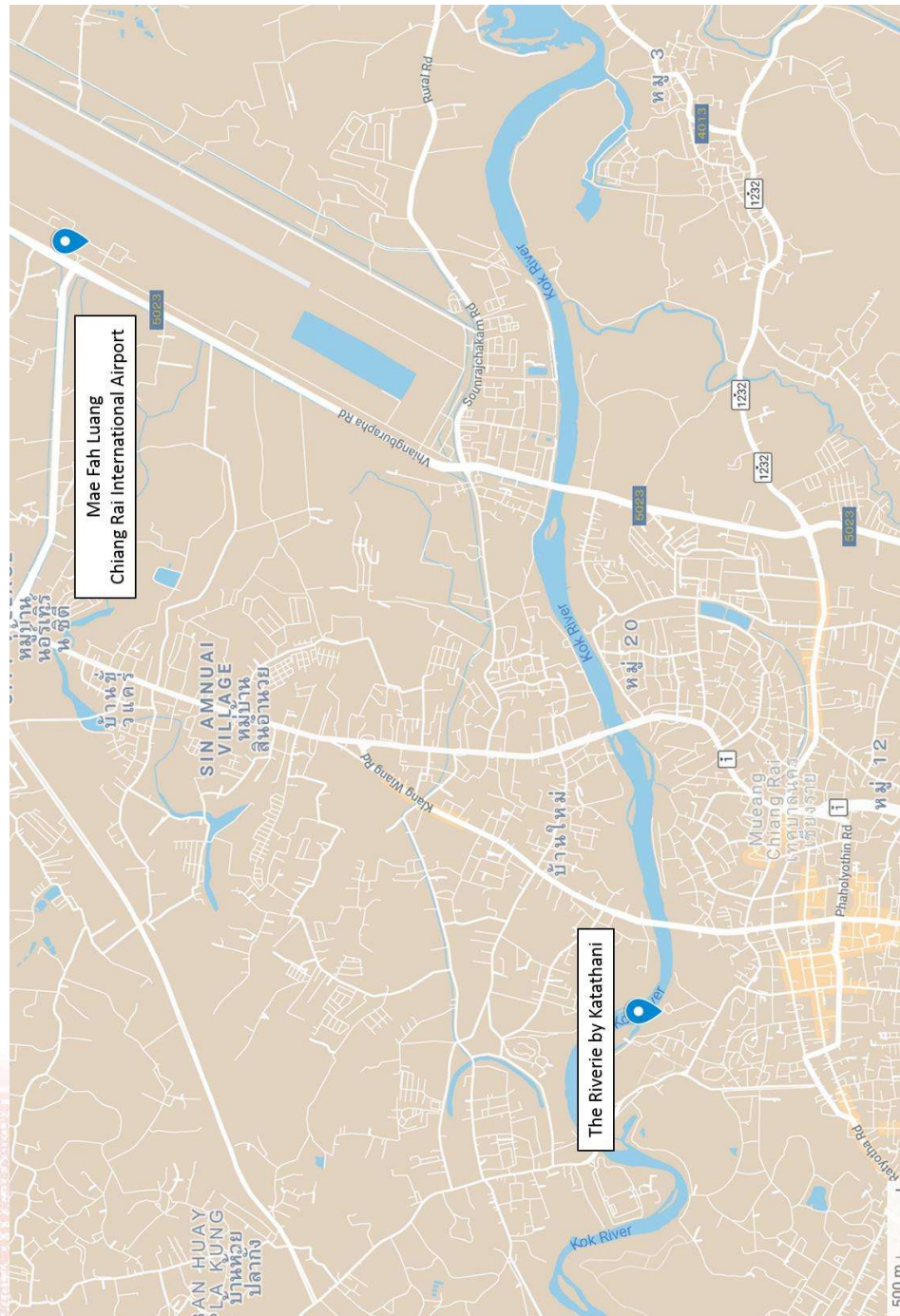
Posters

Posters should not exceed A0 size. Poster boards and tape to secure your posters will be available. Posters should be placed on display in the *Doi Tung Foyer* prior to the start of the conference or no later than the beginning of morning break (10:15 – 10:45) on Monday 4th November. Posters should be removed by 14:00 on Wednesday 6th November.

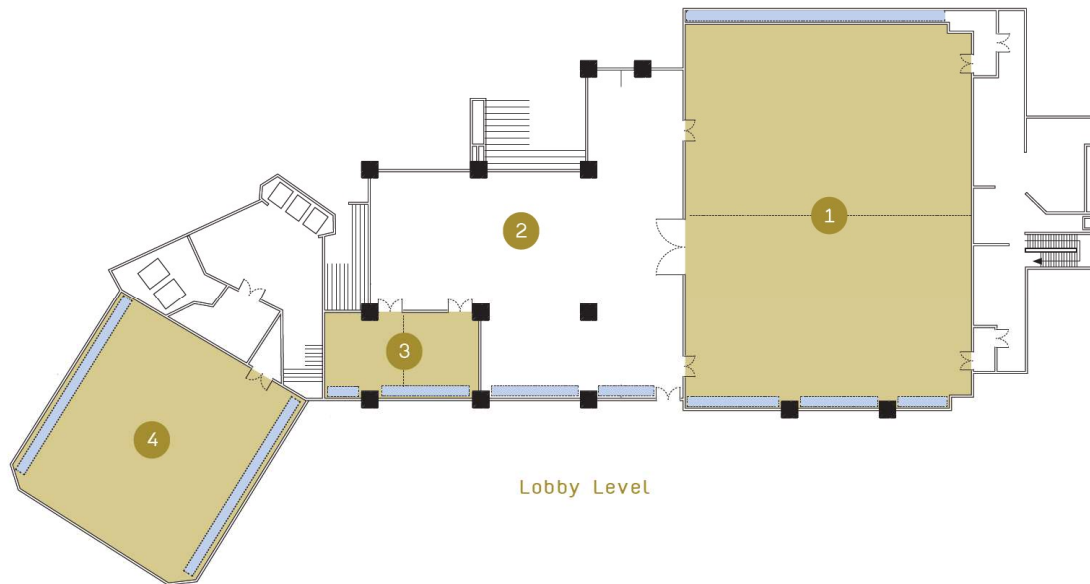
Three poster sessions will be run during the conference. Please make sure you are by your poster during the correct session below.

Session	Day	Time	Posters
1	Monday	16:30 – 17:30	Host-pathogen-vector interactions/Pathogenesis/Cell biology <i>Poster #s: 2,27,33,41,47,49,50,52,63,78,79,80,81,90</i>
2	Tuesday	13:00 – 13:30	Infection/Diagnosis <i>Poster #s: 6,30,51,53,60,73,74</i>
3	Wednesday	10:30 – 11:20	Epidemiology/Genomics <i>Poster #s: 11,14,19,20,25,36,48,54,75,91</i>

Area Map



Venue Map



1. Doi Tung
2. Doi Tung Foyer (Posters and Registration/Help Desk from Monday 4th November)
3. Doi Come (Speaker Room)
4. Doi Tong (M-floor)

Conference Events

Welcome Reception

18:00 – 21:30 Sunday 3rd November

Please join us for Welcome Reception drinks and canapés at the *Bell Tower (Hor Rakang)* at *The Riverie by Katathani* from 18:00, weather permitting.

Monday Dinner

18:30 – 22:00 Monday 4th November

Dinner on Monday evening will be hosted at *Hungry Wolf's Restaurant Steak & Ale House* from 18:30. Please feel free to make your own way there (it is a 5-minute walk, please see area map for location). Alternatively, conference staff will also be on hand to walk larger groups over to the restaurant.

Hungry Wolf's Restaurant Steak & Ale House

113/1 Kraisorasi, Tambon Wiang, Amphoe Mueang Chiang Rai, Chang Wat Chiang Rai 57000

Tuesday Afternoon Free Time

13:30 – 18:00 Tuesday 5th November

The afternoon of Tuesday is set aside for optional sightseeing trips of Chiang Rai town and province or simply for delegates to rest and relax. We are aiming to organise three options for delegates to choose from:

- Doi Tung Royal Lodge and Gardens
- Golden triangle, Hall of Opium Museum and Chiang Saen (old Lanna capital)
- Chiang Rai temple and cultural tour including Wat Rong Khun (white temple), Baan Dam (black house) and Wat Rong Sue Ten (Blue Temple)

On the day, further information will be given regarding these trips.

Conference Dinner

18:30 – 22:00 Tuesday 5th November

The Conference Dinner will be held on Tuesday evening from 18:30 at *Chivit Thammada Restaurant*. Transport will be arranged from the hotel to the restaurant from 18:00-18:30 and the return journey to the hotel from 21:30-22:30, details will be announced during the conference.

Chivit Thammada Restaurant

พิกัด 2 179 Bannrongseartean Soi 3, Mueang Chiang Rai District, 57100

Rickettsia TRN Workshop

Thursday 7th November

Members of the Rickettsia Threat Reduction Network are invited to attend the workshop on Wednesday afternoon and Thursday, which will be held in the *Doi Tong* room. Further details will be sent to members of the TRN. Invitation only event.

Visiting Chiang Rai, Thailand

Chiang Rai is a beautiful small city surrounded by hills and agricultural land. The people are friendly and welcoming and most will go out of their way to help you. Thailand is known as the '*land of smiles*'.

People may be shy to speak English with you but most people especially in tourist places/selling goods will be able to speak some English.

If you need any help or advice please feel free to ask any of the local staff who will be happy to help.

We're looking forward to welcoming you to Chiang Rai!

Arrival

Chiang Rai Mae Fah Luang International Airport is located northeast of the city centre and around 10km drive from The Riverie Hotel.

Metered taxis are available from the airport if you turn left out of arrivals (past the Dairy Queen) and exit the building. Tell the person on the stand outside where you would like to go and they will organise a taxi for you. An airport surcharge of 30THB is added to the fare on the meter (which will also start at 40THB).

We will arrange airport transfers for all APRC2 delegates.

Money

The Thai currency is Thai Baht (THB). 1 USD = 30-32 THB.

Large purchases can usually be made by credit card, including meals in restaurants.

Money exchange is available at the airport, local banks and the hotel. All major currencies are accepted. ATMs are also available at the airport and in the city centre.

Tipping

Tips are not expected in cafes, street food stores or metered taxis. Some hotels and air conditioned restaurants will add 7% government tax and 10% service charges (normally stated at the bottom of the menu). If service is not included in the bill in these settings tips would be welcome (around 10%).

Bargaining

Most shops have a fixed price. Food and vegetables stores tend to have fixed prices which are sometimes displayed (they seem to charge visitors and locals the same price). You can bargain at market stores if prices are not displayed especially when buying clothes and gifts. Bargaining should be a friendly, light hearted affair and not like an argument. No one wants to be left with bad feelings.

Communication

The country code for Thailand is +66. SIM cards are available at the airport for use in Thailand. International roaming services are also available.

Climate

November is the start of the cool, dry season in Chiang Rai. Average temperatures range from 20°C (68°F) to 30°C (86°F).

Dress

Cool loose-fitting clothes are recommended.

If you are visiting a temple or other religious sites you should cover your shoulders and your knees.

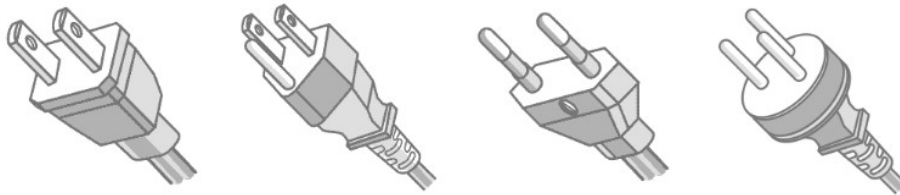
Health

Mosquito bite avoidance: repellent should be used. Covered clothing is advisable when out at dawn/dusk.

Tap water is NOT safe to drink. You should drink bottled water.

Electricity

220V, 50Hz. Plugs normally have 2 round or flat pins:



Culture

It is considered rude to sit with your feet pointing at another person or to touch someone else's head.

It is against the law to speak negatively or disrespectfully about the monarchy.

Monks are highly respected and have priority seating on public transport etc. You should give way to them and women should not touch monks.

Transport

Taxis

Metered taxis are available but you will not normally be able to find them on the street. To order a taxi:

- Use call centres: 053 793555 (but you will need a Thai speaker to help)
- Book via the hotel
- Use GRAB (via mobile phone app or www.grab.com/th/en)



Tuk tuks

Tuk tuks are available at tourist spots but tend to be more expensive than taxis and will offer you a fixed rate which you may be able to negotiate on.

Buses

The city bus runs from the airport to the old bus station 1 in town, central shopping plaza and the newer bus station 2 southwest of the city centre (www.livinginthailand.com/transportation-cr)

Eating and drinking

Thailand is well known for its delicious food and Chiang Rai is no exception. One of its most famous dishes is Kao Soi a coconut based curry with noodles.

A lot of food is cooked to order so vegetarian food and other options are available but state your dietary requirements clearly when ordered because many 'vegetable' dishes will contain some meat or sea food.



Chiang Rai is a large coffee and tea growing area so try some in a local shop. Iced coffee tends to be made very sweet so you can ask for more or less sugar when ordering. Tea tasting is available at the more up-market tea shops.

Ask the local staff for any help or recommendations.

Safety

Chiang Rai is generally a safe city but please take the usual precautions; do not carry large volumes of money or valuables with you. If out in the evening stay in well-lit areas and go with someone if you do not know your way around.

Keep a photocopy of your passport with you. Legally, visitors are meant to carry their original passport with them at all times.

Emergency numbers

- Tourist police 1155
- Emergencies e.g. fire, ambulance 191
- The Riverie Hotel +66 53 607 999
- Chiang Rai Clinical Research Unit +66 52 029842
- Dr Tri Wangrangsimakul, Head of CCRU and APRC2 Organiser +66 83 0745931
- APRC2 Secretariat +66 81 8471125

Travel Award Recipients

Travel awards are kindly sponsored by DTRA/BTRP and cover travel, registration and accommodation for awardees.

Sargis Aghayan	<i>Yerevan State University, Armenia.</i>
Sharanjeet Atwal	<i>Mahidol-Oxford Tropical Medicine Research Unit, Thailand. & Rutgers University, USA.</i>
Sandhya Dhawan	<i>Mahidol-Oxford Tropical Medicine Research Unit, Thailand.</i>
Ivo Elliott	<i>University of Oxford, UK.</i>
Remil Galay	<i>University of the Philippines Los Baños, Philippines.</i>
Shrijana Gurung	<i>New STNM MS Hospital, Sikkim, India.</i>
Veronique Hefter	<i>Philipps University Marburg, Germany.</i>
Siraj Khan	<i>Indian Council of Medical Research, NE Region, India.</i>
Sander Kujiipers	<i>Amsterdam Universitaire Medische Centra, Netherlands.</i>
Rawadee Kumler	<i>The Office of Disease Prevention and Control 12, Songkhla, Thailand.</i>
Fang Shiang Lim	<i>University of Malaya, Malaysia.</i>
Van Lun Low	<i>University of Malaya, Malaysia.</i>
Jonas Mehl	<i>Philipps University Marburg, Germany.</i>
Khin Myint	<i>Eijkman Institute for Molecular Biology, Indonesia.</i>
Siribun Panapruksachat	<i>Lao-Oxford-Mahosot Hospital-Wellcome Trust Research Unit, Lao PDR.</i>
Daniel Pavanelo	<i>University of São Paulo (USP), Brazil.</i>
Vanheuang Phommadeechack	<i>Lao-Oxford-Mahosot Hospital-Wellcome Trust Research Unit, Lao PDR.</i>
Weerawat Phuklia	<i>Lao-Oxford-Mahosot Hospital-Wellcome Trust Research Unit, Lao PDR.</i>
Kartika Saraswati	<i>Mahidol-Oxford Tropical Medicine Research Unit, Thailand & Eijkman-Oxford Clinical Research Unit, Indonesia.</i>
Manutsanun Sumonwiriya	<i>Armed Forces Research Institute of Medical Sciences (AFRIMS), Thailand.</i>
Piyanate Sunyakumthorn	<i>Armed Forces Research Institute of Medical Sciences (AFRIMS), Thailand.</i>
Melbourne Talactac	<i>Cavite State University, Philippines.</i>
Tshokey Tshokey	<i>JDWNR Hospital (JDWNRH) and Khesar Gyalpo University of Medical Sciences of Bhutan (KGUMSB), Bhutan.</i>
Khamsing Vongphayloth	<i>Institut Pasteur du Laos, Lao PDR.</i>

APRC2 Program Overview

Sunday 3rd November, 2019		
14:00 – 21:00	Registration & Help Desk	<i>Hotel reception</i>
17:00 – 18:00	Historical Lecture – Allen Richards	<i>Doi Tung</i>
18:00 – 21:00	Welcome Reception	<i>Bell Tower/ Hor Rakang</i>

Monday 4th November, 2019		
08:30 – 17:00	Registration & Help Desk	<i>Doi Tung Foyer</i>
08:30 – 08:35	Welcome – Nicholas Day (MORU)	<i>Doi Tung</i>
08:35 – 10:20	Session 1. Epidemiology/Diagnosis	
	<i>Plenary Speaker</i> – Thomas Weitzel	
10:20 – 10:45	Morning Break & Poster Preview	<i>Doi Tung Foyer</i>
10:45 – 12:30	Session 2. Pathogenesis, pathophysiology, cell biology/Genomics, transcriptomics, proteomics	<i>Doi Tung</i>
12:30 – 14:00	Lunch Break & Poster Preview	<i>Blossom Restaurant</i>
14:00 – 15:00	Session 3. Infection and Diagnosis	<i>Doi Tung</i>
15:05 – 16:20	<i>Parallel Session</i>	
	Session 4a. Epidemiology	<i>Doi Tung</i>
	Session 4b. Pathogenesis, pathophysiology, cell biology/Genomics, transcriptomics/Diagnosis	<i>Doi Tong (M-floor)</i>
16:20 – 17:30	Afternoon Break	
	Poster Session 1 – Host-pathogen-vector interactions/Pathogenesis/Cell biology	<i>Doi Tung Foyer</i>
17:30 – 18:30	Business meeting (<i>Invitation only</i>)	<i>Doi Come</i>
18:30 – 22:00	Dinner: Hungry Wolf's	

Tuesday 5th November, 2019		
08:30 – 14:00	Registration & Speaker Desk	<i>Doi Tung Foyer</i>
08:30 – 10:15	Session 5. Ecology/Epidemiology/Host-pathogen-vector interactions	<i>Doi Tung</i>
	<i>Plenary Speaker</i> - Serge Morand	
10:15 – 10:45	Morning Break & Poster Preview	<i>Doi Tung Foyer</i>
10:45 – 12:00	<i>Parallel Session</i>	
	Session 6a. Host-pathogen-vector interactions/Epidemiology	<i>Doi Tung</i>
	Session 6b. Epidemiology/Diagnosis	<i>Doi Tong (M-floor)</i>
12:00 – 13:30	Lunch Break	<i>Blossom Restaurant</i>
	Poster Session 2 – Infection/Diagnosis	<i>Doi Tung Foyer</i>
13:30 – 18:00	Free afternoon	
18:30 – 22:00	Conference Dinner: Chivit Thammada Restaurant	

Wednesday 6th November, 2019		
08:30 – 12:00	Registration & Speaker Desk	<i>Doi Tung Foyer</i>
08:30 – 10:20	Session 7. Epidemiology/Infection/Diagnosis/Treatment	<i>Doi Tung</i>
	<i>Plenary Speaker</i> - Yupin Suputtamongkol	
10:20 – 11:20	Morning Break	<i>Doi Tung Foyer</i>
	Poster Session 3 – Epidemiology/Genomics	<i>Doi Tung Foyer</i>
11:20 – 12:25	<i>Parallel Session</i>	
	Session 8. Immunity and prevention/Epidemiology/ Diagnosis	<i>Doi Tung</i>
	Session 8b. Epidemiology/Disease	<i>Doi Tong (M-floor)</i>
12:25 – 12:40	Closing Remarks (Young Investigators Awards, Invitations)	<i>Doi Tung</i>
12:40 – 14:00	Lunch Break	<i>Blossom Restaurant</i>
14:30	Business Meetings	<i>Doi Tong (M-floor)</i>

