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Original Article

Prevalence of Ectoparasites and the Seroepidemiology of Murine Typhus in Murine-like Animals at International Ports in Taiwan, 2004-2011

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Abstract

Murine typhus, one of the forth category National Notifiable Communicable Diseases in Taiwan, is caused by the *Rickettsia typhi*, and is mainly transmitted by fleas, *Xenopsylla cheopis*, that infest murine-like animals. In this article, we addressed the prevalence of ectoparasites of murine-like animals at major international ports in Taiwan and did the serum epidemiology study in murine-like animals between November 2004 and 2011. The total number of murine-like animals underwent examination was 4,260, and 2,358 (55.35%) were *Rattus norvegicus*. On the other hand, the total number of ectoparasites captured from murine-like animals was 4,469, and 2,103 (45.04%) were *Xenopsylla cheopis*. The average seropositive rate of murine typhus among these murine-like animals in international ports was 8.22%, while the seropositive rates in Kaohsiung Port and Taichung Port were 26.12% and 18.09 %, respectively; both were higher than the average. In both ports, the linear correlation between the *Xenopsylla cheopis* index and the seropositive rate of murine typhus in *Rattus norvegicus* was not significant.

Keywords: international ports, murine typhus, *Xenopsylla cheopis*, *Rattus norvegicus*

Introduction

In order to prevent communicable disease transmitted via transportation vehicles such as ships and airplanes, the International Health Regulations (IHR) were established by the World Health Organization (WHO) in 2005. In response, Taiwan Centers for Diseases Control (Taiwan CDC) revised the Regulations on Governing Quarantine at Ports. All ships, planes, cargos and staff on board are to be examined on arrival. Because rats are major reservoirs of many communicable diseases which are often transmitted by their ectoparasites, both rats and their ectoparasites are important targets of quarantine examinations. Murine typhus is a good example.

The pathogen of murine typhus belongs to the family of Rickettsiaceae. Different rickettsia can cause different types of typhus. For example, endemic typhus, or murine typhus is caused by *Rickettsia typhi*, while epidemic typhus is caused by *R. prowazekii*. Murine typhus can be transmitted by rats and their ectoparasites, especially *Xenopsylla cheopis*. The most common rodent vectors are commensal rats such as *Rattus norvegicus* and *Rattus rattus*, both belong to the *Rattus* genus. *R. typhi* can enter the midgut of fleas when they are sucking the blood of rats carrying the rickettsia. The fleas become transmission vectors and cause infection in healthy rats, and result in a rat-flea-rat cycle [1]. These fleas will carry the rickettsia life long and are also able to spread the pathogen to their offspring via transovarial transmission [2]. Human can get infection because of wounds contaminated by the feces of infected fleas, or inhalation of their dry feces. All the infected rats can remain healthy while some of the infected human will become ill.

In addition to *X. cheopis*, *R. typhi* have been isolated from other fleas such as *Nosopsyllus nicanus* and *Ctenocephalides felis*, lice such as *Polyplax spinulosa*, and mites such as *Ornithonyssus bacoti* [1]. These arthropods play minor roles in transmission of human murine typhus, but the bloodsucking mites and lice could be important in maintaining the cycle of *R. typhi* between rats [3].

Typical symptoms of murine typhus include sudden onset of headache, chills, malaise, fever, and generalized pain. Dull red rash could be found initially at upper trunk, which eventually involve all the body since day 5 or day 6; they rarely appear on a patient’s face, palms, or soles. Septicemia is common. Indirect fluorescent antibody test, IFA, is the most frequent used laboratory diagnostic method. In Colombia, 120 patients presenting with fever, headache, and chills have been tested using IFA to compare the difference of antibody titers between patients in acute stage and recovering stage, to determine if definite diagnosis of murine typhus could be made [4]. In Taiwan, the case number of murine typhus during 2004-2011 was between 21 to 63, according to the statistics of Taiwan CDC. Most of the patients lived in Kaohsiung City, Changhua County, and Pingtung County. International ports can become habitats of commensal rats easily if without adequate control. To prevent importation of *R. typhi* from these international ports into Taiwan, Taiwan CDC launched a program to closely monitor the number of *X. cheopis* carried by different kinds of rats and the infection status of the rats. Control and preventive measures about murine typhus were drawn up accordingly.

