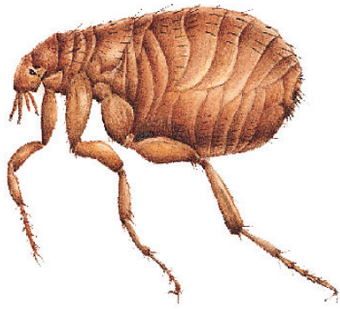


Rickettsial infections in *Xenopsylla cheopis* collected from four islands in Indonesia



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Introduction

- *Xenopsylla cheopis*, an important plague vector, is also vector of murine typhus and potentially several other rickettsiae
- Murine typhus, caused by *Rickettsia typhi*, can be transferred from animal to human by infected flea most commonly the oriental rat flea, *Xenopsylla cheopis*

Rickettsial Diseases in Indonesia

- Indonesia has one of the highest prevalence levels for antibody to *R. typhi* among people in the world

Richards *et al.* 2002. Am. J. Trop. Med. Hyg. 66:431-434.

- Studies in Java found people living in urban areas had lower prevalence of antibody to *R. typhi* than less urban areas with lower concentrations of people

Richards *et al.* 2002. Am. J. Trop. Med. Hyg. 66:431-434.

- Although murine typhus is considered an urban disease it has been noted among rural inhabitants

(Gipsen *et al.* 1951. Docum. Neerl. Indonesia Morb. Trop. 3:60-66, Kouwenaar W. and J. W. Wolff. 1993. Proceedings of the Pacific Science Congress of the Pacific Science Association. 633-637, Richards *et al.* 1997. Am. J. Trop. Med. Hyg. 57(1):91-95.)

Rickettsial Diseases in Indonesia

- Murine typhus has been found in many areas throughout Indonesia including Java, Sumatra, Bali, Muna, and Irian Jaya

(Gipsen *et al.* 1951. Docum. Neerl. Indonesia Morb. Trop. 3:60-66, Kouwenaar W. and J. W. Wolff. 1993. Proceedings of the Pacific Science Congress of the Pacific Science Association. 633-637, Richards *et al.* 1997. Am. J. Trop. Med. Hyg. 57(1):91-95.)

- Evidence of spotted fever group (SFG), *R. rickettsii* and *R. conorii*, in the Gag Islands

Richards *et al.* 2003. Am. J. Trop. Med. Hyg. 68(4):480-484.