APRC2 Detailed Program

Sunday, 3rd November, 2019

14:00 – 17:00	Conference Registration & Speaker Desk	<i>Hotel Reception</i>
17:00 – 18:00	Historical Lecture <i>Session Chair: Tri Wangrangsimakul</i> Allen Richards (#16) Historical perspective and current status of the worldwide presence of orientiae and scrub typhus	<i>Doi Tung</i>
18:00 – 21:00	Welcome Reception	<i>Bell Tower/ Hor Rakang</i>

Monday, 4th November, 2019

08:30 – 17:00	Conference Registration & Speaker Desk	<i>Doi Tung Foyer</i>
08:30 – 08:35	Welcome Nicholas Day, Director of MORU	<i>Doi Tung</i>
08:35 – 10:20	Session 1. Epidemiology/Diagnosis <i>Session Chair: Nicholas Day</i> <i>Plenary Speaker</i> Thomas Weitzel Emerging rickettsial infections in South America - An update from Chile Stephen Graves and John Stenos (#45) An update on rickettsiae and rickettsial infections in Australia Sazaly Abubakar (#21) Serological evidence of Q fever, scrub typhus, spotted fever and typhus fever among Orang Asli populations of the Peninsular Malaysia Siraj Khan (#38) Spatiotemporal distribution of scrub typhus in Northeast India, 2014-2019 Philippe Parola (#86) MALDI-TOF MS for rapid identification of arthropod vectors of rickettsial agents	<i>Doi Tung</i>

10:20 – 10:45	Morning Break <i>Poster preview</i>	<i>Doi Tung Foyer</i>
10:45 – 12:30	Session 2. Pathogenesis, pathophysiology, cell biology/Genomics, transcriptomics, proteomics <i>Session Chair: Matthew Robinson</i> Jason Carlyon (#69) <i>Orientia tsutsugamushi</i> utilizes multiple mechanisms to antagonize NF-κB Christian Keller (#77) Differential recognition of live versus dead <i>Orientia tsutsugamushi</i> by endosomal RNA receptors Jeanne Salje (#5) Distinct developmental stages during the intracellular life cycle of <i>Orientia tsutsugamushi</i> Benjamin Makepeace (#28) An integrated <i>Rickettsia africae</i> chromosome in the nuclear genome of <i>Amblyomma variegatum</i> , vector of African tick-bite fever Constanza Martínez-Valdebenito (#32) Molecular description of <i>Candidatus Orientia chiloensis</i> , a novel <i>Orientia</i> species causing scrub typhus in Chile Elizabeth Batty (#76) Target enrichment sequencing: a new tool to investigate <i>Orientia tsutsugamushi</i> genomics	<i>Doi Tung</i>
12:30 – 14:00	Lunch Break <i>Poster preview</i>	<i>Blossom Restaurant</i>
14:00 – 15:00	Session 3. Infection and diagnosis <i>Session Chair: Tri Wangrangsimakul</i> Katia Abarca (#26) Demographic, clinical, and laboratory features of South American scrub typhus in southern Chile, 2015-2019 Khin Myint (#59) <i>Rickettsial felis</i> with central nervous system involvement in Indonesia Abraham Goorhuis (#64) Searching for, and finding the hidden treasure: Rickettsial disease among Dutch international travellers	<i>Doi Tung</i>

Cecilia Kato (#65)
Enhanced laboratory diagnosis of rickettsial diseases and scrub typhus at the acute stage of illness

15:05 – 16:20

**Session 4a (Parallel Session).
Epidemiology**

Doi Tung

Session Chair: Allen Richards

Pierra Mazzega (#12)
Power laws of scrub typhus incidence in Thailand (2003-2018)

Remil Galay (#15)
Molecular detection of *Rickettsia* spp. in dogs and *Coxiella burnetii* in ruminants in the Philippines

Tamalee Roberts (#37)
Differential spatiotemporal dynamics of scrub typhus and murine typhus in the Lao PDR

Kartika Saraswati (#71)
Mapping scrub typhus in Indonesia: Preliminary results

Rachel Greer (#24)
Importance and challenges of public engagement work on scrub typhus in Chiang Rai

**Session 4b (Parallel Session).
Pathogenesis, pathophysiology, cell biology/Genomics,
transcriptomics/Diagnosis**

Doi Tong (M-floor)

Session Chair: Jeanne Salje

Sharanjeet Atwal (#62)
Peptidoglycan in Rickettsiales species

Piyanate Sunyakumthorn (#68)
Dissemination of *Orientia tsutsugamushi* in the rhesus macaque scrub typhus model

Kentaro Kasama (#9)
Genomic features of *Rickettsia heilongjiangensis* revealed by intraspecies comparison and detailed comparison with *Rickettsia japonica*

Weerawat Phuklia (#55)
Investigations of mutations in 16S rRNA gene sequences, a possible doxycycline target of *Orientia tsutsugamushi*

Ratree Takhampunya (#58)
Metagenomic approach to characterizing disease epidemiology in a disease-endemic environment

16:20 – 17:30	Afternoon Break	<i>Doi Tung Foyer</i>
	Poster Session 1 Host-pathogen-vector interactions/Pathogenesis/ Cell biology	<i>Doi Tung Foyer</i>
17:30 – 18:30	Business meeting <i>Invitation only</i>	<i>Doi Come</i>
18:30 – 22:00	Dinner <i>Hungry Wolf's Restaurant</i>	

Tuesday, 5th November, 2019

08:00 – 14:00	Conference Registration & Speaker Desk	<i>Doi Tung Foyer</i>
08:30 – 10:15	Session 5. Ecology/Epidemiology/Host-pathogen-vector interactions <i>Session Chair: Benjamin Makepeace</i> <i>Plenary Speaker</i> Serge Morand Integrating environmental changes in disease ecology of scrub typhus Ivo Elliott (#10) The clinical epidemiology of scrub typhus in humans, chiggers and rodents Kevin Macaluso (#43) The biology of emerging <i>Rickettsia felis</i> rickettsiosis Chi-Chien Kuo (#35) Invasive plants facilitated by socioeconomic change shelter vectors of scrub typhus	<i>Doi Tung</i>

	Tshokey Tshokey (#17) <i>Rickettsia</i> and ticks in Bhutan	
10:15 – 10:45	Morning Break <i>Poster preview</i>	<i>Doi Tung Foyer</i>

10:45 – 12:00

Session 6a (Parallel Session).

Doi Tung

Host-pathogen-vector interactions/Epidemiology

Session Chair: Chi-Chien Kuo

Daniel Pavanelo (#40)

Rickettsia rickettsii modulates the quantity but not the composition of *Amblyomma aureolatum* midgut microbiota

Gerardo Acosta-Jamett (#46)

First identification of trombiculid mites (Acari: Trombiculidae) on rodents captured on Chiloé Island, an endemic region of scrub typhus in southern Chile

Chanakan Suwanbongkot (#56)

Infection and transmission dynamic of spotted fever group *Rickettsia* in *Amblyomma Maculatum*

Khamsing Vongphayloth (#67)

The hard tick of Laos: an update checklist of species record

Matthew Robinson (#66)

Sero-survey of market vendors associated with the bushmeat trade in Lao PDR

Session 6b (Parallel Session).

Doi Tong (M-floor)

Epidemiology/Diagnosis

Session Chair: Cecilia Kato

Ju Jiang (#44)

Detection of *Rickettsia* species in *Haemaphysalis flava* from the Republic of Korea

Nor-Azlina A.A. (#82)

Molecular Prevalence of *Ehrlichia canis* and *Anaplasma platys* in Stray Dogs and their ticks in peninsular Malaysia

Sandhya Dhawan (#18)

Review of diagnostic cut-offs for murine typhus IgM and IgG IFAs

Sander Kuijpers (#29)

InBios Scrub Typhus Detect IgG and IgM ELISA kits for the diagnosis of scrub typhus acquired in Chile: proposed cut-off values

Vanheuung Phommadeechack (#72)

Review of factors affecting rickettsial isolations at Mahosot Hospital, Lao PDR

12:00 – 13:30	Lunch Break	<i>Blossom Restaurant</i>
	Poster Session 2 Infection/Diagnosis	<i>Doi Tung Foyer</i>
13:30 – 18:00	Free afternoon <i>Further details on optional trips will be provided</i>	
18:30 – 21:30	Conference Dinner <i>Chivit Thammada Restaurant</i>	

Wednesday, 6th November, 2019

08:30 – 10:45	Conference Registration & Speaker Desk	<i>Doi Tung Foyer</i>
08:30 – 10:20	Session 7. Epidemiology/Infection/Diagnosis/Treatment <i>Session Chair: Stephen Graves</i>	<i>Doi Tung</i>

Plenary Speaker

Yupin Suputtamongkol

Scrub typhus and acute undifferentiated fever in Asia

Manisha Biswal (#88)

Rickettsia conorii and *Rickettsia typhi*: not uncommon causes of acute febrile illness in India

Stuart Blacksell (#57)

Biosafety and biosecurity requirements for *Orientia* spp. diagnosis and research: Recommendations for risk-based biocontainment, work practices and the case for reclassification to Risk Group 2

J. Stephen Dumler (#13)

High-throughput screening of modulators of cellular calcium metabolism as potential drugs against rickettsia-induced microvascular dysfunction

Paul Newton (#31)

Prospective, open-label, randomized trials of doxycycline versus azithromycin for the treatment of uncomplicated murine typhus and scrub typhus

Tri Wangrangsamakul (#23)

Scrub typhus and the misconception of doxycycline resistance

10:20 – 11:20 **Morning Break** *Doi Tung Foyer*

Poster Session 3 *Doi Tung Foyer*
Epidemiology/Genetics

11:20 – 12:25 **Session 8a (Parallel Session).** *Doi Tung*
Immunity and prevention/Epidemiology/Diagnosis
Session Chair: Paul Newton

Nam-Hyuk Cho (#7)
Immunization with a recombinant antigen composed of conserved blocks from TSA56 provides broad genotype protection against scrub typhus

Roman Ganta (#61)
Rickettsia rickettsii whole cell antigen vaccine confers protection against Rocky Mountain spotted fever

John Stenos (#70)
Evidence of Q fever and rickettsial disease in Chile

George Varghese (#87)
New insights into the diagnosis of scrub typhus

Session 8b (Parallel Session). *Doi Tong (M-floor)*
Epidemiology/Diagnosis
Session Chair: Stuart Blacksell

Ram Raghavan (#84)
Potential geographic distribution of *Amblyomma americanum* (Acari: Ixodidae) in New Zealand

Carol Devamani (#83)
Risk factors for scrub typhus, murine typhus and spotted fever seropositivity in urban areas, rural plains, and peri-forest hill villages in South India: cross-sectional study

Wolf Schmidt (#85)
Antibody response following scrub typhus infection: Clinical cohort study

Jasleen Kaur (#89)
Prevalence and genetic diversity of *Orientia tsutsugamushi* in scrub typhus patients in North India

12:25 – 12:40	Closing Session Young Investigators Awards (oral & poster) Invitations Provisional plans for APRC3 TTP10 24 th -28 th August 2020, Danube Delta, Romania International Rickettsia Meeting 24 th -27 th August 2020, Lausanne, Switzerland	<i>Doi Tung</i>
12:40	Conference Closed	
12:40 – 14:00	Lunch Break	Blossom Restaurant
14:30 – 17:00	Business Meetings	<i>Doi Tong (M-floor)</i>

Abstracts

Oral Presentations

Sunday, 3rd November 2019

Historical Lecture

Abstract 16: Historical perspective and current status of the worldwide presence of orientiae and scrub typhus.

Richards AL¹

¹Department of Preventive Medicine and Biostatistics, Uniformed Services University of the Health Sciences, Bethesda, Maryland, USA

Scrub typhus and its etiological agents have been around a very long time, maybe since the dawn of time. As suggested by Andersson *et al.*, the *Rickettsia*, closes relative to *Orientia*, phylogenetically are related to mitochondria and furthermore a rickettsial ancestor may have predated the mitochondria. Human historical reference to scrub typhus was only first described in Chinese literature in Zhouhofang in 313 BC and then not again until 1810 when Hakuju Hashimoto described tsutsuga, a noxious harmful disease in the Niigata prefecture. This was followed by first account of Tsutsugamushi disease from Japan reported in Europe literature by Theobald Palm in 1878. Other clinicians and scientists in Sumatra, Malaysia and India reported on a disease most likely to have been scrub typhus in the early 1900s. All of these initial reports about scrub typhus were from the Tsutsugamushi Triangle. It was not until the 21st century that evidence of scrub typhus outside of Asia-Australia-South Pacific Island region was published. This report will describe in more detail the history of scrub typhus, its epidemiology, the diverse causative agents, and their ecology.

Monday, 4th November 2019

Session 1: Epidemiology/Diagnosis

Abstract 45: An update on rickettsiae and rickettsial infections in Australia.

Stephen Graves¹ and John Stenos¹

¹Australian Rickettsial Reference Laboratory

Australia is an island continent with a wide range of rickettsiae and rickettsial infections. These including *Orientia tsutsugamushi* (scrub typhus) in northern tropical Australia; three different Spotted Fever Group rickettsiae that cause human infections, *Rickettsia australis* (Queensland Tick Typhus), *R. honei* (Flinders Island Spotted Fever) and *R. felis* (cat flea typhus); and a Typhus Group rickettsia, *R. typhi* (murine typhus).

Febrile returned travellers to Australia are regularly shown to have rickettsial infection.

Coxiella burnetii (Q Fever) is widespread with approximately 1 in every 20 Australian exposed.

There has not yet been any recognition of human infections with *Anaplasma* spp or *Ehrlichia* spp in Australia.

There are important animal rickettsial infections in Australia, including *A. platys* in dogs and *A. marginale* in cattle.

Newly described Australian rickettsiae will be discussed, many associated with endogenous Australian ticks and their native vertebrate hosts. Pathogenicity for humans is rarely known.

Abstract 21: Serological evidence of Q fever, scrub typhus, spotted fever and typhus fever among Orang Asli populations of the Peninsular Malaysia

Chee Sieng Khor¹, Jing Jing, Khoo¹, Brian L. Pike², Sazaly AbuBakar^{1,3}

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The prevalence of Q fever, scrub typhus, spotted fever and typhus fever among the indigenous people of the Peninsular Malaysia, also known as the Orang Asli, is rarely reported. The Orang Asli communities, many of which are located within the forest fringe areas are particularly at high risk due to the close contact with wildlife and arthropod vectors such as ticks and fleas. Here, the seroprevalence of Q fever, spotted fever and typhus fever among the selected Orang Asli communities in Peninsular Malaysia was determined. 477 Orang Asli sera were obtained from 6 selected Orang Asli villages. Presence of IgG antibodies reactive against the rickettsial agents was determined using commercially available ELISAs. IgG antibodies reactive against Q fever, scrub typhus, spotted fever and typhus fever agents were detected in 9%, 18%, 15% and 5% respectively of the total population tested, with varying degrees of exposures in different villages. The highest prevalence for Q fever, scrub typhus, spotted fever and

typhus fever was observed in villages in Kelantan (24%), Perak (26%), Kedah (32%) and Johor (12%) respectively.

It is important now to expand the surveillance efforts to identify the source of infections, especially among the wildlife and arthropod vectors, and to determine the risk factors for these diseases. With human activities reaching closer to the forested areas brought about by rapid change of land use and development in Malaysia, it becomes important to understand the transmission of these diseases to control the spread to the greater public.

Abstract 38: Spatiotemporal distribution of scrub typhus in Northeast India, 2014-2019

Siraj Khan¹, Anisha Shah¹, Himanshu Medhi¹, Jahnabi Saikia¹ and Trishna Bora¹

¹Medical Entomology, Arbovirology and Rickettsial Disease Division, Indian Council of Medical Research, Regional Medical Research Centre, Northeast Region, Dibrugarh, Assam, India,

Following re-emergence of scrub typhus (ST) in Northeast (NE) India during 2010, there has been an increasing incidence of the disease across the 8 states of NE region of India. Among these 8 NE states, Assam has been the worst affected, with ST being identified as an important etiology of acute febrile illness (AFI) and acute encephalitis syndrome (AES). However, the spatiotemporal dynamics of ST and the potential epidemiological risk factors remains to be characterized. The present study aimed to explore the disease dynamics in terms of its distribution and dispersion in Assam over five and a half years during January 2014 - June 2019, to identify the disease reporting clusters and locate the high risk zones. Month-wise case series of ST were analyzed for temporal distribution. Spatiotemporal distribution pattern was analyzed by the Inverse Distance Weighting Interpolation (IDW) analysis and Kernel density (KD) estimation method. The Getis-Ord $G_i^*(d)$ Z score was used to identify the intensity of hotspot (CI-95%) of the study area. During the study period, a total of 421 ST cases were identified. Majority of the cases were reported between June-August each year. Spatiotemporal analyses revealed varied clusters over the years, with predominant clusters in 3 Upper Brahmaputra valley districts (Dibrugarh, Tinsukia and Sivasagar) of Assam. Hot spot mapping showed a diffused pattern identifying 4 hot spots in the Upper Brahmaputra valley. This study identified the ST clusters in Assam, which could be targeted with public health interventions to check the spread of the disease.

Abstract 86: MALDI-TOF MS for rapid identification of arthropod vectors of rickettsial agents

Philippe Parola¹

¹University Hospital Institute Méditerranée Infection, Marseille, France

In the last few years, matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) has been used for the protein-profiling identification of bacteria and other microorganisms. This has led to a revolution in clinical microbiology. More recently, MALDI-TOF MS has been recently applied by our group for the identification of several arthropod vectors, such as ticks, fleas, mosquitoes from laboratory, collection or field specimens. It has also been used to differentiate

arthropods infected or not by rickettsiae. This communication aims to present our most recent developments of this technology in the field of rickettsial diseases.

Session 2: Pathogenesis, pathophysiology, cell biology/Genomics, transcriptomics, proteomics

Abstract 69: *Orientia tsutsugamushi* utilizes multiple mechanisms to antagonize NF- κ B

Jason Carlyon¹, Tanaporn Wangsanut¹, Sarika Gupta¹ and Haley Adcox¹

¹Virginia Commonwealth University

The obligatory intracellular lifestyle of rickettsial pathogens primes them for immunodetection and consequently eliciting innate immune signaling. As the ensuing cell-mediated immunity contributes to both controlling disease and immunopathogenesis, understanding how these bacteria modulate innate immune signaling is critical. The transcription factor, NF- κ B, is a tightly regulated initiator of the antimicrobial response. Typically, I κ B α and p105 bind to and sequester the NF- κ B p50:p65 heterodimer in the cytoplasm. Canonical activation of NF- κ B involves IKK-mediated phosphorylation of I κ B α and p105, which leads to their ubiquitination and proteasomal degradation and hence induces NF- κ B nuclear translocation. A portion of ubiquitinated p105 is processed into NF- κ B p50. *Orientia tsutsugamushi* has evolved to counter innate immune signaling. Indeed, we previously reported that two *O. tsutsugamushi* ankyrin repeat-containing effectors called Ank1 and Ank6 facilitate NF- κ B p65 nuclear export. Here, we present that their ability to do so is linked to three tandemly-arranged C-terminal motifs – the PRANC (originally identified in poxviral Ank proteins), F-box (a eukaryotic-like domain), and ISR (interspacer region between the N-terminally located ankyrin repeats and PRANC) – each of which contributes to recruiting individual components of the host cell SCF1 ubiquitination ligase complex. As a complementary strategy, *O. tsutsugamushi* also elevates p105 levels by as much as six-fold in bacterial dose- and protein synthesis-dependent manners even in the presence of TNF α , an otherwise robust NF- κ B activator. Overall, these data demonstrate that *O. tsutsugamushi* uses a multi-pronged approach to negatively regulate NF- κ B.