Materials and Methods

1. Locations of rat trapping and timing of investigation

a. Locations: Keelung Harbor, Taoyuan International Airport, Taichung Harbor, Mailiao Harbor, Kaohsiung Harbor, Kaohsiung International Airport, Hualien Harbor, Suao Harbor, Shueitou Port and Liaoluo Port of Kinmen , and Fuwo Port in Matsu

b. Timing: From November 2004 to December 2011

2. Sampling and management of the captured rats and their ectoparasites

a. Rat trapping process

ⅰ. House shrews (*Suncus murinus*) share the same habitats with commensal rats. Both are small mammals that can easily be captured at ports. Although house shrews look like rats, they belong to the Order Insectivora while rats belong to Order Rodentia. Commensal rats and house shrews are called murine-like animals in combination. In terms of the so called *Rattus rattus*, the nomenclature has been revised and the species is now called *Rattus tanezumi* [5].

ⅱ. Rat trapping at international ports was arranged three days every month. At least 20 to 30 cages were placed at any location with trace of murine-like animals in each port.

ⅲ. Different baits were used to lure more different kind of murine-like animals.

ⅳ. Cages were checked the next morning. The trapped murine-like animals were stored in double-layered plastic bags.

b. Identification of murine-like animals, serum sampling, and collection of ectoparasites

ⅰ. Intra-abdominal injection of 0.1 to 0.4 ml of Zoletil 50 was used to anesthetize the captured murine-like animals according to their body size. After adequate anesthesia, blood was collected by cardiopuncture, kept one hour under room temperature, and centrifuged for 10 minutes with speed fixed at 3,000 rpm. The separated sera were divided into tubes marked with serial numbers and stored in the refrigerators under –20^{o} C.

ⅱ. The body length, tail length, sex, and body weight of the murine-like animals were recorded. Their species were identified; demographics, captured date and locations were registered.

ⅲ. Ectoparasites of the murine-like animals were collected using hairbrush, tweezers, and flea combs and stored in 70% alcohol. Their species were identified in the laboratory according to morphology.

3. Seroprevalence of *Rickettsia typhi*

a. The serum samples of the murine-like animals, positive control and negative control were diluted by 0.01M phosphate buffered saline (PBS), making a 1:40 dilution.

b. The diluted samples and controls were dropped on the *Rickettsia typhi* IFA slides (FOCUS Diagnostics, USA). The slides were incubated in a humid chamber for 30 minutes under 37^{o} C.

c. Slides were washed by PBS solution, soaked in PBS solution for 10 minutes, washed by distilled water, and then air-dried.

d. The fluorescent marked antibody solution, FITC - Goat Anti-mouse IgG + A + M (H + L chain) (Zymed Laboratories Inc., USA), was diluted to 1/40; 2 μl of the diluted solution was dropped on each slide and placed in a water bath for 30 minutes at 37^{o} C.

e. The slides were taken out and washed by PBS solution, soaked in PBS solution, preserved in dark place for 10 minutes, washed by distilled water, and air-dried.

f. PBS solution containing glycerin was dropped and sealed by cover glasses. Fluorescent microscopy was used to observe the samples magnified to 400X of the original size.

4. Identification of ectoparasites of murine-like animals

a. Ticks: Ticks were identified under stereomicroscope Leica M165C and classified according to references 6,7, and 8.

b. Fleas: Fleas preserved in 70% alcohol were taken out and soaked in water for 30 minutes, soaked with 5% KOH solution and baked for 16 to 24 hours at 45℃ . After cooling down, they were put in the water for 30 minutes, reacted with 10% acetic acid for 5 to 10 minutes, and dehydrated with 40% alcohol for 15 minutes, 70% alcohol for 2 hours and 100% alcohol for 2 hours twice. The samples were preserved in clove oil overnight and fixed with Canada balsam. Fleas were identified under microscope Olympus BX50 and classified according to references 9 and 10.

c. Mites: Mites preserved in 70% alcohol were taken out and soaked in water for 30 minutes three to four times. The samples were then sealed with Berlese fluid (ASCO Laboratory, UK) and observed using microscope Olympus BX50. The species were identified according to reference 11.

5. Statistical analysis

Two-way ANOVA test analyzing the association between ectoparasites collected from different ports, the number of murine-like animals, and the seropositive rates of murine typhus was done using Statistica (Stat soft In., Tulsa, Oklahoma, USA). Tukey HSD was used for post hoc tests.

In addition, to clarify the association between the *Xenopsylla cheopis* indices and the seropositive rates of murine typhus of *R. norvegicus*, the correlation between the seropositive rates and the fleas indices collected from ports with more *X. cheopis*, including Taichung Harbor and Kaohsiung Harbor were analyzed using linear correlation model. The number of fleas was transformed in flea index by dividing it to the number of *R. norvegicus*.