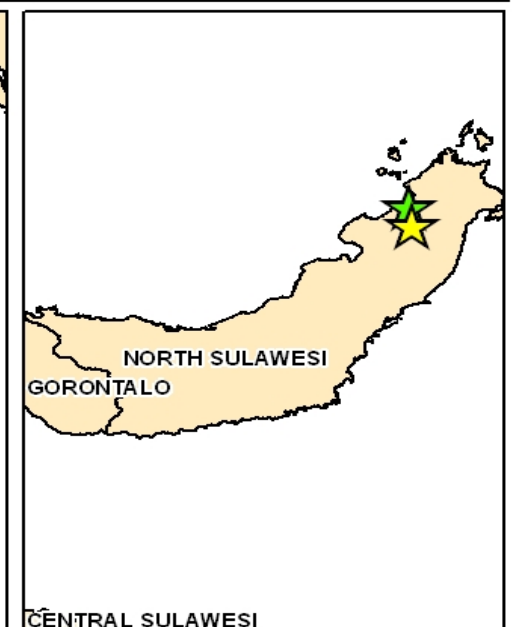
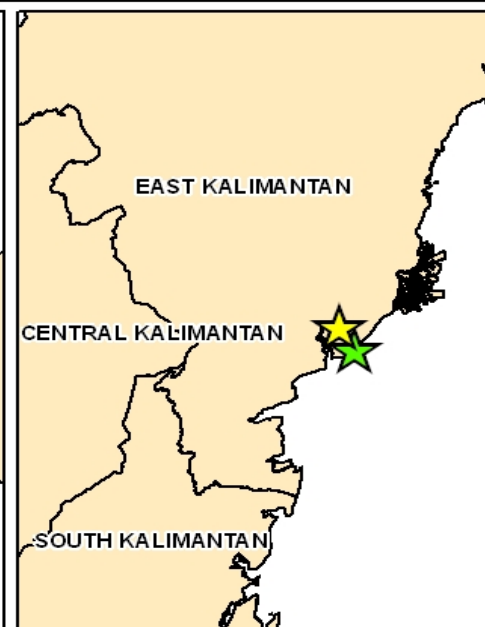
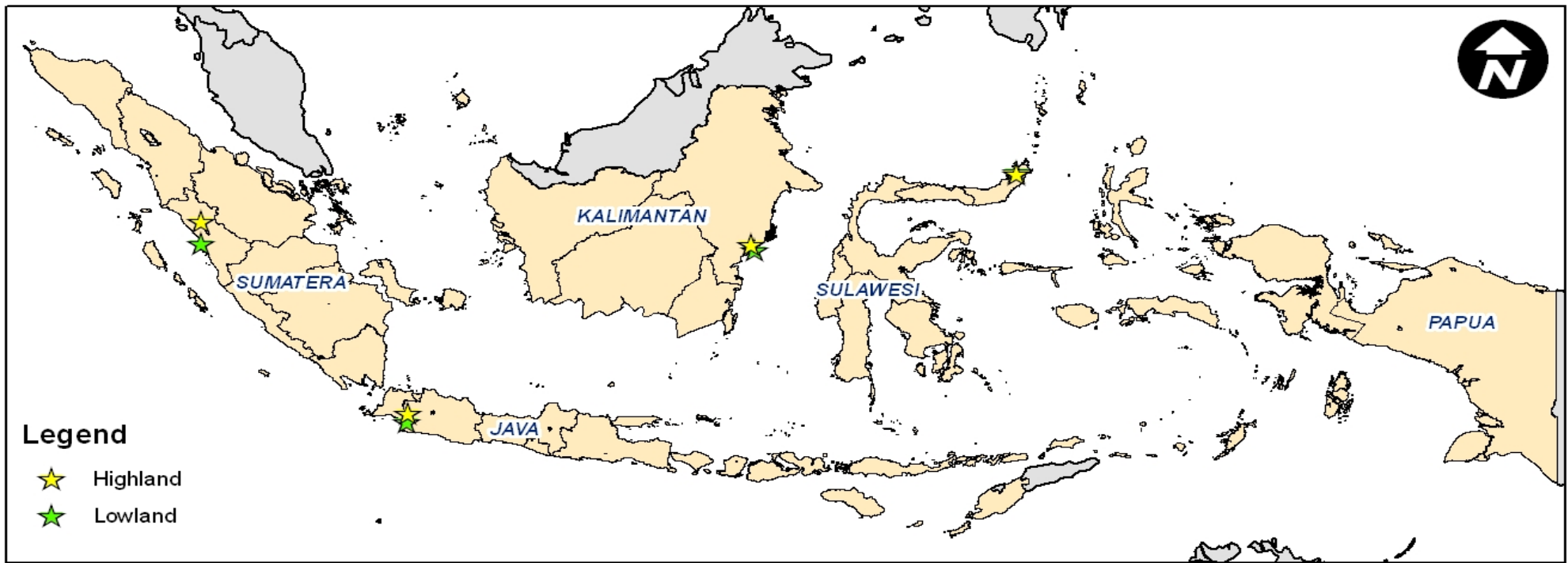
- *Rickettsia felis* and *R. typhi* were identified in rat fleas

Jiang *et al.* 2006. EID. 12 (8):1281-1283.