Abstract 77: Differential recognition of live versus dead *Orientia tsutsugamushi* by endosomal RNA receptors

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Orientia tsutsugamushi enters the cell through the endocytic pathway, but soon after escapes the endosome to replicate in the cytoplasm. Live *Orientia* are potent inducers of pro-inflammatory cytokines (TNF- α , IL-6) and type I interferons (IFNs). We and others observed that heat inactivation of *Orientia* reduced its capacity to induce IFN- β , but not pro-inflammatory cytokines, suggesting a specific recognition mechanism for live in contrast to dead bacteria.

In mouse bone marrow-derived dendritic cells (BMDC) infected with *Orientia*, inhibition of endosome acidification by chloroquine or bafilomycin A abrogated the induction of IFN- β and TNF- α , suggesting that endosomal maturation is required for immune activation. BMDC from triple-knockout mice lacking the Toll-like receptors (TLR) 3, 7 and 9 showed reduced transcription of IFN- β and TNF- α , upon both infection and stimulation with heat-inactivated *Orientia*, compared to the wildtype. Using single TLR-deficient BMDC, we demonstrated that TLR7, but not TLR3 or 9, was required for induction of IFN- β and TNF- α mRNA upon infection with live *Orientia*. Contrarily, heat-inactivated *Orientia* were recognized by TLR3 and TLR13, but not TLR7, suggesting differential receptor usage for recognition of RNA from *Orientia*. Interestingly, BMDC deficient for the cytoplasmic adaptor protein MAVS showed no reduced cytokine transcription, suggesting that RIG-I is not involved in *Orientia* RNA recognition in BMDC.

Altogether, these results suggest RNA recognition significantly contributes to inflammation induced by *Orientia*, with TLR7 recognizing live *Orientia*, and TLR3 and TLR13 involved in RNA recognition of heat-inactivated organisms. TLR usage for endosomal RNA recognition thus depends on the viability of *Orientia*.

Abstract 5: Distinct developmental stages during the intracellular life cycle of *Orientia tsutsugamushi*

Suparat Giengkam¹, Jantana Wongsantichon¹, Sharanjeet Atwal², Yanin Jaiyen¹, Piyanate Sunyakumthorn³, Radoslaw Sobota⁴ and Jeanne Salje²

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⁴ASTAR

The rickettsial bacterium *Orientia tsutsugamushi* is the causative agent of the severe vector-borne human disease scrub typhus. *O. tsutsugamushi* is an obligate intracellular bacterium that escapes from the endolysosomal pathway shortly after entry and replicates directly in the host cell cytoplasm. Unlike many other obligate intracellular bacteria (*Coxiella burnettii*, *Chlamydia* spp., *Anaplasma* spp.) *O. tsutsugamushi* does not cycle between two morphologically distinct developmental stages. Bacterial changes over the approximately 7-day cellular infection of *O. tsutsugamushi* have not been described in detail. Here, we use a combination of light microscopy, gene expression analysis and proteomics techniques to show that *O. tsutsugamushi* transitions between five distinct developmental stages and we characterise their differences in morphology, infectivity and metabolic activity. These results provide a framework for future studies on the host-pathogen cell biology of this important but understudied human pathogen.

Abstract 28: An integrated *Rickettsia africae* chromosome in the nuclear genome of *Amblyomma variegatum*, vector of African tick-bite fever

Alistair Darby¹, Alaa Al-Khafaji¹, Mark Whitehead¹, Catherine Hartley¹, Glen Robinson¹, Stuart Armstrong¹, Aleksandra Beliavskaia¹, Germanus Bah², Naftaly Githaka³, Lesley Sakyi¹ and Benjamin Makepeace¹

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Amblyomma variegatum, the tropical bont tick, is one of the most important ticks transmitting pathogens to humans and livestock in sub-Saharan Africa and the Caribbean. It is the primary vector of two bacteria: *Ehrlichia ruminantium*, the aetiological agent of heartwater in ruminants, and *Rickettsia africae*, which causes African tick-bite fever in humans. Unusually for a pathogenic *Rickettsia* sp., the prevalence of *R. africae* in *A. variegatum* has been reported to be close to fixation. We confirmed this using specimens collected from the Adamawa Region of Cameroon, where 95.3% of ticks removed from cattle were positive by qPCR. The Tick Cell Biobank maintains two *A. variegatum* cell lines (AVL/CTVM13 and AVL/CTVM17) that were found to be positive by PCR for several rickettsial genes; Sanger sequencing confirmed that these genes were of *R. africae* origin. Unexpectedly, no microscopic or proteomic evidence of a bacterial infection in these cell lines was evident, while tetracycline treatment of cultures over two months had no significant effect on *R. africae* DNA signal. We extracted high molecular-weight DNA from AVL/CTVM17 cells and an adult *A. variegatum* from a colony maintained in Nairobi. Using Chromium 10x libraries, we sequenced these genomes and obtained an assembly of ~6 Gb for the tick (the first from an *Amblyomma* sp.) and a lower-coverage assembly for the cell line. Both these genomes contained an almost complete, integrated *R. africae* chromosome. This finding has significant implications for the epidemiology of *R. africae* and suggests that other tick genomes may contain integrated rickettsial DNA.

Abstract 32: Molecular description of *Candidatus Orientia chiloensis*, a novel *Orientia* species causing scrub typhus in Chile

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Background: Scrub typhus is an important and potentially fatal vector-borne rickettsiosis. Until recently, the infection was limited to the “tsutsugamushi triangle” in Pacific-Asia, caused by *Orientia tsutsugamushi*. In 2006, two individual patients acquired scrub typhus in regions outside Asia-Pacific; one in the United Arab Emirates, caused by *Candidatus O. chuto*; the other on Chiloé Island in southern Chile, associated to an unknown *Orientia* species. Since 2015, our group confirmed further autochthonous scrub typhus cases on Chiloé Island and continental Chile. This study presents a genetic characterization of the Chilean isolates, suggesting that they represent a new species, *Candidatus Orientia chiloensis*.

Methods: We extracted total DNA from eschars of 18 patients with scrub typhus acquired in southern Chile. 16S rRNA (rrs) and 47-kDa (htrA) genes were amplified by PCR, sequenced, phylogenetically analyzed, and compared to different strains of *O. tsutsugamushi* and to *Candidatus O. chuto*.

Results: Nucleotide sequences of rrs and htrA showed 99.7-100% identity between the 18 Chilean isolates. Nonetheless, significant sequence diversity was observed in comparison to *O. tsutsugamushi* (3.5% rrs gene; 11.2% htrA gene) and *Candidatus O. chuto* (3% rrs gene; 14.8% htrA gene). For both genes, the phylogenetic analysis grouped the Chilean specimens in a single clade outside the other *Orientia* species.

Conclusion: Considering the high genetic divergence and geographic distance of Chilean isolates to the known *Orientia* species, our results suggest a novel species within the *Orientia* genus. Due to the initial discovery of this pathogen on Chiloé Island, we propose the name *Candidatus Orientia chiloensis*.

Abstract 76: Target enrichment sequencing: a new tool to investigate *Orientia tsutsugamushi* genomics

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Whole-genome sequencing is a powerful tool to explore the epidemiology of bacterial pathogens, but routine sequencing of rickettsial species is still challenging due to the difficult and laborious nature of bacterial culture. We present a target enrichment sequencing method designed to capture the genome of *Orientia tsutsugamushi* and allow for direct sequencing from human and animal samples without the need for culture. This method uses whole-genome amplification, sequence probes designed to the known diversity of *Orientia tsutsugamushi* and Illumina sequencing to generate sequence data which can be compared to a reference genome. Using this method, we sequenced the genomes of *O. tsutsugamushi* directly from chigger samples, and demonstrate that the method can be used to investigate the phylogeography of *O. tsutsugamushi* and look at how genomes cluster geographically, between chiggers isolated from the same individual, and between genomes collected from diverse rodent and chigger host species. We outline improvements to the laboratory and computation methods which will be required to make direct-from-sample sequencing for *Orientia tsutsugamushi* a routine procedure.

Session 3: Infection and Diagnosis

Abstract 26: Demographic, clinical, and laboratory features of South American scrub typhus in southern Chile, 2015-2019

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Introduction. Within the last years, endemic scrub typhus (ST) was confirmed in patients from Chiloé Island and other regions in southern Chile. Here we report the demographic, clinical, and laboratory features of cases diagnosed from 2015 to 2019.

Methods. Since 2015, our group implemented surveillance for patients with suspected ST in Chile, defined as fever plus ≥ 1 of following criteria: eschar, rash, thrombocytopenia, and transaminitis. Demographic data were obtained using a standardized questionnaire. Cases were diagnosed by PCR (eschar material/buffy coat) and/or IgG seroconversion.

Results. Among 70 included patients, ST was confirmed in 40 cases (39 PCR positive, 1 seroconversion, 13 both). 49% were acquired on Chiloé Island and 51% on mainland Chile. 71% were male; the median age was 42 years (range 17-72). All occurred during the summer months and were associated to outdoor activities. Infections were acquired in 3 regions (distance $>1,930$ km). Apart from fever, patients presented with eschar (98%), headache (93%), maculopapular rash (90%), myalgia (85%), night sweats (73%), regional lymphadenopathy (32%), and impaired consciousness (22%). Lab abnormalities were elevated CRP (94%) and transaminases (71%), thrombocytopenia (42%), and leukopenia (39%). 45% of patients required hospitalization. All recovered without sequelae, 90% after treatment with doxycycline, 5% with azithromycin, and 5% spontaneously.

Conclusion. South American ST mostly manifested as an acute febrile disease with eschar and/or rash, accompanied by other non-specific symptoms; typical lab alterations include elevated CRP and transaminases. Although almost half of patients required hospitalization, severe or fatal cases have yet not been registered.

Abstract 59: *Rickettsia felis* with central nervous system involvement in Indonesia

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Rickettsia felis has recently emerged worldwide as a cause of human illness. Typically causing mild, undifferentiated fever, it has been implicated in several cases of neurological disease. Although rickettsial etiology of acute febrile cases is well documented in Southeast Asia, there is limited information on central nervous system (CNS) rickettsioses. CSF specimens from patients >15 years old admitted to Kandou General Hospital, Manado, North Sulawesi from August 2015–February 2017 with presumed CNS infections were screened for *O. tsutsugamushi* and *Rickettsia* spp. using qPCR targeting 47-kDa and 23S rRNA genes. Positive samples were characterized using semi-nested PCR targeting the 17-kDa, ompB, and gltA genes. In addition, sera and CSF specimens were tested for rickettsial antibodies with scrub typhus, spotted fever group, and typhus group IgM ELISA.

Two out of 38 available CSF samples were positive for *Rickettsia* DNA as determined by genus-specific qPCR, both from fatal cases. DNA from both cases had 100% sequence homologies to the *R. felis* reference genes 17-kDa and ompB, and 99.91% to gltA. No evidence was found of other possible pathogens in either the CSF or blood. Moreover, positive IgM was found in 8 of 30 specimens (7 serum and 1 CSF) for typhus group, and 1 of 35 CSF specimens for spotted fever group. *R. felis*, which is usually associated with non-specific febrile illness, was found in the CSF of two fatal CNS infections. Further studies are urgently needed as morbidity and mortality could be considerably reduced through recognition, timely and available pharmacotherapy.

Abstract 64: Searching for, and finding the hidden treasure: Rickettsial disease among Dutch international travelers

Sophie de Vries¹, Louise van Eekeren¹, Hans van der Linden¹, Benjamin Visser¹, Martin Grobusch¹, Jiri Wagenaar², Marga Goris¹ and Abraham Goorhuis¹

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Background. Rickettsial disease (RD) is a prevalent and underestimated cause of febrile illness worldwide. In this study, we attempted to quantify the underestimation at our clinic, by reviewing past cases of febrile illness in travelers who had tested negative for leptospirosis, a disease that initially has many similarities with RD.

Methods. We performed a retrospective analysis in Dutch travelers who had returned from Asia, Africa or the Americas between 2010-2017 with a febrile illness and who had tested negative for leptospirosis. Immunofluorescence assays were performed for *Orientia tsutsugamushi* (scrub typhus), and Typhus Group, and Spotted Fever Group RD. For cases with available single or convalescent samples, patient charts were reviewed. In case of a fitting medical history, cases were deemed confirmed or suspected.

Results. We included sera of 97 patients, 17 of which (17.5%) had a convalescent sample available, and 80 (82.5%) only a single sample. Among the 17 with a convalescent sample, there were 11 (64.7%) laboratory confirmed cases (seroconversion or a ≥ 4 -fold titer rise). Among the 80 with only a single

sample, 21 (26.3%) laboratory suspected cases (single IgM or IgG titers above the cut-off values) were identified. Clinically, RD was the likely diagnosis in 8/17 (47.1%) patients with a convalescent sample and in 8/80 (10%) patients with only a single sample.

Conclusions. In this study, RD was an important and poorly recognized cause of illness, especially among patients of which convalescent samples were available. Prospective, research should be conducted to reduce this under-estimation.

Abstract 65: Enhanced laboratory diagnosis of rickettsial diseases and scrub typhus at the acute stage of illness

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Introduction: Rickettsial disease and scrub typhus infections occur worldwide in all continents except Antarctica. These diseases range from mild to severe/lethal. Early clinical symptoms are nonspecific and may include fever, headache, and malaise, with or without the observation of a rash or eschar. Nonspecific symptoms cause difficulties in clinical diagnosis and appropriate treatment may not be administered. Current molecular assays are capable of efficiently detecting genomic DNA targets at a limit-of-detection of ~9 copies/5µL of blood, or 1,800 genome copies/mL of blood for reproducible detection, well above the low level of bacteremia observed at ~100 copies/mL. Methods: Highly sensitive reverse transcriptase real-time PCR assays were developed and validated for *Rickettsia* genera (RCKr) and *Orientia tsutsugamushi* (OTSr) detection. Results: RCKr has high analytical specificity and >100X the sensitivity in contrived samples. RCKr testing of acute clinical blood samples averaged 5.1 CT values lower than the current DNA real-time PCR assay. The differences in CT values were even greater when testing fatal case samples, where average CT values were 7.6 values lower than DNA detection assays. Ten-fold serial dilutions revealed RCKr increases detection sensitivity 100 to 100,000X. *Orientia tsutsugamushi* testing of non-fatal acute clinical samples showed an average of 4.7 values lower than current DNA detection. Conclusions: Detection of *Rickettsia* and *Orientia tsutsugamushi* in acute blood samples historically has been elusive. These findings represent important steps in the pursuit of more accurate diagnosis of Rickettsial diseases and scrub typhus. Further validation is being conducted with larger sets of patient samples.

Session 4a: Epidemiology

Abstract 12: Power laws of scrub typhus incidence in Thailand (2003-2018)

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The database of the Thai Ministry of Public Health lists more than 102,000 individual cases of scrub typhus between January 2003 and December 2018. Prior to any modelling, this study characterizes the most relevant and robust properties of these data. Through a spatial or temporal aggregation, several distributions of the data are highlighted which resemble power laws.

First, a maximum likelihood approach is used to determine the exponent of the data probability density function (pdf). A Kolmogorov-Smirnov statistic test quantifies the validity of the hypothesis of an underlying power law, given the noise level of the data and their limited number. The best candidates to fit the data pdf are truncated power law, stretched exponential and log-normal distributions.

Two Taylor's power laws linking data variances and means are also found with different exponents when performing data aggregation in space (Thailand-level monthly incidences) or in time (province-level 16-years period incidences). The exponents are estimated with various approaches: ordinary least squares, geometric mean regression, Perry's third method, and a bi-objective L1-norm optimization. Because of the lack of knowledge of the data error distribution, these methods provide different but coherent and acceptable exponent estimates.

These three power laws show an abrupt change of their exponent value that also have to be explained by candidate models. Finally, a critical reflection is proposed by comparing various types of modelling approaches able to account simultaneously for these robust properties of the incidence of scrub typhus in Thailand.

Abstract 15: Molecular detection of *Rickettsia* spp. in dogs and *Coxiella burnetii* in ruminants in the Philippines

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Tick-borne pathogens (TBPs) such as *Rickettsia* spp. and *Coxiella burnetii* are known to be zoonotic. Infection in animals may be unnoticed, but are usually severe and potentially fatal to humans. The

tropical climate of the Philippines is very conducive to thriving of ticks which primarily affects cattle and dogs. However, there is very little knowledge on the occurrence of zoonotic TBPs in the Philippines. This study investigated the occurrence of *Rickettsia* spp. in dogs and *C. burnetti* in large ruminants using nested PCR (nPCR). Blood samples were collected from a total of 258 dogs through veterinary clinics in cities and municipalities, and 520 cattle and water buffaloes from four provinces. After DNA extraction, nPCR targeting *Rickettsia* gltA gene and nPCR targeting *C. burnetti* com1 gene was performed for blood DNA of dogs and large ruminants, respectively. A positive band for *Rickettsia* spp. was observed in blood samples from 6 (2.3%) dogs. Interestingly, some of these dogs also tested positive for other TBPs, and there are some that did not show any clinical signs at the time of sample collection. Meanwhile, 10 blood samples from large ruminants showed a positive band for *C. burnetii*. Sequence analysis of positive amplicons revealed high identity with reported isolates of *Rickettsia japonica* and *C. burnetii*. To our knowledge, this is the first molecular evidence of *Rickettsia* spp. in dogs and *C. burnetii* in ruminants in the Philippines. Further studies are needed to determine their geographic distribution and raise awareness on potential public health implications.

Abstract 37: Differential spatiotemporal dynamics of scrub typhus and murine typhus in the Lao PDR

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Scrub typhus and murine typhus are endemic to South and South East Asia and are major causes of treatable febrile illness. In this study we analysed the annual patterns of incidence of scrub typhus and murine typhus in Lao PDR (Laos) and the spatiotemporal dynamics from reported patients.

Samples were submitted to Mahosot Hospital Microbiology Department from consenting febrile patients between 2004 and 2017 with suspected rickettsial infection and tested against IgM RDT. The results were analysed with demographic, clinical, weather and geographical data.

Over the 13 year period, 1,337/8,150 (16.4%) scrub typhus and 1,283/6,269 (17%) murine typhus patients were identified. The proportion of scrub typhus patients with an eschar identified was 10%. The majority of patients came from Vientiane Capital followed by Vientiane Province. There was a peak in number of patients with murine typhus in 2009 and 2010 while there was a dramatic increase in scrub typhus cases in 2011 from 88 cases in 2010 to 206 in 2011. Scrub typhus patient presentations peaked in the wet season months of July and August while murine typhus was most frequent in the hot, dry months of March to May. The full differential spatiotemporal results for both murine typhus and scrub typhus will be presented in relation to weather and habitat and be discussed in terms of interventions to reduce risk of acquiring these infections and for their management.

Abstract 71: Mapping scrub typhus in Indonesia: Preliminary results

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Scrub typhus is an acute and potentially fatal febrile illness caused by the obligate intracellular bacteria *Orientia tsutsugamushi*. Although scrub typhus cases have been documented in Indonesia, definitive knowledge concerning the distribution and risk of scrub typhus in Indonesia is scant. We executed a mapping effort employing two-pronged approach, i.e. geographic placement of reported evidence, and a seroprevalence study of samples collected at varied sites. This study aimed to confirm the presence and distributions of *Orientia tsutsugamushi* and its vector, *Leptotrombidium* mites, at certain locales in Indonesia. Literature searches were performed in electronic databases to locate existing evidence, along with searches of the 'grey' literature. Data from the last five decades of studies were extracted, yielding 99 data points across 14 out of 34 provinces in Indonesia. *Orientia tsutsugamushi* transmission/occurrence was documented in at least four major islands: Borneo, Java, Sumatra, and Papua. There were 11 data points of human seroprevalence estimates, with seropositivity ranging from 0% to 20%. *Leptotrombidium* mites of seven distinct species have been documented at five major islands: Borneo, Java, Sulawesi, Sumatra, and Papua. Seroprevalence surveys utilising enzyme-linked immunosorbent assay (ELISA) for IgG and IgM antibody against scrub typhus will be performed on archived serum samples from four locations on three islands: Sumatra, Java, and Bali. A great deal more work in Indonesia remains to be done to inform even the most basic understanding of the distribution and burden of this infection, and our efforts detailed here begin to fill those many wide gaps in understanding.