Results

1. The number of murine-like animals and the seropositive rates of murine typhus

The numbers of murine-like animals, the number and proportion of seropositive for murine typhus were listed in Table 1. Seven species of 4,260 murine-like animals were captured, including *Rattus norvegicus*, *Suncus murinus*, *Rattus losea*, *Bandicota indica*, *Rattus tanezumi*, *Apodemus agrarius*, and *Micromys minutus*. Most of the captured animals were *R. norvegicus* (2,358, 55.35%), followed by *S. murinus* (1,049, 24.67%), *R. losea* (529, 12.43%), and *B. indica* (244). Most of the *B. indica* were captured at Taoyuan International Airport. Among the ports, the number of captured murine-like animal was highest in Kinmen (925) and Kaohsiung Harbor (850); the number of captured *R. norvegicus* on the other hand, was highest in Kaohsiung Harbor (689) and Suao Harbor

(333). Using two-way ANOVA test to analyze the association between different ports and species of murine-like animals, we found significant difference and interaction (F port = 10.90, $p<0.001$; F murine-like animals = 64.1, $p<0.001$; F port × murine-like animals = 9.25, $p<0.001$). *R. norvegicus* and *S. murinus* were the most common murine-like animals (Tukey HSD, $p<0.05$). The number of *R. norvegicus* in Kaohsiung Harbor surpassed that in other ports (Tukey HSD, $p<0.05$). Considering the seropositive rates of all murine-like animals for murine typhus, the average was 8.22% while the seropositive rate was highest in *R. norvegicus* (13.4%), followed by *R. tanezumi* (5.26%). Among the ports, animals captured in Kaohsiung Harbor and Taichung Harbor was found to have the highest seropositive rates of murine typhus (26.12% and 18.09%). A significant difference could be found between different harbors and seropositive rates of murine typhus of different murine-like animals (F murine-like animals =16.87, $p<0.001$, F ports = 5.51, $p<0.001$, F port × murine-like animals = 3.98, $p<0.001$). The seropositive rate was highest in *R. norvegicus* (Tukey HSD, $p<0.05$).

2. The number and distribution of the ectoparasites of murine-like animals

Ectoparasites collected from murine-like animals included two fleas, *X. cheopis* and *Nosopsyllus nicanus*; three ticks, *Ixodes granulatus*, *Haemaphysalis bandicota* and *Rhipicephalus haemaphysaloides*; three mites, *Laelaps nuttalli*, *L. sedlaceki*, *L. echidninus*; and lice.

Table 1. Numbers of captured murine-like animals, numbers and seropositive rates of animals for murine typhus at international ports between November 2004 and 2011

	Keelung Harbor	Taoyuan Int'l Airport	Taichung Harbor	Mailiao Harbor	Kaohsiung Harbor	Kaohsiung Int'l Airport	Suao Harbor	Hualien Harbor	Kinmen	Matsu	Total
Rattus norvegicus	2/268 (0.75)*	0/169 (0)	50/208 (24.04)	16/240 (6.67)	221/689 (32.08)	19/88 (21.59)	3/333 (0.9)	2/73 (2.74)	3/175 (1.71)	0/115 (0)	316/2,358 (13.4)
Suncus murinus	0/33 (0)	1/85 (1.18)	0/80 (0)	3/82 (3.66)	0/150 (0)	0/148 (0)	0/0 (0)	0/49 (0)	1/340 (0.29)	0/84 (0)	5/1051 (0.48)
Rattus losea	0/0 (0)	1/93 (1.08)	5/15 (33.33)	0/2 (0)	0/0 (0)	0/0 (0)	0/0 (0)	0/6 (0)	10/410 (2.44)	0/3 (0)	16/529 (3.02)
Bandicota indica	0/1 (0)	0/4 (0)	0/0 (0)	2/5 (40.00)	1/11 (9.09)	0/7 (0)	0/1 (0)	1/42 (2.38)	0/0 (0)	0/5 (0)	4/76 (5.26)
Rattus tanezumi	0/0 (0)	9/237 (3.80)	0/1 (0)	0/0 (0)	0/0 (0)	0/1 (0)	0/0 (0)	0/5 (0)	0/0 (0)	0/0 (0)	9/244 (3.69)
Apodemus agrarius	0/0 (0)	0/1 (0)	0/0 (0)	0/0 (0)	0/0 (0)	0/0 (0)	0/0 (0)	0/0 (0)	0/0 (0)	0/0 (0)	0/1 (0)
Micromys minutus	0/0 (0)	0/1 (0)	0/0 (0)	0/0 (0)	0/0 (0)	0/0 (0)	0/0 (0)	0/0 (0)	0/0 (0)	0/0 (0)	0/1 (0)
Total	2/302 (0.66)	11/590 (1.86)	55/304 (18.09)	21/329 (6.38)	222/850 (26.12)	19/244 (7.79)	3/334 (0.9)	3/175 (1.71)	14/925 (1.51)	0/207 (0)	350/4,260 (8.22)