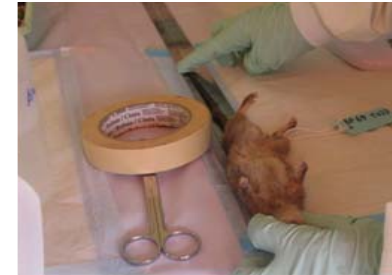
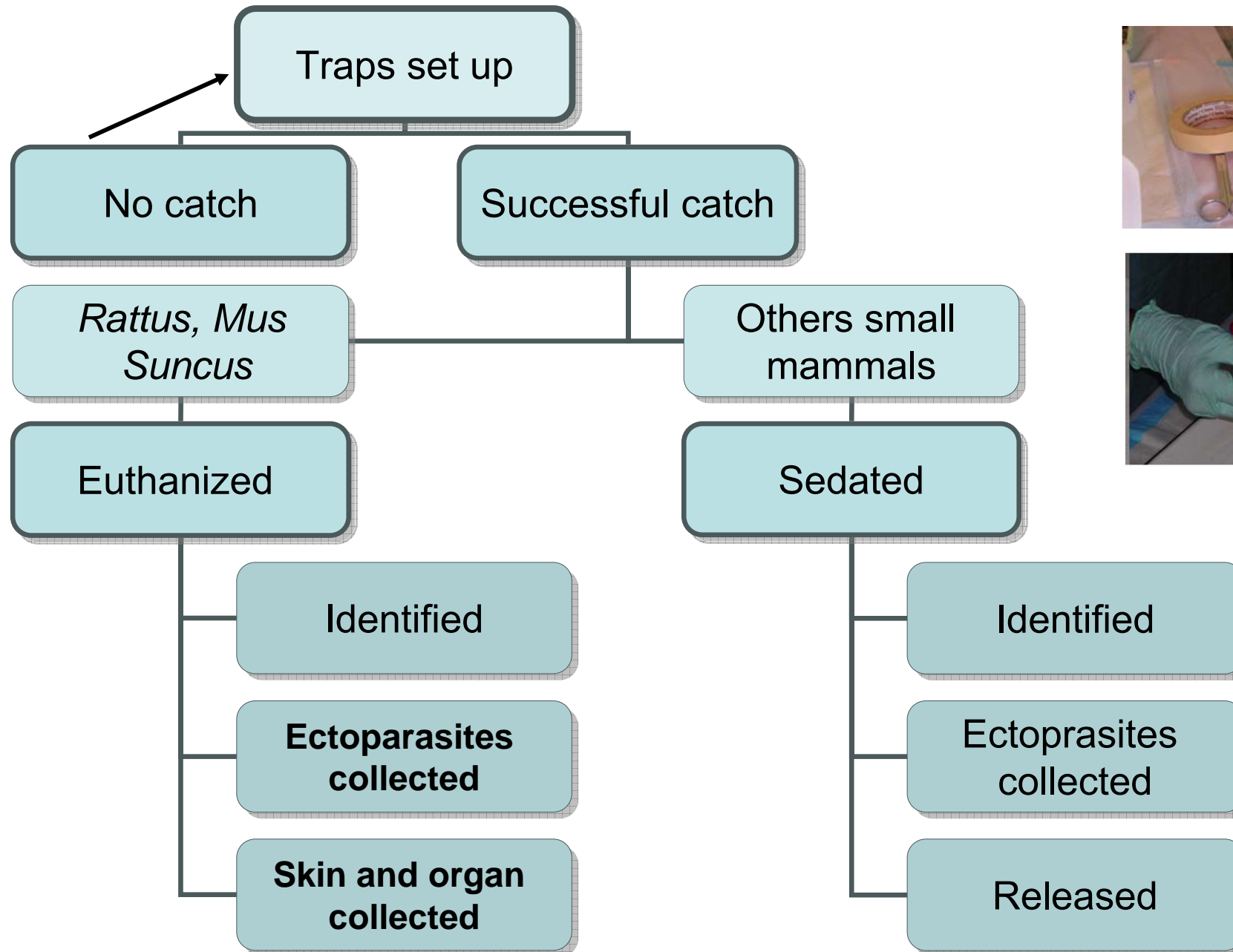
Objective