Abstract 24: Importance and challenges of public engagement work on scrub typhus in Chiang Rai

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Scrub typhus is an important disease which disproportionately affects the poor and hill tribe populations in northern Thailand. Simple measures such as avoiding resting on the ground and washing after time spent in high risk habitats can help to reduce the risk of infection but the public's and healthcare workers' knowledge of scrub typhus can be limited, even in endemic areas.

Public engagement plays an important role in global health through the dissemination of knowledge and research benefits which can result in improved health outcomes. Public engagement is also important in the research context and can help to improve trust between researchers and communities, support research processes such as informed consent and achieve recruitment targets as well as increasing the impact and adoption of research findings.

In this presentation we will describe a typical scrub typhus research participant from Chiang Rai, northern Thailand, discuss the effects of research participation, and their understanding of research and scrub typhus drawing on data from local clinical and qualitative studies. We will then give an overview of the challenges of public engagement within these populations, areas where changes to current policies are required and examples of recent public engagement initiatives.

Session 4b: Pathogenesis, pathophysiology, cell biology/Genomics, transcriptomics, proteomics/Diagnosis

Abstract 62: Peptidoglycan in Rickettsiales species

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Peptidoglycan is found in the cell envelope and is necessary for shape determination and protection against osmotic stress. Obligate intracellular bacteria grow and divide directly in the cytoplasm or within membrane-bound vacuoles in eukaryotic cells. Given the immunogenic nature of peptidoglycan, bacteria may be under selective pressure to reduce its abundance. Peptidoglycan has been well studied in the Chlamydiales, but not in the Rickettsiales.

We have explored the selective pressure of the obligate intracellular lifestyle on the cell wall of bacteria by performing a comparative analysis of peptidoglycan in multiple Rickettsiales species. A bioinformatics analysis led us to classify bacteria into three groups: classical peptidoglycan species, negative peptidoglycan species and a new group, intermediate peptidoglycan species which possess most genes required for peptidoglycan biosynthesis but lack bifunctional class A penicillin binding proteins. This third group may produce a distinct type of peptidoglycan that is low in abundance and difficult to detect.

We have used a combination of approaches including in vivo labelling using D-alanine probes, growth inhibition assays, a NOD1 reporter assay and structural studies on the resistance of bacterial cells to osmotic stress. Using these methods we have studied the presence and abundance of peptidoglycan-like structures in *Orientia*, *Rickettsia*, *Ehrlichia*, *Wolbachia* and *Anaplasma* species.

Our bioinformatics and experimental results allow us to classify the peptidoglycan status of Rickettsiales species which likely reflect differences in cell tropism and infection cycle and may have an impact on the mechanisms of virulence by this family of important human and animal pathogens.

Abstract 68: Dissemination of *Orientia tsutsugamushi* in the Rhesus Macaque Scrub Typhus Model

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Scrub typhus is an acute febrile illness caused by *Orientia tsutsugamushi*, an obligate intracellular bacterium, which can be transmitted to vertebrate hosts by the bite of larval *Leptotrombidium* mites (chiggers). Eschars, ulcers with black necrotic crusts, often develop at the bite sites where bacteria propagate and then spread to other tissues. In this study, we examined the dissemination of *O. tsutsugamushi* in rhesus macaques after the inoculation of *O. tsutsugamushi*. Three groups (each n=4) of rhesus monkeys were intradermally injected with *O. tsutsugamushi* Karp strain at 107.8 MuLD50. Blood and tissue samples (including injection site (eschar), lymph nodes, liver, kidney, lung, and heart) were collected on days 6, 12, and 18 post inoculation (pi) for bacterial determination using a qPCR assay and immunohistochemistry. Bacteremia was detected on day 6 pi and peaked on day 12 pi. All monkeys developed classic eschar lesions at the injection site within 7 days, with the highest bacterial count detected on day 12 pi. Double-labelling IHC staining was utilized to identify host immune cells and *O. tsutsugamushi* organisms in the eschar samples. The bacteria were co-localized with antigen presenting cells (HLADR+), dendritic cells (HLADR+, CD-SIGN+ and CD1a+), and monocyte/macrophage (CD68+, CD14+) similar to observations in human eschars. These novel findings give insight into the early host response to *O. tsutsugamushi* inoculation, and show the usefulness of the rhesus macaque scrub typhus model for future immunological and vaccine efficacy studies.

Abstract 9: Genomic features of *Rickettsia heilongjiangensis* revealed by intraspecies comparison and detailed comparison with *Rickettsia japonica*

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Rickettsia heilongjiangensis is a causative agent of far eastern spotted fever (FESF). The first Japanese case of FESF was identified in Miyagi prefecture in 2008, and *R. heilongjiangensis* were isolated from *Haemaphysalis concinna* ticks collected in a suspected geographic area of infection. The intra-species genome diversity of Rickettsiae has been poorly investigated while our recent analysis revealed the extreme low genomic diversity in *R. japonica*, the agent of Japanese spotted fever which is a close relative to *R. heilongjiangensis*. To investigate the genome diversity of *R. heilongjiangensis* and understand the genetic relationship between Japanese and Chinese *R. heilongjiangensis* strains, we sequenced four *R. heilongjiangensis* strains (three were isolated in Miyagi, Japan and one was isolated in Inner-Mongolia, China), and performed genomic comparison between these strains and the type strain (strain 054) of *R. heilongjiangensis* isolated in Heilongjiang province of China. Although Japanese strains were isolated in 2008, 2009 and 2012, respectively, their genomes were identical, showing no genomic variations. In addition, only 81 SNP and 13 InDel sites were identified among the five strains, despite the

facts that their origins significantly differed geographically and temporally. These results imply the very low genomic diversity of *R. heilongjiangensis* as observed in *R. japonica*. The results of a fine genome comparison of *R. heilongjiangensis* and *R. japonica* using high quality whole genome sequences will be also presented.

Abstract 55: Investigations of mutations in 16S rRNA gene sequences, a possible doxycycline target of *Orientia tsutsugamushi*

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O. tsutsugamushi is the causative agent of scrub typhus. Although, conventionally treatable with doxycycline, doxycycline-resistant cases were reported in Chiang Rai, Northern Thailand, in 1996. In *Escherichia coli*, mutations were found in the well-defined 16S rRNA doxycycline target gene in antibiotic-resistant strains. We aimed to investigate mutations in the *O. tsutsugamushi* 16S rRNA gene, as a possible doxycycline target. The binding site regions in the 16S rRNA gene from 36 clinical isolates of *O. tsutsugamushi* from Laos and Thailand were sequenced; we found gene mutations in 16S rRNA in all isolates, however not at the same binding sites as described for *E. coli*. All investigated sequences had ATC at positions 967, 968 and 969 adjacent to the known doxycycline binding site in *E. coli*. Using an in silico binding analysis tool to determine doxycycline binding affinity to this region of *O. tsutsugamushi*, we found high binding affinity. Whether this higher ribosomal binding affinity to doxycycline associates with improved susceptibility to doxycycline in *O. tsutsugamushi* compared to *E. coli* warrants functional assays.

O. tsutsugamushi Karp, AFC-3 and AFSC-4 strains were investigated as to whether susceptible strains can become resistant under antibiotic pressure. Quantitative PCR was used to quantify the bacterial DNA load of *O. tsutsugamushi* cultured with incremental doxycycline concentrations. The results demonstrated that the AFSC-4 strain replicated to higher bacterial loads under doxycycline pressure compared to the other strains. This data indicated that AFSC-4 isolate might exhibit tolerance rather than resistance to doxycycline, which might affect clinical outcomes.

Abstract 58: Metagenomic approach to characterizing disease epidemiology in a disease-endemic environment

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In this study, we used a metagenomic approach to analyze bacterial communities from diverse populations (humans, animals, and vectors) to investigate their role as causative agents of disease in human and animal populations. Wild rodents (N=325) and ectoparasites (N=428) were collected in Nan province, Thailand and Wangdue Phodrang District, Bhutan. Four hundred whole blood samples of undifferentiated febrile illness (UFI) patients were obtained from a hospital in Nan province, Thailand and hospitals in 8 districts, Bhutan. The bacterial 16S rRNA (V3-V4) was amplified and sequenced with Illumina. Real-time PCR and Sanger sequencing were used to confirm the next-generation sequencing (NGS) results.

Several pathogens were detected by NGS in all populations studied. In Nan province, Thailand the most common pathogens identified included *Bartonella* spp., *Rickettsia* spp., *Leptospira* spp., and *Orientia tsutsugamushi*. Interestingly, *Anaplasma* spp. was detected in patient, rodent and tick populations. The same *O. tsutsugamushi* genotypes were shared among UFI patients, rodents, and chiggers in a single district indicating that the chiggers were likely responsible for transmitting to people. NGS Study in Bhutan also revealed several pathogens in UFI patients including *Orientia* spp., *Rickettsia* spp., *Leptospira* spp., and *Bartonella* spp. NGS results from rodents and ectoparasites indicated that rodents were infected with *Anaplasma* spp., *Bartonella* spp., *Ehrlichia* spp., and *Rickettsia* spp., while *Bartonella* spp. was detected in 2 pools of ectoparasites.

Using a metagenomic approach, we have demonstrated that there is active circulation and transmission of several pathogens in the environment that is causing febrile illness in human populations.

Tuesday, 5th November 2019

Session 5: Ecology/Epidemiology/Host-Pathogen-Vector Interactions

Abstract 10: The clinical epidemiology of scrub typhus in humans, chiggers and rodents

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Modern techniques were developed to revisit fundamental aspects of the epidemiology and ecology of scrub typhus in Thailand and Laos. Vector chiggers and small mammal hosts were collected, identified and tested for *Orientia tsutsugamushi*, the causative agent and mapped using GIS. The complex ecological interactions of infected and uninfected vectors and hosts with habitats and seasons were investigated. A low-input targeted enrichment sequencing method was developed and applied to a subset of positive samples. Over 18 months, 244 small mammals and ~17,000 chiggers were tested resulting in 290 *O. tsutsugamushi* PCR positive samples. Sixty-nine positive human samples were collected. Overall 8.6% of individual chiggers and 25.9% of chigger pools tested positive. No consistent high-risk area of infection was identified within our study sites (~9km²). High-risk sites were associated with a lower diversity of chigger species, higher proportion of recognized vector species and a higher mean number of chiggers attached to hosts. The end of the dry season was most strongly associated with *O. tsutsugamushi* positivity. Phylogenetic clustering was evident among samples collected from the same sites, although strains with greater genetic differences also appear to co-exist even on the same host. In Thailand and Laos, human infections rise dramatically during the rainy season. However, corresponding proportions of infected chiggers remained stable, suggesting that human behaviour plays a critical role. Improving our understanding of risk behaviour could yield relatively simple interventions to reduce disease acquisition through public health education.

Abstract 43: The biology of emerging *Rickettsia felis* rickettsiosis

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Rickettsia felis is an emerging pathogen of the transitional group of *Rickettsia* species and recognized as a cause of febrile illness with a worldwide distribution. Similar to the tick-borne spotted fever group of *Rickettsia*, *R. felis* can be transmitted to vertebrate hosts during arthropod bloodmeal acquisition. However, consistent with the flea-borne typhus group of *Rickettsia*, viable *R. felis* has been detected in flea feces and potential transmission of *R. felis* exists via infectious vector feces. Infection of the flea host occurs in a temporal fashion and flea responds to rickettsial infection. Additionally, the transmission of insect-borne *Rickettsia* requires an environmentally stable form that likely contains distinct characteristics essential for vertebrate infection. Ongoing studies utilize in vitro and in vivo systems to elucidate the vector-pathogen interactions and the unique biological properties of rickettsial

agents that persist outside of the host cell and facilitate environmental stability. Identifying the determinants of vector infection will contribute to the understanding of pathogenesis and development of diagnostic assays.

Abstract 35: Invasive plants facilitated by socioeconomic change shelter vectors of scrub typhus

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Although socioeconomic change can have significant downstream effect on human risks to vector-borne diseases via a change in land use and vegetative community, particularly facilitating the invasion of exotic plants, related studies remain very scarce. Scrub typhus is emerging around the globe and is transmitted by chigger mites, with small mammals as the primary hosts. We investigated how an invasion of *Leucaena leucocephala* plant after extensive abandonment of farmlands driven by industrialization in Penghu Islands of Taiwan affected abundance of chiggers by trapping small mammals in three types of habitats (invasion site, agricultural field, human residence site) every two months for a year. We found that the invasion sites sheltered more chiggers than the other two habitats; moreover, chiggers maintained higher abundance in early winter and its population was more stable across seasons in invasion sites, suggesting that the invasive sites could be a temporary refuge for chiggers and might help mitigate the negative influence of unfavorable climate. Infective rates of etiologic agents of scrub typhus in chiggers was also higher in invasion sites. Invasion sites harbored more *Rattus losea* rat, on which the infested chiggers were more well fed than those from the most commonly trapped species (*Suncus murinus* shrew). This study highlights an important but largely neglected issue that socioeconomic change can have unexpected consequence for human health mediated particularly through invasive plants, which could become a hotspot for emerging infectious diseases but are usually very hard to be eradicated.

Abstract 17: *Rickettsia* and ticks in Bhutan

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At the commencement of this study, there was no data on ticks and *Rickettsia* in Bhutan. The current study collected blood samples (human and animal) and ticks from domestic animals in Bhutan. Samples were analyzed at the Australian Rickettsial Reference Laboratory and ticks were morphologically identified at the US National Tick Collection Centre, Georgia. Rickettsial antibodies were detected by an Immunofluorescence assay and DNA by qPCR.

An estimated 5% of acute undifferentiated febrile illnesses in patients attending 14 Bhutanese hospitals were caused by a *Rickettsia*; 4.4% by Spotted Fever Group (SFG) and 0.4% by Typhus Group (TG). In healthy Bhutanese population, 15.7% and 3.5% had evidence of past exposure to SFG and TG respectively. SFG exposure significantly increased with age and farming. In domestic animals, seropositivity against SFG and TG were 36% and 15% respectively. Seropositivity differences between animal species appeared to be significant. Two-hundred ticks from 155 domestic animals were identified; the commonest tick species being *Rhipicephalus microplus*, *R. haemaphysaloides*, *Haemaphysalis* sp. near *ramachandrai*, *H. tibetensis*, *H. bispinosa*, *Haemaphysalis* sp, *Haemaphysalis* sp. near *davisi*, *R. sanguineus*, *H. shimoga*, *H. hystricis*, *Ixodes ovatus* & *Amblyomma testudinarium*. Twenty-nine (15%) of the 188 ticks subjected to a qPCR to detect rickettsial DNA were positive and the *Rickettsia* belonged to three major groups: *R. sibirica*, *R. raoultii* and *R. japonica* in phylogenetic tree.

As the first report, this represents baseline data for Bhutan to prompt further research. Health services should be equipped to manage rickettsial infections by developing diagnostics and clinical guidelines.

Session 6a: Host-pathogen-vector interactions/Epidemiology

Abstract 40: *Rickettsia rickettsii* modulates the quantity but not the composition of *Amblyomma aureolatum* midgut microbiota

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Amblyomma aureolatum is an important vector of *Rickettsia rickettsii* – the causative agent of Rocky Mountain spotted fever – in São Paulo, Brazil. Intriguingly, bacteria only reach the tick salivary glands whether the midgut (MG) is infected, pointing out the tick MG as an important barrier to infection. In addition, the tick microbiota is known to exert an effect on the establishment of pathogen infection. To better understand the relation between *R. rickettsii* and the resident microbiota, the aim of this work was to analyze the quantity and the composition of the MG bacterial microbiota of *R. rickettsii*-infected and noninfected *A. aureolatum* ticks. The total number of bacteria within the tick MG was determined by qPCR using primers for the V2 variable region of the bacterial 16S rRNA. Data showed that the MG of noninfected *A. aureolatum* harbors around four times more bacteria than *R. rickettsii*-infected ticks. Regarding the composition of MG microbiota, DNA was amplified for V3 and V4 variable regions of 16S rRNA and sequenced by Illumina MiSeq. Data were analyzed with QIIME2 pipeline and compared with Silva 16S rRNA. *A. aureolatum* MG microbiota is mostly composed by bacteria of the genus *Francisella*, and *R. rickettsii*-infected ticks, despite presenting a lower bacterial load, do not present changes in this composition. These results point out the influence of *R. rickettsii* in the quantity, but not in the composition of *A. aureolatum* MG microbiota.

Abstract 46: First identification of trombiculid mites (Acari: Trombiculidae) on rodents captured on Chiloé Island, an endemic region of scrub typhus in southern Chile

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Background: Scrub typhus (ST) is an emerging vector-borne zoonosis, caused by *Orientia* spp., which, in Asia-Pacific, is transmitted by trombiculid mites (chiggers). Endemic ST has recently been discovered in southern Chile. Still, the reservoir(s) and vector(s) of the infection outside Asia-Pacific are unknown. The aim of the present work was to study the prevalence of chiggers on rodents captured on Chiloé Island in southern Chile.

Methods: During austral summer 2018, rodents were live-trapped in 6 sites in rural areas on Chiloé Island, previously identified as probable hot spots of scrub typhus. Rodents were thoroughly examined for chigger infestation. Mites were identified by optical and electronic microscopy following nomenclature and methodology of Brennan & Goff (1977).

Results: During 4,713 trap-nights, 244 rodents of 7 species were captured, most abundantly *Abrothrix olivacea* (76%). Chiggers were detected in all 6 sites and infested all rodent species, with an overall infestation rate of 55%. We identified trombiculids of three genera; *Colicus* was the most abundant (93%), prevalent in 5/6 sites, followed by *Quadrasetta* (7%) and *Paratrombicula* (7%), both identified in a single site.

Conclusions: We firstly describe rodent-associated chiggers in a region with endemic scrub typhus in southern Chile. Identified mites belonged to the genus *Colicus* and two other neotropical genera, infesting a high rate of rodents with low host specificity. Since the study was performed in hot spots of scrub typhus, these results suggest a possible role as vectors and reservoir of this emerging infection.

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Abstract 56: Infection and Transmission Dynamic of Spotted Fever Group Rickettsia in *Amblyomma maculatum*

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Tick vectors are capable of transmitting several rickettsial species to vertebrate hosts resulting in varying levels of disease. Studies have demonstrated the transmissibility of both rickettsial pathogens and novel *Rickettsia* species with unknown pathogenicity to hosts during tick bloodmeal acquisition; however, the quantitative nature of transmission remains unknown. We tested the hypothesis if infection severity is a function of rickettsial load delivered during transmission, then a more virulent SFG *Rickettsia* species is transmitted at higher levels during tick feeding. Using *Rickettsia parkeri* or *Candidatus Rickettsia andeanae* infected *Amblyomma maculatum* cohorts, a qPCR assay was employed to quantify rickettsiae in tick salivary glands, saliva, and vertebrate hosts over the duration of tick feeding. Compared to *Ca. R. andeanae*, significantly greater *R. parkeri* were present in tick saliva, salivary glands, and in the vertebrate hosts at the feeding site. IFA in tick salivary glands demonstrates the presence of both rickettsial species and IHC identified localized *R. parkeri*, but not *Ca. R. andeanae*, at tick attachment site. To further test this relationship, we generated a *R. parkeri* that lacks the functional *sca4* gene exposed to *A. maculatum*. Preliminary data suggests a reduction of bacterial infection in *R. parkeri* mutants, compared to the wild type, in the tick saliva and organs. The specific factors that contribute to the generation of a sustained rickettsial infection and subsequent disease are yet to be elucidated, but

the results of this study suggest that rickettsial load in ticks and rickettsiae present during transmission may be an important element.