- To identify *Rickettsia* species occurring in *X. cheopis* and its host distribution in Indonesia

Study sites



Methods



Trapping Methods

- Two locations at each site to obtain a variety of small mammals and rickettsial species :
 - Highland
 - Lowland
- Three Zones at each location:
 - Zone 1: Urban
 - Zone 2: Semi urban
 - Zone 3: Crops, plantation, secondary forest
 - Zone 4: Primary forest, jungle

Methods

Ectoparasites collected

Micro Eppendorf tube
(frozen in liquid nitrogen)

Laboratory

Identified

Tested by PCR



Flea index

- Mean number of fleas collected per rodent

$$\text{Flea index} = \frac{\Sigma \text{ of fleas collected}}{\Sigma \text{ of rodent captured}}$$

- Flea index followed by laboratory testing can be use to describe disease burden

Results

West Java

West Java								
	Lowland				Highland			
Mammal Species	Total # Individuals	Total <i>X. cheopis</i>	Flea Index	Range	Total # Individuals	Total <i>X. cheopis</i>	Flea Index	Range
<i>Rattus tanezumi</i>	17	15	0.88	0-3	15	26	1.73	0-9
<i>Rattus exulans</i>	0	0	0	0	2	0	0	0
<i>Suncus murinus</i>	24	17	0.71	0-4	10	8	0.80	0-4
<i>Rattus tiomanicus</i>	1	0	0	0	1	0	0	0
TOTAL	42	32	0.76	0-4	28	34	1.21	0-9

Results

West Sumatra								
	Lowland				Highland			
Mammal Species	Total # Individuals	Total <i>X. cheopis</i>	Flea Index	Range	Total # Individuals	Total <i>X. cheopis</i>	Flea Index	Range
<i>Rattus tanezumi</i>	51	3	0.06	0-2	43	87	2.02	0-20
<i>Leopoldamys sabanus</i>	0	0	0	0	3	0	0	0
<i>Rattus tiomanicus</i>	4	0	0	0	0	0	0	0
<i>Tupaia glis</i>	3	0	0	0	0	0	0	0
TOTAL	58	3	0.05	0-2	46	87	1.89	0-20

Results

North Sulawesi

	Lowland				Highland			
Mammal Species	Total # Individuals	Total <i>X. cheopis</i>	Flea Index	Range	Total # Individuals	Total <i>X. cheopis</i>	Flea Index	Range
<i>Rattus tanezumi</i>	43	10	0.23	0-3	37	2	0.05	0-1
<i>Rattus exulans</i>	1	2	2.00	0-2	2	0	0	0
<i>Suncus murinus</i>	56	2	0.04	0-1	0	0	0	0
<i>Rattus norvegicus</i>	33	12	0.04	0-4	0	0	0	0
<i>Mus musculus</i>	5	0	0	0	2	0	0	0
<i>Rattus tiomanicus</i>	1	0	0	0	0	0	0	0
<i>Maxomys rajah</i>	0	0	0	0	4	0	0	0
TOTAL	139	26	0.19	0-4	45	2	0.04	0-1

Results

East Kalimantan

	Lowland				Highland			
Mammal Species	Total # Individuals	Total <i>X. cheopis</i>	Flea Index	Range	Total # Individuals	Total <i>X. cheopis</i>	Flea Index	Range
<i>Rattus tanezumi</i>	12	1	0.08	0-1	28	0	0	0
<i>Rattus exulans</i>	4	0	0	0	11	0	0	0
<i>Suncus murinus</i>	32	6	0.19	0-3	1	0	0	0
<i>Rattus norvegicus</i>	32	13	0.41	0-7	0	0	0	0
<i>Leopoldamys sabanus</i>	0	0	0	0	1	0	0	0
<i>Maxomys rajah</i>	0	0	0	0	1	0	0	0
<i>Rattus laticaudatus</i>	0	0	0	0	5	0	0	0
<i>Rattus tiomanicus</i>	0	0	0	0	8	0	0	0
<i>Rattus whiteheadi</i>	0	0	0	0	6	0	0	0
<i>Sundasciurus lowii</i>	0	0	0	0	5	0	0	0
<i>Viverra zibetha</i>	0	0	0	0	2	0	0	0
TOTAL	80	20	0.25	0-7	68	0	0	0