Abstract 67: The hard tick of Laos: an update checklist of species record

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Ticks and tick-associated pathogens are poorly known, especially rickettsial pathogens transmitted by ticks. An investigation of Laotian ticks and tick-borne pathogens was launched in 2012 and continued into 2019 through collaboration between the U.S. NMRC-Asia, the IP-Laos, and the LOMWRU (Laos) to investigate the geographical distribution of putative tick vectors of bacterial and arboviral diseases in Laos. Herein we present results of a faunal study of tick species collected during our surveys and we provide an updated checklist of tick records from Laos. A total of 17,803 ticks representing larval (50.24%), nymphal (45.42%) and adult (4.34%) life stages were collected from dragging and animals. Five genera comprising at least 25 species, including 14 new records and 2 new species, were identified from adult specimens. These collections, together with the literature to date, provide evidence for the occurrence of at least 32 ixodid tick species, representing six genera, in Laos. These results served as a framework for further molecular investigations of putative tick vectors and their pathogens in Laos. In this regard, it should be noted that errors in tick identification, especially in the case of the larval-nymphal stages and also difficult genus *Haemaphysalis*, can lead to the erroneous reporting of tick species as disease vectors. As there are no morphological identification keys available for larval and nymphal stages and very little is known about ticks in Southeast Asia and particularly in Laos. Therefore, further studies on taxonomy, both morphological and molecular, are urgently needed.

Abstract 66: Sero-survey of market vendors associated with the bushmeat trade in Lao PDR

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Wildlife trade is thought to be the biggest driver of emerging infectious diseases. Of those currently classed as emerging pathogens, 60% are classed as zoonotic and nearly 70% originate in wildlife. Along with collections of wildlife specimens from wildlife trade markets across Lao PDR as part of a pathogen surveillance program, we carried out a longitudinal sero-survey of market vendors to determine if those in close-contact with wildlife on sale were at risk of becoming infected with pathogens we had identified within the bushmeat. Over a period of one year we screened 150 market vendors from three wet markets at three intervals for the presence of IgG against Typhus group (TG) and scrub typhus group (STG) rickettsia and *Leptospira* spp. Of these vendors, 54 (36%) sold wildlife meat (with or without domesticated meat and/or vegetables). Thirty market vendors were IgG positive for STG at least once over the year; whilst 36 were IgG positive for TG and 22 were IgG positive for *Leptospira* spp. Fourteen market vendors sero-converted over the year. Twelve market vendors were IgG-positive for two pathogens whilst three were IgG-positive for all three. This preliminary investigation shows that market vendors are at risk of becoming infected with pathogens identified in bushmeat sold at these markets. However, we do not know whether they were infected at the markets or elsewhere. Further research with controls from the community not working in wet markets is needed to better understand the risk and inform mitigation strategies in markets and their communities.

Session 6b: Epidemiology/Diagnosis

Abstract 44: Detection of *Rickettsia* species in *Haemaphysalis flava* from the Republic of Korea

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Haemaphysalis flava is one of the most abundant tick species in the Republic of Korea, as well as in neighboring countries of Japan and China. *H. flava* is more commonly associated with forest habitats and parasitizes birds, small-large animals, and, incidentally, humans. *H. flava* carries several pathogens of medical and veterinary importance, including tick-borne encephalitis virus, Lyme disease borreliae and the severe fever with thrombocytopenia syndrome (SFTS) virus. However, the information on *Rickettsia* spp. associated with *H. flava* has been very limited. A total of 105 *H. flava* were collected from 14 collection sites during a tick survey in Jeju Island 2016 and assessed for rickettsial agents. Fifty-two (49.5%) of individual *H. flava* were positive by the *Rickettsia* genus-specific qPCR assay (Rick17b). To further identify the rickettsial agents, multilocus sequence typing using partial sequences of 6 genes (*rrs*, *gltA*, 17 KDa antigen gene, *ompA*, *ompB*, and *sca4*) were attempted, but not all gene fragments were amplified using primers currently available. Phylogenetic analysis of a 1,400 bp fragment of *ompB* showed that one of the *Rickettsia* molecular isolates (*R. sp.* Hf-144) was closest in similarity (96.0%) to *Candidatus Rickettsia tasmanensis* strain T152. Moreover, a 66 bp insertion in the *ompB* sequence makes this isolate unique among rickettsiae. A SYBR Green qPCR assay (*RspHf*) was developed targeting this specific *ompB* sequence. Among the *Rickettsia* positive ticks tested, *R. sp.* Hf-144 was identified in 2 (1.9%) samples by *RspHf*. Further investigations are needed to more clearly characterize these rickettsial agents within *H. flava*.

Abstract 82 Molecular Prevalence of *Ehrlichia canis* and *Anaplasma platys* in Stray Dogs and Their Ticks in Peninsular Malaysia

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The present study was designed to investigate some of the most important tick-borne haemopathogens of dogs in Peninsular Malaysia. Blood samples were collected from 220 dogs and 140 tick samples, randomly selected from 10 animal shelters located in Peninsular Malaysia. DNA were extracted and

amplified by conventional PCR using species-specific primers that amplify the 400bp fragment of the 16SrRNA gene for *Ehrlichia canis* and the 500bp fragment of the 16SrRNA gene for *Anaplasma platys*. The results showed that 35% (n=76) of the collected samples were infected with at least one of the two hemopathogens, of which *Ehrlichia canis* predominated with a prevalence of 20% (n=43; CI=0.1465-0.2554) and *Anaplasma platys* with prevalence rates of 12% (n=26; CI=0.0801-0.1701). Single infection of haemopathogens is common (n = 53; 24%), while co-infection of *Anaplasma platys* and *Ehrlichia canis* was also observed (n = 12; 6%). The occurrence of infection with this two tick-borne haemopathogens was also observed in the sampled dogs (n = 2; 1%). For tick samples, 3.57% (n=5) of the collected samples were infected with at least one of the two haemopathogens. *Ehrlichia canis* and *Anaplasma platys* both show a similar prevalence (n = 2; 1.43%) In addition, no co-infection of tick-borne haemopathogens in ticks obtained in the present study. The study presented for the first time extensive molecular detection of tick-borne haemopathogens with *Ehrlichia canis* as the main contributor as well as the presence of co-infection of haemopathogens in shelter dogs of Peninsular Malaysia.

Abstract 18: Review of diagnostic cut-offs for murine typhus IgM and IgG IFAs

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Background

Murine typhus is a neglected but widespread infectious disease that causes acute fever. Diagnosis is commonly made through serological techniques, or detection of the causative agent *Rickettsia typhi* by PCR or in vitro isolation. The IFA is the “gold standard” and a decisive means of detecting IgM or IgG antibodies. However, lack of standardisation in IFA methodology raises concerns regarding the validity of cut-offs used in studies.

Methodology/Principal Findings

A PubMed search and manual screening of reference lists identified 78 studies that used IFA or IIP antibody cut-offs to diagnose murine typhus, 39 of which were case series. Overall, 45 studies (57.7%) provided little to no explanation as to how the cut-off was derived. Variation was seen locally in the cut-off titers used, but a four-fold or greater increase was often used as a diagnostic reference standard. The cut-offs also varied depending on the antibody target. No consensus was found in determining a cut-off, or for a single value diagnostic cut-off.

Conclusions/Significance

There is a lack of consensus in the determination of a single value cut-off. It may be possible to make an accurate diagnosis based on a four-fold or greater rising titer, if an adequate region-specific evidence base is available. Further studies will need to be performed at each geographic location to identify

region-specific cut-offs, while taking into consideration background antibody levels to distinguish between healthy and infected patients.

Abstract 29: InBios Scrub Typhus Detect IgG and IgM ELISA kits for the diagnosis of scrub typhus acquired in Chile: proposed cut-off values

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Introduction: Routine diagnosis of scrub typhus (ST) mostly relies on serology. A recent commercial ELISA has been successfully evaluated in endemic countries in Asia-Pacific, but its use in other regions is hampered by the absence of cut-off values. Here we propose cut-offs for the application of these new assays in patients with suspected ST acquired in Chile.

Methods: Cut-offs were determined for IgM/IgG kits (Scrub Typhus Detect, InBios, Seattle, USA), using panels of negative serum samples from ST endemic regions in Chile, which were obtained during past projects. For IgM, negative controls derived from patients diagnosed as ST negative by PCR and IgG serology within our ongoing surveillance and a fever study on Chiloé Island. For IgG, samples derived from a seroprevalence survey from Chiloé Island and were confirmed as seronegative by IFA (Fuller Laboratories) and an in-house ELISA (Naval Medical Research Center). As previously described and proposed by manufactures, cut-offs were calculated as the mean optical density (OD) plus 3 standard deviations (SD).

Results: For IgM, we selected 49 sera. Mean OD was 0.13 (0.03-0.54), SD was 0.10, resulting in a cut-off value of 0.44. For IgG, 171 samples were included. Mean OD was 0.20 (0.05-0.37), with a SD of 0.07, resulting in a cut-off of 0.42.

Conclusion: The determination of cut-off values using negative local serum samples is necessary to provide reliable test results. The values provided by this study can be used to further evaluate the diagnostic performance in patients with possible ST acquired in southern Chile.

Abstract 72: Review of factors affecting rickettsial isolations at Mahosot Hospital, Lao PDR

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In Laos, rickettsial infections account for a significant burden of fevers, especially scrub typhus and murine typhus. Scrub typhus, *Orientia tsutsugamushi* infection, is transmitted through the bite of an infected chigger mite. Murine typhus, *Rickettsia typhi*, is spread via fleas. The Lao-Oxford-Mahosot Hospital-Wellcome Research Unit (LOMWRU), at Mahosot Hospital, has been performing rickettsial isolation since 2008. EDTA blood or buffy coat from febrile patients, positive for either pathogen from IgM rapid diagnostic tests, were used to infect mammalian cell-lines (L929 and Vero cells) using RPMI 1600 medium supplemented with 10% fetal bovine serum, and incubated at 35°C with 5% CO₂. Isolations were attempted for 4-8 weeks. Cultures were checked by IFA every week and positives confirmed by qPCR. A total of 3,227 whole blood samples collected in Vientiane Capital, Luangnamtha and Salavan Provinces between 2008 and 2014 were received for rickettsial isolation. 256 of 3227 (7.9%) samples were cultured; *R. typhi* was isolated in 23/256 (9.0%) and *O. tsutsugamushi* in 228/256 (89.1%). Successful isolations in Vientiane were 166/256 (64.8%), Luangnamtha (66/256, 25.9%) and Salavan (24/256, 9.4%). We evaluated procedural and patient factors to determine factors that may affect the success of isolation. The time between blood collection and inoculation was associated with higher rates of isolation and may relate to time-dependent decline in bacterial viability. In addition, in order to optimize *R. typhi* isolations, we evaluated the effect of varying the temperature of incubation and percentage of fetal bovine serum. Factors to consider when attempting isolations will be discussed.

Wednesday, 6th November, 2019

Session 7: Epidemiology/Infection/Diagnosis/Treatment

Abstract 88: *Rickettsia conorii* and *Rickettsia typhi*: not uncommon causes of acute febrile illness in India

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Background:

Rickettsial disease (RD) are one of the major under-diagnosed cause of arthropod borne acute febrile illness (AFI) presenting with a range of mild self-limiting fever to fatal sepsis. The spotted fever group (SFG) and Typhus group (TG) are the major RD, which commonly caused by *Rickettsia conorii* and *Rickettsia typhi* respectively. The limited availability and role of serological tests in the acute phase of illness warrants rapid reliable molecular methods for diagnosis and epidemiological studies.

Materials and Methods:

Blood samples were collected over 2 months (April to May 2019) from patients of AFI. DNA was extracted and nested PCR using primers specific for both SPG and TG pathogens. The positive amplified products were sequenced for species identification and phylogenetic analysis was performed using MEGA 7.0 software. The demographic details of the RD cases were documented.

Result:

The prevalence of RD among AFI cases was 7% (14/200); *R. conorii* and *R. typhi* were identified as the causative agents in 4% and 3% of AFI cases. The median age of the RD cases is 22 years (range 2-65). The median duration of fever is 3 days (range 1-12). The RD cases presented with respiratory symptoms or signs (44.44%), jaundice (22.22%), abdominal pain (22.22%), diarrhea (22.22%), vesicular rash (11.11%), vomiting (11.11%), loss of appetite (11.11%) and headache (11.11%), and leukocytosis (88.88% with mean count 22750/mm³) and thrombocytosis (33.33%). The patients were treated empirically with piperacillin-tazobactam (66.66%), clindamycin (44.44%), cefotaxim (33.33%), meropenem (33.33%), metronidazole (33.33%), doxycycline (22.22%), azithromycin (22.22%), ceftriaxone (11.11%) and amoxicillin-clavulonic acid (11.11%). The mortality among the RD cases was 11.11%. The phylogenetic analysis is shown in Fig 1 and 2.

Discussion and conclusion:

The pilot study shows that spotted fever and murine typhus are not uncommon in AFI cases in north India. The febrile episodes are usually transient, usually mild, and without typical presentation or documented tick exposure history in the hospitalized patients. Empirical antibiotic therapy given to AFI patients might not cover for these organisms. The study emphasizes that rapid molecular methods like PCR should be a part of the algorithm for the diagnosis of AFI in India.

Abstract 57: Biosafety and biosecurity requirements for *Orientia* spp. diagnosis and research: Recommendations for risk-based biocontainment, work practices and the case for reclassification to Risk Group 2.

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Scrub typhus is an important arthropod-borne disease causing significant acute febrile illness by infection with *Orientia* spp.

Using a risk-based approach, this review examines current practice, the evidence base and regulatory requirements regarding matters of biosafety and biosecurity, and presents the case for reclassification from Risk Group 3 to Risk Group 2 along with recommendations for safe working practices of risk-based activities during the manipulation of *Orientia* spp. in the laboratory.

We recommend to reclassify *Orientia* spp. to Risk Group 2 based on the classification for RG2 pathogens as being moderate individual risk, low community risk. We recommend that low risk activities, can be performed within a biological safety cabinet located in a biosafety level (BSL) 2 core laboratory using standard personal protective equipment. But when the risk assessment indicates, such as high concentration and volume, or aerosol generation, then a higher biocontainment level is warranted. The majority of animal activities involving *Orientia* spp. would still require animal BSL3 biocontainment.

Abstract 13: High-throughput screening of modulators of cellular calcium metabolism as potential drugs against rickettsia-induced microvascular dysfunction

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Background: Tick-borne rickettsiae cause severe disease in up to >20% of patients, even if treated. The pathophysiology of severe disease is increased microvascular endothelial cell (MEC) barrier permeability. Control of cellular Ca²⁺ metabolism is essential for MEC barrier function. Using *in vitro*

human MEC, we showed spotted fever *Rickettsia* and *Borrelia burgdorferi* caused Ca^{2+} -dependent disruption of barrier integrity. Methods: We tested >20 pharmacologic modulators of Ca^{2+} signaling for the ability to prevent MEC dysfunction with *Anaplasma phagocytophilum*, *Ehrlichia chaffeensis* and *Rickettsia parkeri*. We used human brain MEC (HBMEC) in 96-well microelectrode arrays with static electric cell impedance sensing (ECIS) to monitor barrier integrity with treatments. Cellular and bacterial toxicity was examined using cell viability and regrowth, respectively. At maximum resistance, HBMEC were pre- or sham-treated with drugs and then with *A. phagocytophilum*-infected HL-60 cells, *E. chaffeensis*-infected THP-1 cells, or cell free *R. parkeri* at 10-50 MOI. Results: Drug concentrations <10 μM or <10 $\mu\text{g/mL}$ were nontoxic for HBMEC. Toxicity for ex vivo human neutrophils was observed at $\geq 1 \mu\text{M}$ for 3 drugs. Toxicity against *A. phagocytophilum* was demonstrated at concentrations $\geq 1 \mu\text{M}$ for 3 drugs. Over 72 hours, membrane-active chelators DPb99 and DP460, and the calcium channel blocker benidipine, in a dose-dependent manner, stabilized and prevented pathogen-induced MEC integrity disruption at low, non-toxic drug concentrations. Conclusions: Drugs known to affect Ca^{2+} flux stabilized rickettsia-induced vascular permeability without directly impacting rickettsial viability. These data provide support for host-directed therapeutics for rickettsial disease, and perhaps others that promote vascular permeability.

Abstract 31: Prospective, open-label, randomized trials of doxycycline versus azithromycin for the treatment of uncomplicated murine typhus and scrub typhus

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Murine typhus and scrub typhus are both common causes of fever in and around Vientiane, Laos. Murine typhus is a global but neglected disease without randomised clinical trials to guide antibiotic therapy. Scrub typhus is also neglected and its known global distribution is expanding. Its management has the benefit of some clinical trials, but none from Laos. Doxycycline is commonly used but without objective evidence for optimum treatment duration. Azithromycin is a potential alternative.

We conducted two linked prospective, open, randomised trials was conducted in non-pregnant, consenting inpatient adults with rapid diagnostic test evidence for uncomplicated murine typhus and scrub typhus. Patients were randomised to seven (D7) or three days (D3) oral doxycycline or three days oral azithromycin (A3). Primary outcome measures were fever clearance time (FCT) and frequencies of treatment failure and relapse.

For the murine typhus trial, 216 patients (72 per arm) were enrolled; 158 (73.2%) patients had serology/PCR-confirmed murine typhus; 52 (24.1%) were *R. typhi* PCR-positive. Treatment failure risk was greater following regimen A3 (22.5%, 16/71) compared to D3 (4.2%, 3/71) or D7 (1.4%, 1/71)($p < 0.0001$). The FCT, for *R. typhi* PCR-positive patients, was significantly higher in patients following A3 than D3 (1.9 fold,) and D7 (1.6 fold)($p = 0.021$). No patients returned with PCR-confirmed *R. typhi* relapse. The equivalent data for the scrub typhus trial are being analysed and will be presented. The clinical responses to the trial antibiotics will be compared between murine typhus and scrub typhus patients from the same community.

Abstract 23: Scrub typhus and the misconception of doxycycline resistance

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Scrub typhus, a mite-borne infectious disease caused by the obligate Gram negative intracellular bacteria *Orientia tsutsugamushi*, is a major cause of acute undifferentiated fever in the Asia Pacific region. The disease is potentially fatal but risk can be mitigated by early recognition and treatment with antibiotics such as doxycycline, chloramphenicol or azithromycin. In the mid-1990s, clinicians from northern Thailand informed researchers of a worrying observation; some scrub typhus patients appropriately treated were failing to respond to treatment with fatal consequences in a few.

Subsequently, a small clinical study was performed in Chiang Rai in which researchers were able to culture clinical *Orientia tsutsugamushi* isolates including AFC-3. Antibiotic susceptibility testing (AST) revealed AFC-3 to be highly doxycycline resistant. Around the same period, another study investigating the in vitro susceptibility of *Orientia tsutsugamushi* Karp strain and AFSC-4 isolate, cultured from a patient from western Thailand with poor response to doxycycline treatment, concluded that AFSC-4 was resistant to doxycycline. These reports were controversial at the time and a lack of independent verification has subsequently led to growing uncertainty regarding doxycycline resistance and what factors truly determine treatment outcome.

In this presentation, I shall review the existing evidence for drug resistant *Orientia tsutsugamushi*, assess the quality of evidence for doxycycline resistance focusing on the AST assays used and describe new evidence refuting the findings of doxycycline resistance. By doing so, I hope to provide a reasoned explanation for the findings of the original 1990s studies and outline the determinants of treatment outcome in scrub typhus.

Session 8a: Immunity and Prevention/Epidemiology/Diagnosis

Abstract 7: Immunization with a recombinant antigen composed of conserved blocks from TSA56 provides broad genotype protection against scrub typhus

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Scrub typhus is an acute febrile disease caused by *Orientia tsutsugamushi* infection. Recently, the rise of scrub typhus events in endemic countries of the Asia-Pacific region, as well as new emergence in previously unrecognized areas including South America and Africa, have become a public health problem. Despite the wide range of approaches explored during the last seventy years, an effective prophylactic vaccine is not yet available. Here, we developed a novel recombinant antigen derived from conserved regions of 56 kDa type-specific antigen (TSA56), a major outer membrane protein responsible for genetic heterogeneity and antigenicity, and evaluated it as a protective vaccine antigen. Our findings demonstrate that immunization with conserved blocks of TSA56 (cTSA56) not only provides protective immunity against lethal challenges with the homologous genotype, but also confers significantly better protection against heterologous genotypes than TSA56. Adoptive transfer of CD4+ or CD8+ T cells from immunized mice provided significantly enhanced protection against lethal challenge, whereas immune B cells failed to do so, indicating that cellular immunity against the conserved epitopes plays a protective role. Moreover, immunization with a 10-mer peptide mixture, screened from CD8+ T cell epitopes within the conserved region of TSA56, provided enhanced protection against lethal challenge with *O. tsutsugamushi*. Therefore, this novel recombinant antigen is a promising candidate for scrub typhus vaccine against a wide range of *O. tsutsugamushi* genotypes.

Abstract 61: *Rickettsia rickettsii* whole cell antigen vaccine confers protection against Rocky Mountain spotted fever

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Rocky Mountain Spotted Fever (RMSF) can be a fatal tick-borne disease in people and dogs. The disease continues to threaten the health of people in the USA and several countries in north, central and south Americas. The rickettsial agent of RMSF, *Rickettsia rickettsii*, is transmitted by several tick species; *Dermacentor andersoni*, *Rhipicephalus sanguineus*, and *Amblyomma americanum*. RMSF clinical signs range from fever, headache, nausea, vomiting, muscle pain, lack of appetite, and rash. The disease can quickly progress into a life-threatening illness in untreated patients resulting in high fatality rates ranging from 30-80%. Further, recent data during the last two decades suggest that the reported cases remain high in people, particularly in parts of North America. Vaccines are currently not available to prevent RMSF in dogs or people. In support of developing a vaccine, we established canine model for

RMSF and then evaluated two experimental vaccines; a subunit vaccine containing two recombinant outer membrane proteins (RCA) and a whole cell inactivated antigen vaccine (WCA), to confer protection against virulent *R. rickettsii* infection challenge. WCA offered a complete protection from the RMSF, while animals offered RCA remained highly susceptible to the disease, which is similar to non-vaccinated *R. rickettsii*-infected group. WCA also reduced the pathogen loads to nearly undetected levels in the blood, lungs, liver, spleen and brain, and induced bacterial antigen-specific immune response. This study provides the first evidence of the protective ability of WCA against RMSF in dogs. The implications of these data and future perspectives will be discussed.

Abstract 70: Evidence of Q fever and rickettsial disease in Chile

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In July 2017 in the Los Lagos Region, southern Chile, a group of farm workers presented with fever, cough, pneumonia, nausea and diarrhoea. The aetiology of these infections were unknown but a zoonosis was suspected given the outbreak cluster was associated with a dairy farm. The sera was sent to a Canadian laboratory for Q fever serology and some positive results were returned. This was further investigated at the Australian Rickettsial Reference Laboratory where the Q fever serology was confirmed together with the presence of a rickettsial diseases. Given that these diseases hadn't been reported in Chile previously a conservative serological approach was utilised to increase diagnostic specificity by increasing the cut-off titres used in Australia by fourfold. The Q fever and rickettsial titre cut-off were increased from 1:50 to 1:200 and 1:128 to 1:512 respectively. In addition a fourfold increase in antibody titres between paired sera was also used to confirm these diagnoses. Using this strategy we can confidently report the presence of both these infectious diseases in Chile.

Abstract 87: New insights into diagnosis of scrub typhus

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Diagnosis of scrub typhus, caused by the bacterium *Orientia tsutsugamushi*, is challenging because of the overlap of its non-specific symptoms with other infections coupled with the lack of sufficient data on the performance of diagnostic tests. We have evaluated the following tests on blood samples for scrub typhus and calculated the sensitivity, specificity, positive predictive value, and negative predictive value: (1) Quantitative PCR using 47kDa gene (qPCR); (2) Conventional PCR using 56kDa gene (cPCR); (3) Loop-mediated isothermal amplification assay (LAMP assay); (4) Immunofluorescence assay (IFA); (5) Enzyme-linked immunosorbent assay (ELISA); (6) Weil-Felix test (WF test); and (7) Immunochromatographic Rapid Diagnostic Test (RDT) on 316 well characterised cases and controls. ELISA and RDT detecting IgM antibodies had excellent discriminative potential with sensitivities and specificities of 92%, 94% and 92%, 92% respectively. The sensitivity and specificity of IFA were found to be 95% and 74% respectively. IgM serology had a false positivity rate of 7% with other acute febrile illnesses such as dengue,

leptospirosis and spotted fever due to the nonspecific binding of the pentavalent IgM antibody. qPCR exhibited excellent sensitivity (97%) and perfect specificity. ELISA and RDT detecting IgM antibodies have excellent sensitivity and specificity while the sensitivity of IFA is suboptimal for the diagnosis of scrub typhus. Given its perfect specificity and superior sensitivity, qPCR is preferred for diagnostic confirmation in reference laboratories particularly for diagnosis of early disease of less than 6 days duration.

Session 8b: Epidemiology/Diagnosis

Abstract 84: Potential Geographic Distribution of *Amblyomma americanum* (Acari: Ixodidae) in New Zealand

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Abstract: The accidental introduction of ticks to New Zealand and their subsequent establishment is a significant concern. The potential geographic distribution of *Amblyomma americanum*, a leading tick species in N. America that transmit rickettsial pathogens to humans and animals was modeled for New Zealand using the maximum entropy (MaxEnt) approach. Several hundred models were calibrated across the native range of *A. americanum* in North America using present-day climatic conditions and occurrence data from museum collections. Subsequently, the best-fitting model was projected onto New Zealand using both present-day and future climates modelled under two greenhouse gas emissions scenarios, Representative Concentration Pathways (RCP) 4.5 (low) and RCP 8.5 (high). The final model was selected based on partial Receiver Operating Characteristic (ROC) tests, the omission rate and the lowest Akaike Information Criterion (AIC). The projected New Zealand distribution was broadly similar to that of *Haemaphysalis longicornis*, New Zealand's only livestock tick, but with a more extensive predicted suitability. The climate change predictions for 2050 under both low and high RCP scenarios projected only moderate increases in habitat suitability along the mountain valleys in the South Island. Mobility-oriented Parity analysis showed that the predictions for the South Island mountains, the West Coast and North Island mountains for present day and future climates were extrapolative and should be regarded with considerable caution. In conclusion, this analysis shows that given the opportunity and license *A. americanum* could and would successfully establish in New Zealand.

Abstract 83: Risk factors for scrub typhus, murine typhus and spotted fever sero-positivity in urban areas, rural plains, and peri-forest hill villages in South India: cross-sectional study

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Scrub typhus and spotted fever group rickettsioses are thought to be common causes of febrile illness in India, while murine typhus is rarely tested for. This cross-sectional study explored the risk factors associated with scrub typhus, tick-borne spotted fever and murine typhus sero-positivity in three different geographical settings, urban, rural and hill villages in Tamil Nadu, South India.

We enrolled 1353 participants living in 48 clusters. The study included a questionnaire survey and blood sampling. Blood was tested for *Orientia tsutsugamushi* (scrub typhus), *Rickettsia typhi* (murine typhus) and spotted fever group rickettsiae IgG using ELISA. The sero-prevalence of scrub typhus, spotted fever and murine typhus were 20.4%, 10.4% and 5.4%. Scrub typhus had the highest prevalence in rural areas (28.1%) and spotted fever was most common in peri-forested areas (14.9%). Murine typhus was more common in rural (8.7%) as compared to urban areas (5.4%) and absent in peri-forested hill areas. Agricultural workers had a higher relative risk for scrub typhus especially in urban areas. For Murine typhus, proximity to a water body and owning a dog were found to be major risk factors. The main risk factors for spotted fever were agricultural work and living in proximity to a forest. Urban, rural plains and hill settings display distinct epidemiological pattern of *Orientia* and *Rickettsia* infections. While scrub typhus and spotted fever were associated with known risk factors in this study, the findings suggest a different ecology of murine typhus transmission compared to other studies done in Asia.

Abstract 85: Antibody response following scrub typhus infection: Clinical cohort study

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Background: This study was conducted to estimate the IgM and IgG antibody response following scrub typhus infection and to explore whether clinical and demographic characteristics modified IgM and IgG antibody response and the clinical course.

Methods: We enrolled 198 patients with acute scrub typhus and followed them up for up to two years. We collected 501 bloods samples at varying intervals, which were tested for IgM and IgG using ELISA. The IgM and IgG ELISA optical density (OD) was analysed using quantile regression.

Results: In half of cases, IgM OD values fell below 1.0 after 82 days following fever onset, with 90% of patients falling below this threshold after 231 days. After one year, 50% of cases had predicted IgG OD values of 2.1 or higher. We found stronger IgM and IgG responses in patients with complicated infection. Patients with a high initial IgG OD values had a slower IgG decline over time compared to those with a low initial IgG OD, and were at an increased risk of complications (18/36= 50% vs. 28/91= 30.8%, risk ratio= 1.63, 95%CI 1.04 / 2.55, p= 0.035). This association was robust to adjusting for age (risk ratio 1.50, 95%CI 0.96 / 2.33, p= 0.072). IgM and IgG responses were not associated with eschar presence.

Conclusion: Repeated infection may lead to IgG antibodies persisting over many years. High initial IgG levels as a proxy for IgG levels prior to an acute infection may be associated with a higher risk of complicated scrub typhus infection.

Abstract 89: Prevalence and genetic diversity of *Orientia tsutsugamushi* in scrub typhus patients in North India

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Scrub typhus is a zoonosis caused by *Orientia tsutsugamushi*. It has emerged in recent years to become one of the most common causes of acute febrile illness in India. We aimed to study the prevalence and genetic diversity of *O. tsutsugamushi* in scrub typhus patients presenting to our tertiary care hospital in north India. A total of 590 samples (blood, eschar and CSF) were received in the Neglected Bacterial Diseases Laboratory, Medical Microbiology Department, PGIMER, Chandigarh for scrub typhus PCR, between 1st April 2017 to 31st March 2018. DNA was extracted by phenol chloroform isoamyl alcohol protocol and subjected to nested PCR and conventional PCR targeting 56 kDa outer membrane protein and 16S rRNA gene of *Orientia tsutsugamushi* respectively. The purified amplicons were then subjected to DNA sequencing using ABI 3130 sequencer, BDT 3.1 version cycle sequencing kit. The DNA sequences were subjected to BLAST search and a phylogenetic tree was constructed using MEGA version 7. Out of 590 samples processed N-PCR was positive in 52 (8.8%) and C-PCR in 20 (3.4%). By N-PCR, positivity in eschar was 46.7% while that in blood was 9.3%. No CSF sample was positive. Maximum sequences showed close identity with Karp followed by Gilliam prototypes, while two clustered with Boryong reference strains.

Poster Presentations

Monday, 4th November 2019

Poster session 1: Host-pathogen-vector interactions/Pathogenesis/Cell biology

Abstract 2: Diversity and distribution of tick-borne bacteria and parasites in Armenia

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Ticks are vectors of human and animal disease, and variation in the distribution and prevalence of pathogens in ticks can have implications for human health. We conducted a large-scale study to detect bacterial and parasitic agents in ticks in Armenia. We collected 209 ticks from different regions in Armenia between June and October 2017. Ticks were collected from dogs, cows, sheep, goats, and human clothes. Samples were tested using high-throughput microfluidic single-cell qPCR and 42 genospecies of bacteria and parasites were detected. We used GIS to determine biotic and abiotic factors governing the prevalence of different pathogens and applied statistical analyses to determine correlations between the prevalence of pathogens in ticks and hosts, location, and environmental variables. Of 209 ticks, 175 (84%) were positive for at least one of the target pathogens. The prevalence of pathogens varied significantly among hosts: the highest prevalence was observed in ticks from calves and cows (90% of which were positive for a pathogen), followed by ticks from sheep (85%). Interestingly, sheep had the highest co-infection rate (59%), followed by calves (55%) and cows (48%). Among co-infections with different pathogens, we found only one statistically significant association (*Francisella tularensis* and *Rickettsia massiliae*). Pathogen prevalence was also highly variable by location; *Anaplasma* species prevalence was significantly correlated with slope, elevation, and temperature, and similar patterns were observed among other pathogens. The results of this large-scale survey helped expand our understanding of pathogen distribution, prevalence and spatial variation in prevalence and environmental determinants.

Abstract 27: Description of *Colicus* sp. nov. (Trombiculiformes: Trombiculidae) from a scrub typhus endemic region in southern Chile

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Background: Scrub typhus (ST) is endemic in Asia-Pacific, where it is maintained and transmitted by trombiculid mites. Recently, the disease was discovered within a wider geographical range, including Chile, but reservoirs and vectors in the new endemic regions are unknown. This work describes a new species of *Colicus*, isolated from rodents, captured on Chiloé Island in southern Chile.

Methods: Trombiculids were collected from rodents captured in 6 probable ST hotspots on Chiloé Island. Specimens were stored in 96% ethanol, cleared in Nesbitt's solution, mounted in Berlese's medium and examined by optical microscopy. Morphological identification followed the key and nomenclature of Brennan and Goff (1977) and Goff *et al.* (1982), respectively.

Results: *Colicus* mites from Chiloé were morphologically distinct to the known 18 species of the genus, described from different neotropical regions. Its scutal setation formula PL>AL>AM was only shared with *C. pichindensis*. However, it differed from *C. pichindensis* by the palpal setation formula B/B/BbB, the presence of two genulas I, and by its row pattern of dorsal setae. The new species differed from *C. sinpretasus* by lacking pretarsal II and distinct formulas of scutal (PL>AM>AL) and palpal setation (B/B/NNB).

Conclusions: These first trombiculids of the genus *Colicus* in Chile represent a new species. We suggest naming it in honor of Eloísa Díaz Insunza (1866-1950), first Chilean female medical student and woman working as a medical doctor in South America. Its abundance in hotspots of ST on Chiloé Island suggests a possible role as vectors of this emerging infection.

Abstract 33: Molecular detection of *Orientia* spp. in trombiculid mites collected from rodents on Chiloé Island, Chile

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Background: Until recently, scrub typhus (ST) was only endemic in Asia-Pacific, where it is caused by *Orientia tsutsugamushi* and maintained and transmitted by trombiculid mites. Since recently, the disease has been discovered within a wider geographical range, including Chile, but reservoirs and vectors in the new endemic regions are unknown. This study analyzed, if *Orientia* DNA was present in

rodent-associated trombiculid mites collected on Chiloé Island, a scrub typhus endemic region in southern Chile.

Methods: Trombiculid mites were collected from rodents captured in 6 probable ST hotspots on Chiloé Island and identified using morphological criteria. Three genera of chiggers were identified, most abundantly *Colicus*, a neotropical genus, first described in Chile. DNA of mite pools (consisting of a single genus from single rodent) was extracted and tested by real-time PCR (qPCR) against *rrs* gene (16S rRNA). In selected positive pools from each site, hemi-nested PCR was used to obtain amplicons for sequencing. *Orientia* sequences were phylogenetically compared to those of Chilean clinical isolates and strains from other regions.

Results: A total of 133 mite pools were tested; of those 21 (15.8%) were positive by qPCR and all were *Colicus*. *Orientia* sequences obtained from positive mite specimens by hemi-nested PCR were 100% identical to those of Chilean ST cases from the same region.

Conclusions: Our findings demonstrate that a significant proportion of *Colicus* mites from ST hotspots on Chiloé Island harbor *Orientia* sp., which are molecularly identical to clinical isolates, suggesting that these mites serve as the local vector of ST.

Abstract 41: The Tick Cell Biobank: resources for research on rickettsial pathogens and symbionts

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The Tick Cell Biobank (TCB) is the world's only dedicated culture collection for cell lines derived from ticks and other arthropods. As well as establishing, storing and distributing these cell lines, the TCB provides training in their maintenance to recipient scientists, and carries out genotypic and phenotypic characterisation studies. Over the past few decades, many of the >50 tick cell lines in the TCB have been used in a wide variety of studies on isolation, propagation and characterisation of known and novel rickettsial pathogens and symbionts and their interaction with vector cells. The TCB houses a small collection of *Anaplasma*, *Ehrlichia* and *Rickettsia* spp. that can be propagated in tick cell lines, and several primary cultures and cell lines derived from other arthropods including *Lutzomyia* and *Phlebotomus* sand flies, *Culicoides* biting midges and *Aedes* and *Culex* mosquitoes. To facilitate uptake of tick cell line technologies by scientists in South-East Asia, an Outpost of the TCB is being set up in Malaysia at TIDREC, University of Malaya, Kuala Lumpur. From early 2020, the TCB Asia Outpost will offer a panel of popular and regionally-relevant tick cell lines and locally-delivered training, and aims to generate novel cell lines from Asian tick species, to support and enhance tick and tick-borne pathogen research in the region.

Abstract 47: Molecular detection of *Orientia* spp. in wild rodents from Chiloé Island, southern Chile

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Background: Scrub typhus is a vector-borne zoonosis caused by *Orientia* spp. and transmitted by infected mites. Wild rodents are imported hosts of these mites in Asia-Pacific (*Leptotrombidium* spp.), where various species of rodent hosts have been found infected with *Orientia*. Scrub typhus was recently discovered in Chile, but transmission and maintenance of the Chilean *Orientia* species is unknown. The aim of this work was to study *Orientia* spp. in rodents trapped in sites associated with scrub typhus cases in southern Chile.

Methods: Between January to March 2018, rodents were trapped in 6 sites on Chiloé Island. Males and juvenile females were euthanized and spleen, liver, and lungs were removed. DNA from organs was extracted and analysed by real-time PCR (qPCR) for a fragment of the 16S rRNA gene specific to *Orientia* spp.

Results: Of 156 captured rodents analysed, 7 species were identified and the two most commonly collected were *Abrothrix olivacea* and *Irenomys tarsalis*. Molecular detection of *Orientia* spp. among rodent tissue DNA samples showed that 38% (59/156) belonging to 5 rodent species were qPCR positive for *Orientia*. Prevalence rates were 41% (45/111) in *A. olivacea*, 44% (10/23) in *I. tarsalis*, 13% (1/8) in *Geoxus valdivianus*, 29% (2/7) in *Abrothrix sanborni*, and 50% (1/2) in *Rattus norvegicus*. *Orientia* sp detection was similar between parasitized-rodents versus no-parasitized-rodents, 38.5% and 36%, respectively.

Conclusions: Our study suggests that various species of wild rodents on Chiloé Island in southern Chile are infected with *Orientia* spp.

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Abstract 49: Detection of Anaplasmataceae agents and co-infection with other tick-borne protozoa in dogs and *Rhipicephalus sanguineus sensu lato* ticks

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Anaplasmosis and ehrlichiosis are of serious health concern worldwide for animals and humans. In the present study, we report the occurrence of *Anaplasma platys* and *Ehrlichia canis* in dogs and *Rhipicephalus sanguineus* sensu lato (s.l.) ticks from Peninsular Malaysia using a nested polymerase chain reaction assay based on amplification of the 16S rRNA gene. *Anaplasma platys* was detected from dogs and ticks with prevalence rates of 3.3% (8/240) and 2.9% (4/140), respectively. On the other hand, 12.9% (31/240) of the dogs and 0.7% (1/140) of the ticks were tested positive for *E. canis*. Additionally, co-infections of *A. platys* and *E. canis* with *Babesia* or Hepatozoon protozoa were also noted in this study. Double infection (*E. canis* + *B. gibsoni*) was observed in tick, whereas triple infections (*E. canis* + *A. platys* + *B. vogeli* and *E. canis* + *A. platys* + *H. canis*) were found in dogs. This study represents the first evidence of *A. platys* DNA in *R. sanguineus* s.l. in Malaysia.