Discussion

- Flea index at the West Java and West Sumatera highland sites were greater than lowland
- Flea index at the North Sulawesi and East Kalimantan lowland sites were greater than highland

Molecular Characterization of Rickettsiae

- Fleas were pooled by site, lowland/highland, zone, and species of mammal host
- DNA isolation: QIAamp DNA Mini kit® (Qiagen)
- Real time PCR
 - Pools were extracted and screened with Rickettsia genus-specific qPCR assay targeting the 17kDa common antigen gene
 - Positive results were tested by the SFG-specific qPCR assay and species-specific *R. felis* and *R. typhi* qPCR assay, target different region of ompB
 - Nested PCR to detect SFG and typhus group rickettsiae

Results

- PCR testing of 37 pools:
 - 6 positive pools for *Rickettsiae* spp.
 - 4 (10.8%) *R. typhi*
 - 1 (2.7%) *R. felis*
 - 1 (2.7%) *R. felis* and another SFG (not *R. felis*)

qPCR Analysis

Site	Location	Zone	<i>R. typhi</i>	<i>R. felis</i>	<i>R. Rickettsii</i> (SFG)	R17K	Small Mammal Species
West Java	Highland	2	+			+	<i>Rattus tanezumi</i>
West Java	Lowland	2	+			+	<i>Rattus tanezumi</i>
West Java	Lowland	3	+			+	<i>Suncus murinus</i>
North Sulawesi	Lowland	1		+		+	<i>Rattus tanezumi</i>
East Kalimantan	Lowland	1		+	+	+	<i>Suncus murinus</i>
East Kalimantan	Lowland	2	+			+	<i>R. norvegicus</i>

Discussion

- This is the first report of *R. felis* in *X. cheopis* in Indonesian locations other than Java. It is also the first report of *R. typhi* in Kalimantan.
- One pool of *X. cheopis* collected from East Kalimantan showed evidence of double infection with *R. felis* and other unidentified SFG (not *R. felis*)
 - This pool is being sequenced to determine specific infection

Discussion

- Five *Rickettsia* spp. positive were found in lowland area and only one was found in highland area
- All *R. typhi* were found outside houses (zone 2 and 3) and all *R. felis* were found inside houses (zone 1)
- No *Rickettsia* spp. found in Zone 4 (primary forest, jungle area)

Summary

- PCR data shows 6 of 37 pools tested positive for *Rickettsiae* spp.
- *Rickettsia typhi* positive found in West Java (n=3) and East Kalimantan (n=1)
- *Rickettsia felis* positive found in East Kalimantan (n=1) and North Sulawesi (n=1)
- *Rattus tanezumi* (Asian house rat, ship rat, black rat) and *S. murinus* (Asian house shrew) appear to be the primary hosts for *R. typhi* and *R. felis* infected *X. cheopis*

Summary

- Flea index varies over different islands in Indonesia
- Rickettsiae were found to be predominant in lowland areas rather than highland
- Rickettsia infections should be considered when diagnosing undifferentiated fevers in Indonesia, especially in Java which was found to have the highest flea index and fleas positive for *R. typhi*.

Acknowledgements

- Funded by DoD GEIS
- Indonesian Ministry of Health, NIHRD
- Local health centers (Puskesmas)
 - Simpenan, West Java Province
 - Bukit Tinggi, West Sumatera Province
 - Manado, North Sulawesi Province
 - Balikpapan, East Kalimantan Province