Abstract 50: Evaluation of *Leptotrombidium* chigger mites minimal transmission time for *Orientia tsutsugamushi* utilizing a rhesus macaque scrub typhus model

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Development of a protective vaccine against homologous and heterologous strains of *Orientia tsutsugamushi* (Ot) is crucial for prevention of scrub typhus. A successful vaccine is considered the most effective way to tackle the issue of antibiotic resistance and reduce preventable illnesses and deaths. Nonetheless, no effective vaccine is currently available as a consequence of the inability to develop a broadly protective immune response. This study aims to establish a standardized, reproducible rhesus macaque model of scrub typhus using a natural chigger-challenge method incorporating multiple strains of Ot which is considered the best way to mimic natural route of pathogen(s) transmission and cannot be duplicated by any other challenged model. As the initial phase of the study, we determined the minimum chigger attachment time which could efficiently transmit Ot to the macaque hosts. Preliminary results indicated that Ot transmission via the chigger challenged could occur within 1 hour after attachment which is shorter than transmission times of other Acari. Quantification data revealed that bacteremia was first detected at 250 copies/mL on day10 post-chigger attachment, rose to 2.62x10³ copies/mL at day 16 and thereafter declined until it reached base line level at day 21. The immunological reactions were first detected on day13 post-chigger bite and continued until day38. Serum cytokine analyses showed antigen-specific T cell responses of G-CSF, IL-4, and IL-15 were markedly increased. In addition, we found that inoculation dose from chigger to host was possibly considered as time dependent manner which may impact the development of scrub typhus disease model

Abstract 52: Artificial Infection of *Leptotrombidium* Mite with Human Pathogenic Strain of *Orientia tsutsugamushi* using a Microinjection Method

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Orientia tsutsugamushi (Ot), an obligate intracellular bacterium, is the causative agent of scrub typhus (ST) and is transmitted by the bite of infected trombiculid mite larvae. Ot is inherited from infected mothers to progeny through a process called transovarial and transstadial transmission. Single larvae naturally infected with multiple strains of Ot can be found widely in endemic areas. Multi-strain infection is a consequence of multiple larvae mites co-feeding at the same site on an individual host. To support ST disease model and vaccine development studies, chigger colonies harboring only sole Ot strain infection are required. A single strain colony will prevent confounding of the host immune response to the heterogeneous strains. A capillary-based, microinjection method was used to mechanically transfect Ot strain Karp and Gilliam to *Leptotrombidium chailingraiensis*. The maintenance and transmission of Ot to offspring was followed for 2 generations using an animal-challenge method and infection was verified by molecular and serological detection. Injection at the dorsal surface of the opisthosomal haemocoel or ventral shield in adults or at dorsal or genital region in tritonymphs did not affect oviposition or spermatophore production in adults when compared to the control group. Transmission rate of Ot from adult females to chiggers at the F1 generation was 10.4% (10/96) and 8.2% (17/208) from transfected tritonymphs and transfected adults, respectively. ICR mice developed low levels of serum IgM and IgG to ST infection. However, no lethal infections occurred. Ot infection in adult mites was maintained in colonies up to 20 months after transfection.

Abstract 63: Molecular detection of *Rickettsia* species in ectoparasites collected from Southern Provinces of Cambodia

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Rickettsial diseases are vector-borne infections that have historical significance to military operations and remain a current force health protection threat in Southeast Asia. Arthropod-borne rickettsioses comprise a wide variety of subtypes which are endemic in Cambodia, but there remains very little data on the geographic distribution of the pathogens or their vectors. In collaboration with Department of Health, Ministry of National Defense, ectoparasite surveys were conducted in Koh Kong and Sihanoukville Provinces between July 2017 and June 2018. Ectoparasites were collected from peridomestic animals and from the environment using dragging and flagging methods. Collected ectoparasites were sorted and identified morphologically, then pooled by species, host, and location for molecular detection using *Rickettsia* genus- and species-specific qPCR assays. *Rickettsia*-positive samples not identified by species-specific qPCR were analyzed further using multilocus sequence typing (MLST). A total of 16,255 ectoparasites were collected, consisting of ticks (78.4%), fleas (12.6%), lice (6.6%), and mites (2.4%). *Rickettsia* species were detected in 35.5% (n=173) of the pools screened (n=486) representing 2,846 randomly selected ectoparasites. *Rickettsia asembonensis* was found in 90.6% (146/161) of *Rickettsia*-positive flea pools and 3.7% (6/161) were positive for both *R. asembonensis* and *R. felis*. Additional *Rickettsia* species or genotypes were detected through MLST: *Candidatus R. senegalensis* from *C. felis orientis* fleas, *Rickettsia* sp. close to *R. japonica* and *R. heilongjiangensis* from *Haemaphysalis* ticks. This appears to be the first reports of these rickettsial species in southern Cambodia, suggesting a potential health risk to military and civilians in this region.

Abstract 78: Heat-stable RNA from the Gram-negative bacterium *Orientia tsutsugamushi* is recognized by murine TLR13

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Orientia tsutsugamushi (OT) is the causative agent of scrub typhus, a potentially lethal infectious disease endemic in south-east Asia, affecting about 1 million people annually. After entering phagocytic host cells (e.g. macrophages and dendritic cells) via endocytosis, OT escapes from the endosome to replicate in the vicinity of the nucleus. During infection, OT triggers strong innate immune responses, but the details of its recognition, including receptors and ligands, remain unknown.

We hypothesized that OT-RNA contributes to innate activation of dendritic cells. Indeed, transfection of primary bone marrow-derived dendritic cells (BMDC) from C57BL/6 mice with OT-RNA induced an upregulation of IFN- α and TNF- α . Treatment with RNases abrogated this stimulation. Since toll-like receptor (TLR) 13 is an important sensor of bacterial ribosomal RNA in mice, we investigated its role in endosomal recognition of OT-RNA. By in silico analysis, we found that the TLR13 consensus sequence originally identified in *Staphylococcus aureus* 23S ribosomal RNA (rRNA), is also present in the 23S rRNA of four major OT strains Karp, Kato, Gilliam and Boryong. Thus, BMDC from C57BL/6 wildtype and MyD88- or TLR13-deficient mutants were transfected with OT-RNA or stimulated with heat-killed OT (hkOT), and mRNA transcription of pro-inflammatory cytokines (TNF- α and pro-IL-1 β) and IFN- β was measured. Our data showed a MyD88- and TLR13-dependent upregulation of the NF κ B-dependent

cytokines TNF- α and pro-IL-1 β upon stimulation with hkOT or OT-RNA transfection. Surprisingly, the upregulation of IFN- β mRNA by OT-RNA was TLR13-independent.

These data support a central role for OT-RNA in the induction of inflammation.

Abstract 79: Innovative distance learning method of chigger mites as vectors of scrub typhus: a preliminary study

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Scrub typhus, a tropical infection caused by *Orientia tsutsugamushi*, affects more than one billion people globally with average fatality rate of 6%. Humans, as accidental hosts, are infected through the bite of infected Trombiculid mite larval stage, known as chiggers. Unlike hematophagous arthropods, chiggers feed on hosts' extracellular fluid for survival and to complete their development. *O. tsutsugamushi* are maintained throughout the chigger's lifespan and through various generations by transstadial and transovarial transmission. The knowledge this disease's transmission is essential in designing effective prevention and control strategies. Unfortunately, many personnel in related sectors are still unfamiliar to this disease and its vector. In this study, we aim to improve the knowledge on this topic by developing a distance learning method. Since it is facilitated online, the "students" and "tutors" are not required to be physically present at the same place, thus allowing flexibility. We developed a video containing basic information regarding scrub typhus and its vector and distributed it to 34 participants in related sectors. The knowledge improvement was evaluated by pre- and post-tests questionnaires. Fifty-four percent of participants had prior knowledge on this topic. However, when asked to identify the scrub typhus vector, 76.5% still answered incorrectly. After watching the video, the average score increased 15.3% from the baseline. Most participants (63.6%) showed interest in this topic and learning method. These preliminary results suggested that distance learning method was promising in distributing health-related information. In the future, we plan to improve and test this method in more heterogeneous communities.

Abstract 80: Innate recognition of heat-stable ligands from *Orientia tsutsugamushi* by C-type lectins

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Orientia tsutsugamushi, an obligate intracellular Gram-negative bacterium causing the neglected febrile disease scrub typhus, elicits chemokine and cytokine production by heat-stable ligands via NF- κ B-dependent pathways in phagocytes. It has not been studied how recognition by receptors other than Toll-like (TLR) and NOD-like receptors shape the inflammatory response to *Orientia*. *Orientia* has an atypical cell wall composition with high amounts of neutral saccharides in its outer membrane, which could predispose for recognition by C-type lectin receptors (CLR).

In order to screen for potential CLR ligands, we used purified heat-inactivated *Orientia* in a FACS-based interaction assay involving a library of murine and human CLRs. We identified four mouse candidate receptors binding to *Orientia*. Binding to mouse Mincle was EDTA-sensitive and thus shown to be specific. Mincle was therefore chosen for further investigations.

Bone marrow-derived dendritic cells (BMDC) from C57BL/6 mice stimulated with inactivated *Orientia* showed an increasing, dose-dependent induction of mincle mRNA over time. Mincle however, did not contribute to the production of pro-inflammatory cytokines, since Mincle-deficient BMDC secreted significantly higher levels of TNF- α , IL-6 and IFN- β compared to the C57BL/6 wildtype upon stimulation with *Orientia*. This observation suggests an inhibitory effect of Mincle on NF- κ B-dependent and -independent cytokine production.

Combined, these results point toward an initial upregulation of Mincle by another receptor, e.g. a TLR, and an inhibitory effect of Mincle on cytokine production. We aim to provide further insight into the role of Mincle in the recognition of *Orientia tsutsugamushi*.

Abstract 81: Screening of Rickettsiaceae, Anaplasmataceae, *Candidatus* Midichloriaceae, and Leptospiraceae from ticks collecting from Nakai District, Khammouan Province, Laos

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Vector-borne diseases are regarded as a serious public health concern as they are estimated to be a culprit of one sixth of illnesses worldwide and more than half the world's population might be at risk of the infections. Ticks are one of many important globally distributed vectors that are known to transmit various bacterial diseases to humans: spotted fever, scrub typhus, lyme disease, ehrlichiosis, anaplasmosis, Q-fever, tularemia, and relapsing fever. In Laos, scrub typhus and spotted fever account for 9.6% and 2.6%, respectively, of febrile adult patients admitted to Mahosot hospital. However, compared to other countries, knowledge of the prevalence of tick-borne diseases in Laos is still inadequate and needs to be investigated. One thousand and thirty-eight DNA pools of ticks, collected

from Nakai District, Khammouan Province during February 2016-February 2017, were screened for family Rickettsiaceae, Anaplasmataceae, Candidatus Midichloriaceae, and Leptospiraceae by qPCR and cPCR. We found that 58 (5.6%) samples were Rickettsia spp. positive, 10 (1%) samples were Leptospira spp. positive, 2 (0.2%) samples were positive for *Anaplasma phagocytophilum*, 2 (0.2%) samples were positive for *Ehrlichia chaffeensis*, 22 (2.1%) were identified to the family Anaplasmataceae, and 3 (0.3%) samples were positive for Candidatus Midichloriaceae. Further sequencing of Rickettsia spp. positive samples identified *R. typhi* (1 sample), *R. raoulti* (8 samples), and *R. japonica* (3 samples) which are recognized as human pathogen. These findings highlight the presence of tick-borne bacterial pathogens in Laos and the potential for transmission of clinically relevant pathogens to humans.

Abstract 90: Evaluation of heterogeneity in the composition, abundance and infection prevalence of *Anaplasma marginale* among different Ixodidae ticks throughout the Flint Hills Region of USA

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Abstract: The role of persistently infected ticks as biological magnifiers and their differing ability to harbor *Anaplasma marginale*, causative agent of bovine anaplasmosis in heterogeneous tick habitats in the Midwestern US is poorly understood. The objective of this study was to quantify the composition, abundance, and infection status of different Ixodid ticks throughout the rangeland of the Flint Hills, a dominant cattle-producing region in Kansas. Tick sampling was performed during the months of May through August of 2016 from twenty-three collection sites. Collection sites were productive grazing pastures, with and without grazing during the collection period. Questing ticks of all species and life-stages were collected. In total, 4,976 ticks were collected, including ticks of two genera belonging to three species. The most frequently observed species was *Amblyomma americanum*, 94.2% of the total; *Dermacentor variabilis*, and *Amblyomma maculatum* comprised 4.6%, and 1.2% respectively. The tick species were identified morphologically and through PCR testing. The percentage of infection with *A. marginale* among *D. variabilis* ticks were found to be ♂ = 8.7% and ♀ = 11.35%. No infection was noted among the other ticks or other life-stages. Distribution of infected ticks varied across the landscape type; infected *D. variabilis* ticks were found predominantly in areas with higher habitat fragmentation, and further in landscape dominated by grassland/herbaceous vegetation followed by deciduous forest.

Tuesday, 5th November 2019

Poster Session 2: Infection/Diagnosis

Abstract 6: Molecular detection of tick-borne pathogens in cattle and ticks collected from Cavite, Philippines

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Background: Ticks are obligate ectoparasites that are greatly considered important vectors of disease-causing microorganisms affecting humans and animals. In this study, we determined the presence of selected tick-borne pathogens not routinely screened or diagnosed in the province of Cavite, Philippines using polymerase chain reaction (PCR).

Methods: Extraction of DNA from tick and blood samples were accomplished using commercially available DNA extraction kits or alkali neutralization method. On the other hand, viral RNA was exclusively extracted using commercially available extraction kits.

Results: A total of 1302 ticks were collected and identified as *Rhipicephalus microplus* in the present study. 165 tick samples were used for detection of *Rickettsia* spp., *Anaplasma* spp., and *Coxiella burnetii*, however no positive samples were recorded. For *Babesia* spp. detection, 1 out of the 83 tick samples screened was positive and turned out to be *Theileria equi* based on BLAST analysis. No tick-borne flaviviruses were detected from the 80 tick samples screened. On the other hand, 66 out of 100 blood samples (66%) were positive only for *Anaplasma* spp. detection. Among the *Anaplasma* spp. positive samples, 10 representative sequences were further subjected to BLAST analysis and all the 10 sequences showed 100% homology with *Anaplasma marginale* isolates.

Conclusions: In summary, our findings show that *Anaplasma marginale* is highly prevalent in the local cattle population of Cavite Philippines. Such findings also warrant immediate control of tick population and treatment of the animals to prevent the spread of the disease to nearby susceptible backyard cattle population.

Abstract 30: Scrub typhus in patients presenting with fever in southern Chile: a pilot study in Ancud Hospital

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Introduction: Scrub typhus (ST) is a potentially life-threatening disease in Asia-Pacific, presenting as an acute febrile illness with or without its pathognomonic cutaneous manifestation (eschar, rash). Recently, ST has been discovered in Chile, where the spectrum of its clinical presentation is less understood. This pilot study intended to implement a protocol to identify ST cases among febrile patients in an endemic area of southern Chile.

Method: During February/March 2019, a study protocol was evaluated at the emergency room of Ancud Hospital, Chiloé Island. Patients ≥ 7 years consulting with fever without obvious cause were asked to participate. After informed consent was obtained, blood samples were drawn for routine laboratory exams, *Orientia* serology (IFA, ELISA), and *Orientia* PCR in buffy coat and/or eschar (if present), and a follow-up visit in 2-3 weeks was scheduled for a convalescent serum sample.

Results: Of 43 potential cases, 27 (63%) were enrolled. Of those, 26 were without cutaneous manifestations and PCR (buffy coat) negative. One case with eschar and rash was PCR (eschar) positive. This ST patient had presented 4 days previously (before developing skin manifestations) and was then PCR (buffy-coat) negative. Follow-up and convalescent serum was available for only 8 cases. Except for the PCR positive patient, all of those were serologically negative.

Conclusion: The ST prevalence among the febrile cases of this pilot project was 7.4%. The experiences of the project will help to implement future studies with optimized clinical and diagnostic approaches to better understand the clinical spectrum of ST in southern Chile.

Abstract 51: Retrospective host-pathogen identification using amplicon-based next generation sequencing

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An unusually high percentage flea samples collected during 2015-2016 in Thailand were PCR-positive for *Rickettsia* species. To identify *Rickettsia* pathogens and their arthropod hosts to species, we used amplicon-based next generation sequencing (NGS). All *Rickettsia*-positive pools from country-wide surveillance (2015-2016), were selected for retrospective NGS characterization: rodents (2/1384=0.14%) fleas collected from rodents (1/53=1.89%), fleas pools collected from domestic animals (96/173=56%), and ticks collected from domestic animals (3/167=1.80%). To characterize *Rickettsia* species, citrate synthase (*gltA*) gene was amplified, while Cytochrome Oxidase subunit I (COI) and subunit II (COII) genes were used as target genes for ectoparasite identification. All target gene amplicons were pooled and sequenced with Illumina MiSeq platform. Two *Rickettsia*-positive rodent samples were characterized as

Rickettsia typhi. *R. asembonensis* was found in a rodent flea pool. Interestingly, among 96 *Rickettsia*-positive fleas collected from domestic animals, most of them were found to carry *R. asembonensis* (84%) while *Candidatus R. senegalensis* was detected in only 15%. For ticks from domestic animals, *Candidatus R. senegalensis* and *R. helongjiangensis* was found in 3 of 167 pools (1.80%). The majority of *Rickettsia*-positive fleas (80.9%) collected were identified as *Ctenocephalides felis orientis*, only few of them were *C. felis felis* (19.1%). Among *C. felis orientis* population, 98.6% were *Rickettsia* positive, 81.3% carried *R. asembonensis* and 17.3% had *Candidatus R. senegalensis*.

Abstract 53: Newly cloned and expressed 56 kDa recombinant proteins are broadly reactive to *Orientia*-specific antibodies from infection by various *Orientia tsutsugamushi* strains

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Orientia tsutsugamushi is an obligate intracellular bacterium that causes scrub typhus. Scrub typhus is prevalent mainly in the traditional tsutsugamushi triangle with new cases emerging in Africa, South America and Middle East. While the estimated annual cases are in the millions, it is believed that the case number is underestimated mainly due to its difficulty in proper diagnosis. The indirect immunofluorescence assay (IFA), which often requires paired sera, is still the gold standard of serological diagnosis. A recently developed recombinant-protein-based ELISA (TW ELISA) was evaluated using acute sera from IFA confirmed patients. While this TW ELISA is sensitive and specific in detecting *Orientia*-specific antibodies in patient samples collected in Taiwan, whether the TW ELISA offers broad reactivity with antibodies produced from infection by various *Orientia* strains has not been demonstrated. We reported here that TW ELISA is broadly reactive to *Orientia*-specific antibodies from infection by 14 *Orientia* strains are originally isolated from regions including Burma, Japan, Thailand, Malaysia, and Australia. A comparison of TW ELISA with another recombinant-protein-based ELISA (NMRC ELISA) showed similar titers in IgG detection and higher titers in IgM detection. A limited number of patient samples were also tested by TW ELISA and NMRC ELISA with comparable results. We conclude that the TW ELISA is broadly reactive to *Orientia*-specific antibodies and may be suitable for diagnosis of scrub typhus in endemic areas. Additional evaluation of the performance of the TW ELISA using patient samples collected from different endemic areas is needed to ensure its clinical utility.

Abstract 60: Q fever among acute undifferentiated fever patients in Vietnam

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Coxiella burnetii, the causative agent of Q fever in humans, has worldwide distribution with the exception of New Zealand. There are multiple reports of *C. burnetii* in arthropods in Southeast Asia, and an increasing number of cases of Q fever in Thailand. To date, there are no reports of Q fever in Vietnam. As part of a larger surveillance study for rickettsial diseases in 8 ecological regions of Vietnam peripheral blood monocytes (PBMCs) were collected from febrile patients in 27 hospitals spanning 26 provinces throughout Vietnam. The PBMCs were screened by quantitative real-time PCR (qPCR) targeting the IS1111 element found in *C. burnetii*. The IS1111 element is a typical insertion sequence encoding only a transposase that is found in multiple copies in the genome of *C. burnetii* with reports of more than 20 copies in some strains. However, the IS1111 element is also found in *Coxiella*-like bacteria, thereby requiring a confirmatory assay to positively identify *C. burnetii*. Samples that tested positive for the IS1111 element, were then confirmed to be *C. burnetii* by testing for the gene for the outer membrane protein, Com1, which is specific to *C. burnetii*. A total of 1,090 patient PBMC samples were tested and out of those 9 samples (0.8%) were positive for the IS1111 element and com1, confirming Q fever in these patients. Additional testing is underway to characterize the *C. burnetii* found in these patients. These preliminary results will raise the awareness of clinicians and communities of Q fever among febrile patients.

Abstract 73: Determination of Optimal Diagnostic Cut-Offs for the Naval Medical Research Center Scrub Typhus IgM ELISA in Chiang Rai, Thailand

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In this diagnostic accuracy study, we evaluated data from 135 febrile patients from Chiang Rai, to determine the optimal optical density (OD) cutoffs for an in-house scrub typhus IgM ELISA. Receiver operating characteristic curves were generated using a panel of reference assays, including an IgM immunofluorescence assay (IFA), PCR, in vitro isolation, presence of an eschar, or a combination of these. Altogether, 33 patients (24.4%) were diagnosed as having scrub typhus. Correlation between positivity by IFA and increasing OD values peaked at a cutoff of 2.0, whereas there was little association between positivity by culture or eschar with increasing ELISA cutoffs—cutoffs of 3.0 and 4.0 were demonstrated to be optimal for the total absorbance of the OD at dilutions 1:100, 1:400, 1:1,600, and 1:6,400, for admission and convalescent samples, respectively. The optimal cutoff at a 1:100 dilution was found to be between 1.85 and 2.22 for admission samples and convalescent-phase samples, respectively. Sensitivities for the cutoffs varied from 57.1% to 90.0% depending on the reference test and sample timing, whereas specificities ranged from 85.2% to 99.0%. We therefore recommend a

cutoff of around 2.0, depending on the sensitivity and specificity desired in clinical or epidemiological settings. The results demonstrate the ELISA to be a valuable diagnostic tool, suitable for use in resource limited endemic regions, especially when used in combination with other diagnostic modalities such as the presence of an eschar.

Abstract 74: Strain-specific detection of *Orientia tsutsugamushi* using multiplex fluorescence in-situ hybridization

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Scrub typhus is an endemic disease in the Asia-Pacific region caused by *Orientia tsutsugamushi*, an obligate intracellular bacterium, which can be transmitted to humans by the bite of larval *Leptotrombidium* mites (chiggers). In nature, co-existence of multiple strains of *O. tsutsugamushi* has been reported in chiggers, and rodent hosts can harbor multiple *Orientia* strains from the attached chiggers. In this study, an advanced genotyping technique was designed to detect different strains of *Orientia* in individual samples. A specific staining method was developed to identify and localize *O. tsutsugamushi* organisms using a fluorescence in-situ hybridization (FISH) approach (DNAScope). The strain-specific probes were designed based on the branched DNA ZZ probes targeting the hypervariable regions of the 56 kDa type specific antigen of each *O. tsutsugamushi* strain, Karp and Gilliam. The Karp DNA probe was visualized by Atto 550 while Gilliam DNA probe was visualized by Alexa Fluor 488. Using multiple FISH staining, we will be able to localize different strains of *O. tsutsugamushi* in the same tissue section. This approach could reduce differences in antibody binding activity in various sample types. Additionally, the technique can provide additional insight into multiple *Orientia* infections in combination with cell phenotyping by conserving contextual information within cells and surrounding structures.

Wednesday, 6th November 2019

Poster session 3: Epidemiology/Genomics

Abstract 11: MinION whole genome sequencing of *Rickettsia typhi* in a resource-limited setting

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The infrastructure challenges and costs of next-generation sequencing have been largely overcome, for many sequencing applications, by Oxford Nanopore Technologies' portable MinION sequencer. However the question remains open whether MinION-based bacterial whole-genome sequencing (WGS) is by itself sufficient for the accurate assessment of phylogenetic and epidemiological relationships between isolates and whether such tasks can be undertaken in resource-limited settings. To investigate this question, we sequenced the genome of an isolate of *Rickettsia typhi*, an important and neglected cause of fever across much of the tropics and subtropics, for which only three genomic sequences previously existed. We prepared and sequenced libraries on a MinION in Vientiane, Lao PDR using v9.5 chemistry and in parallel we sequenced the same isolate on the Illumina platform in a genomics laboratory in the UK. The MinION sequence reads yielded a single contiguous assembly, in which the addition of Illumina data revealed 226 base-substitution and 5,856 in/del errors. The combined assembly represents the first complete genome sequence of a human *R. typhi* isolate collected in the last 50 years and differed from the genomes of existing strains collected over a 90-year time period at very few sites, and with no re-arrangements. Filtering based on the known error profile of MinION data improved the accuracy of the Nanopore-only assembly. However, the frequency of false-positive errors remained greater than true sequence divergence from recorded sequences. While Nanopore-only sequencing cannot yet recover phylogenetic signal in *R. typhi*, such an approach may be applicable for more diverse organisms.

Abstract 14: Endemic Severe Fever with Thrombocytopenia Syndrome in Vietnam

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Severe fever with thrombocytopenia syndrome virus (SFTSV) is a tick-borne virus of the genus Phlebovirus and family Phenuiviridae that can cause a mild to severe febrile illness similar to hemorrhagic fever. Phleboviruses have been found in the Americas, Asia, Africa, and the Mediterranean region. For example, the Heartland virus (HRTV), another tick-borne Phlebovirus, was identified in northwestern Missouri in the United States in 2009 (2). The Maloor virus, a novel bat Phlebovirus closely related to SFTSV and HRTV, was identified in western India, and a Phlebovirus similar to SFTSV and HRTV was also isolated from ticks in Australia. SFTS was first confirmed in China in 2009 and was retrospectively identified in South Korea in 2010 and the western regions of Japan in 2013. SFTS is characterized by an acute high fever, thrombocytopenia, leukopenia, elevated serum hepatic enzyme levels, gastrointestinal symptoms, and multiorgan failure with 16.2 to 30% mortality. Atypical signs and symptoms and asymptomatic infections have also been identified. Most SFTSV infections occur through *Haemaphysalis longicornis*, although SFTSV transmission can also occur through close contact with an infected patient.

In this study, we detected SFTSV infections in Vietnam, which has not been previously documented to have SFTSV or disease cases, suggesting that SFTSV infection may have a much wider global distribution than previously thought.

Abstract 19: Rickettsial bacteria in ticks parasitizing wild boars in an indigenous community in Malaysia as revealed by 16s rRNA gene amplicon sequencing

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The indigenous people in Malaysia, the Orang Asli, routinely hunts for wild boars as a food source. They may be exposed to tick-borne diseases since wild boars are commonly tick-infested. Here, we performed a survey of the bacterial communities associated with ticks parasitizing wild boars from an Orang Asli village using 16s rRNA amplicon sequencing on Ion Torrent PGM platform, to investigate the presence of tick-borne pathogens.

Ticks (n=72) were morphologically identified as *Haemaphysalis hystricis* (n=32), *Dermacentor compactus* (n=15), *Amblyomma testudinarium* (n=13), *Dermacentor steini* (n=10) and *Dermacentor atrosignatus* (n=2). The sequencing data revealed that *Rickettsia* and *Coxiella* were the dominant bacterial taxa in these samples (>1% relative abundance). Other tick-associated rickettsiae (*Ehrlichia*, *Anaplasma*) were also detected at low abundance.

The presence of the rickettsiae was confirmed by PCR amplification of bacteria-specific genes. Phylogenetic analysis of the *Coxiella* 16s rRNA amplicon sequence suggests the presence of *Coxiella burnetii* and *Coxiella*-like bacteria. Phylogenetic analysis of the rickettsial *gltA* sequence revealed the

presence of *Rickettsia* related to spotted fever group rickettsiae, including *Rickettsia raoultii* and *Rickettsia tamurae*. *Ehrlichia* and *Anaplasma*-specific genes were not amplified in the samples.

This study provides the baseline knowledge of the tick-associated rickettsiae present in the *Haemaphysalis*, *Dermacentor* and *Amblyomma* ticks found parasitizing on wild boar in indigenous community. The presence of *Rickettsia* and *Coxiella* in these tick species may explain the previous findings of spotted and Q fever seropositivity among the Orang Asli. Further investigations will be required to fully determine the zoonotic potential of these bacteria.

Abstract 20: Detection of *Rickettsia felis*-like organisms (RFLOs) in fleas from feral dogs and cats in an Orang Asli village in Malaysia

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Feral dogs and cats are commonly found in the villages of the indigenous people in Malaysia, also known as the Orang Asli. Although not considered as pets, frequent interactions between the Orang Asli and the feral dogs or cats expose them to the bites of fleas infesting these animals. This study aims to determine the presence of *Rickettsia* in fleas collected from feral dogs and cats found in an Orang Asli village in the Perak state in Malaysia.

Flea specimens were pooled based on gender and animal host, and used for DNA extraction. Polymerase chain reaction (PCR) amplification of *Rickettsia*-specific partial 16s rRNA gene sequence was performed using previously published primers for the detection of *Rickettsia* DNA in the specimens.

A total of 120 fleas were collected and were morphologically identified as *Ctenocephalides felis*. *Rickettsia*-specific 16s rRNA gene sequence was amplified in 23 out of 30 flea pools. Comparison of the amplicon sequences to NCBI Genbank database revealed the presence of RFLOs, namely *Candidatus Rickettsia asemboensis* (Coverage: 100%, Identity: 99.69%), as well as *Bartonella clarridgeiae* (Coverage: 100%, Identity: 98.71%).

These findings suggest that RFLOs are present in the fleas commonly infesting feral dogs and cats living close to the Orang Asli. Further studies will be necessary to determine the genetic relatedness of these *Rickettsia* to *R. felis* and other RFLOs, and to gain further insight into the zoonotic potential. The detection of a potential agent for cat-scratch disease also suggests the risk of disease exposure among this community.

Abstract 25: The estimated burden of scrub typhus in Thailand from national surveillance data (2003-2018)

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Scrub typhus, a potentially severe zoonotic disease caused by the obligate intracellular Gram negative bacteria *Orientia tsutsugamushi*, remains prevalent throughout the Asia Pacific region. Efforts to estimate the global burden of disease are hindered by limited data and difficulties surrounding diagnosis. In Thailand, scrub typhus is a major cause of fever and is notifiable to the national disease surveillance system. However, this data has not been analysed or reported previously.

We aim to describe the burden of scrub typhus at the national, regional and provincial levels, utilising surveillance data from 2003 to 2018. We studied the disease burden at the district, sub-district and village levels for Chiang Rai province; the province with the highest burden of disease and explored the relationship between disease epidemiology and meteorological and geographical factors.

A total of 103,345 scrub typhus cases were reported to the surveillance system from 2003-2018. More men were affected than women and 15-64 year olds made up the majority of cases (72%). Burden was highest in agricultural workers (45%). Regionally, scrub typhus was most prevalent in the North (53%) and Northeast (31%) while the South and Central regions contributed 12% and 9%, respectively. Disease seasonality was observed in all 4 regions although the degree of seasonality varied. Modelling revealed association between rising incidence of scrub typhus with increasing temperature, rainfall and land use. The burden of scrub typhus in Thailand is high and continues to rise. However, this is likely to be a gross underestimate due to the reporting criteria used.

Abstract 36: Are the bats carriers for *Rickettsia monacensis*?

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Bats are the most diverse and geographically distributed mammals found on all continents except Antarctica. Their role in disease epidemiology has increased in recognition along with the publication of an increasing number of studies showing their susceptibility to several microorganisms, but also their role as natural reservoirs for pathogens including viruses, bacteria and parasites. Lately, alongside viruses, arthropod-borne bacteria are searched in bats and their ectoparasites. Among the most frequently detected are *Bartonella* spp. and *Neorickettsia risticii* in bats' samples and *Bartonella*, *Borrelia* and *Rickettsia* species in their ectoparasites. In line with this idea, the aim of this study was to detect *Rickettsia* species in blood samples in Romania.

A total of 322 bat samples belonging to 20 species originating from 14 caves in Romania were tested for the presence of *Rickettsia* DNA. PCR detection was performed using a group-specific set of primers amplifying a 381bp fragment of the *gltA* gene. Positive results were obtained in 47 samples (14.6%). However, good concentration of DNA allowing the sequencing was obtained only in 18 cases. Sequences analysis confirmed the presence of *R. monacensis* in 16 samples, in *Nyctalus noctula* and *Pipistrellus pipistrellus*, from two caves. Their similarity varied between 97 to 99.4% with *R. monacensis* Romanian, Serbian and Italian strains (Acc. No. JX003686; GQ925822; KY203389). These results suggest for the first time the possible involvement of these bat species in the epidemiology of *Rickettsia*. However, further studies should be performed to establish the role of these species as carriers or hosts.

Abstract 48: Seroprevalence to spotted fever group, typhus group, and scrub typhus group rickettsial antigens among healthy adults in five regions in Chile

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Introduction: In recent years, Chile has evolved to a country with multiple emerging rickettsiae, including *R. felis*, *R. andeanae*, unidentifed spotted fever group rickettsias (in vectors), and *Orientia* species. This survey studied the seroprevalence of spotted fever group rickettsiae (SFGR), typhus group rickettsiae (TGR), and scrub typhus group orientiae (STGO) among healthy adults in 5 regions of Chile.

Methods: We conducted a cross-sectional study in rural and urban settings of 5 regions, from Arica in the far north to Chiloé Island in the south. Healthy adults were included by household-based randomization (urban areas) and convenience (rural regions) sampling. Serum samples were examined by in-house IgG ELISA (Naval Medical Research Center, USA) using *R. conori*, *R. typhi*, and *O. tsutsugamushi* antigens.

Results: The study included 223-289 samples per region, in total 1,289 serum samples (638 urban and 651 rural sites). Seroprevalence rates to SFGR, TGR, and STGO were 5.0% (CI: 4.0-6.4), 1.2% (CI: 0.7-1.9), and 0.4% (CI 0.1-0.9), respectively. SFGR seroprevalence varied regionally from 1.3-8.2% and was significantly higher in rural areas; TGR ranged from 0.4-2.1% in different regions and was slightly higher in rural settings; STGO was very low in all regions (0-0.7%), including the endemic south.

Conclusions: We provide the first evidence of broad human exposure to SFG rickettsiae, especially in rural regions. Our study suggests that TGR are endemic in Chile, although autochthonous cases have not been reported yet. The low STGO seroprevalence might be related to insufficient cross-reactivity of used antigens to the Chilean *Orientia* species.

Abstract 54: Seroprevalence of scrub typhus and rickettsiosis in undifferentiated febrile illness patients from Nan and Tak provinces, Thailand

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Scrub typhus (ST) is a major public health in northern Thailand. A total of 256 UFI patient samples were received from Bo Kluea hospital (n=206) in Nan province, and Phop Phra hospital (n=50) in Tak province, Thailand during 2017-2019. IFA and ELISA assays, were performed to examine seroprevalence of ST, murine typhus (TGR), and spotted fever group Rickettsia (SFGR).

The results of IFA assays (IgM) against ST, TGR and SFGR showed relatively high number of patients with past infection. Patients with IgM antibody against TGR had the highest number accounting for (32.0%), followed by SFGR (27.0%), and ST (19.0%) in Nan province, However, when the same set of sera (n = 206) were tested for their IgG titer, ST seems to dominate the other two diseases with 154 patients (74.8%) having higher IgG titers (1,600 and >6,400) compared to SFGR (n = 26) or TGR (n = 0). The results of IFA assays (IgM) in Tak province showed the high seroprevalence for SFGR (46%) followed by TGR (28.0%), and ST (14.0%). However, their IgG titers showed that TGR was more prevalent than SFGR and ST (n=19 vs 5 and 3, respectively).

Patients were grouped according to their ages and gender to determine how seroprevalence differed among the groups. Our data showed a significantly higher seroprevalence in two age groups (20–40 and 41–60 years old) compared to the other age groups from Nan province. However, the overall prevalence was not different between male and female patients from Nan and Tak provinces.

Abstract 75: Scrub typhus in the Himalayan region – Sikkim: a five year retrospective study

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Scrub Typhus (ST) caused by *Orientia tsutsugamushi* has emerged in the Indian subcontinent as a disease of immense public health importance. Sikkim with a population of 6.11 lakhs is a small landlocked Indian state sharing borders with China, Bhutan and Nepal. After the first report of ST outbreak in 2011, there is increased number of ST cases every year. In order to study the burden, demographics and clinical features of ST, we conducted a retrospective study among acute febrile cases admitted /visited the outpatient between 2014 – 2018. ST was detected by the rapid IgM immunochromatography test (Standard Diagnostics Inc., Kyonggi-do, Korea). 5279 patients were screened for ST and Typhoid. 489 patients were positive for scrub typhus, 212 were positive for Typhoid. 72 of the patients with ST were paediatric patients. 52% of the ST cases were from rural areas while the rest were from urban and semi urban areas. 66.46% of these patients required admission. Hospital files were reviewed for 27 patients with ST; further clinical details for admitted patients have been sought. The average length of stay was 6 days. Common initial diagnosis was viral fever (n-5) and broad spectrum antibiotics were given. In adults with ST, diarrhoea and vomiting was the most common accompanying feature (n-6). Eschar was seen in only 3 paediatric patients, hepatosplenomegaly (n-6) and generalised swelling (n-5) was the common finding in this age group. SGOT was raised in 6 patients and KFT was deranged in 3. All patients responded to treatment with doxycycline.

Abstract 91: Systematic review of scrub typhus study landscape: protocol and preliminary literature search results

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Introduction: One billion people are at risk of scrub typhus. However, compared to its magnitude, evidence to optimise treatments and disease control are sparse. Existing data collected from past clinical trials and longitudinal observational studies could be a source of information to address research priorities and knowledge gaps.

Aim: To conduct a systematic review to assess the characteristics of scrub typhus clinical studies and explore the feasibility to develop a scrub typhus individual participant-level data (IPD) platform.

Methods: Six databases and two clinical trial registries were searched for clinical trials and longitudinal observational studies conducted between 1998 and March 2018. Variables for extraction include treatment tested, patient characteristics, diagnostic methods, geographical location, outcome measures, and statistical methodology.

Results: The literature searches identified 5,163 citations, of which 2,647 unique articles were independently screened by two reviewers. A total of 95 studies (7 clinical trials and 88 observational studies) met the pre-specified inclusion criteria. The studies have been conducted in 11 countries and enrolled a total of 9,010 patients. 390 case series and reports were also identified.

Conclusion: Although there were only a few scrub typhus clinical trials found, there are substantially more data available from observational studies. Meta-analysis using an IPD approach can produce a more representative secondary analysis because it facilitates the use of data from observational studies as well. Understanding the landscape of scrub typhus treatment studies allows assessment of the feasibility of addressing research questions using IPD meta-analysis method and to conduct a research gap analysis.