* The number of animals seropositive for murine typhus / the number of tested animals (positive rate, %)

The numbers of captured ectoparasites in different years were listed in Table 2. Among the 4,669 ectoparasites, 45.04% of them were *X. cheopis* (2103), 29.25% of them were *H. bandicota*.

In our investigation, the number of ectoparasites collected from different ports was differed; highest in Kaohsiung Harbor, followed by Taoyuan International Airport and least in Hualien Harbor. *X. cheopis* could be found in every ports except Suao Harbor; the number of *X. cheopis* was highest in Kaohsiung Harbor (1205), followed by that in Taichung Harbor (613) (Table 3). Although *H. bandicota* was the second common ectoparasite, most were found in Taoyuan International Airport (Table 3). The *X. cheopis* index was highest in Taichung Harbor (2.02), followed by that in Kaohsiung Harbor (1.42). A significant difference in the distribution of different ectoparasites could be found in different ports (F port = 5.29, $p<0.001$, F ectoparasite = 7.38, $p<0.001$, F port × ectoparasite = 5.49, $p<0.001$), indicating that the number of different ectoparasites varied in different ports. The number of ectoparasites of Kaohsiung Harbor and Taoyuan International airport were significantly higher than that of other ports, except Taichung Harbor (Tukey HSD, $p<0.05$). Considering the species of ectoparasites, there was no significant difference in the numbers of *X. cheopis* and *H. bandicota*, while both were significantly higher than the numbers of other species (Tukey HSD, $p<0.01$).

Table 2. Number of ectoparasites collected from murine-like animals between November 2004 and 2011

	X. cheopis	*N. nicanus*	*H. bandicota*	*I. granulatus*	*R. haemaphysaloides*	*L. echidninus*	*L. sedlaceki*	*L. nuttalli*	Lice	Total
2004 (Nov and Dec)	32	0	81	1	0	5	0	15	0	134
2005	242	0	102	1	16	23	0	39	0	423
2006	152	9	209	16	1	17	28	57	8	497
2007	113	4	48	12	1	18	15	18	46	275
2008	309	6	266	1	5	16	48	61	3	715
2009	98	6	587	18	1	64	11	11	3	799
2010	401	6	73	9	50	40	31	95	18	723
2011	756	19	0	13	38	53	65	142	17	1,103
Total	2,103	50	1,366	71	112	236	198	438	95	4,669

Table 3 Ectoparasites captured at different ports between November 2004 and 2011

	X. cheopis	*N. nicanus*	*H. bandicota*	*R. haemaphysaloides*	*I. granulatus*	*L. nuttalli*	*L. sedlaceki*	*L. echidninus*	Lice	Total
Keelung Harbor	70	0	0	0	0	19	23	52	0	164
Taoyuan Int'l Airport	1	9	1,366	3	3	35	10	6	4	1,437
Suao Harbor	0	0	0	0	0	56	3	58	0	117
Kinmen	77	27	0	7	17	0	36	4	14	182
Matsu	2	5	0	0	44	49	32	16	62	210
Taichung Harbor	613	6	0	98	1	39	29	39	0	825
Mailiao Harbor	40	0	0	0	0	37	0	0	0	77
Kaohsiung Harbor	1,205	3	0	1	0	193	62	50	13	1527
Kaohsiung Int'l Airport	94	0	0	0	0	3	2	1	0	100
Hualien Harbor	1	0	0	3	6	7	1	10	2	30
Total	2,103	50	1,366	112	71	438	198	236	95	4,669

3. The linear correlation between the number of *Xenopsylla cheopis* of Taichung Harbor and Kaohsiung Harbor and the seropositive rates of murine typhus of *Rattus norvegicus*

The annual seroprevalence of murine typhus in murine-like animals of ports were listed in Table 4. The seropositive rates in Taichung Harbor and Kaohsiung Harbor were higher than average every year; more *X. cheopis* could be collected in both harbors. The average *X. cheopis* index was 2.44 in Taichung Harbor and 1.74 in Kaohsiung. The monthly number of *X. cheopis* collected from *R. norvegicus* was shown in Figure, showing a peak between January and June, and a descent between July and December. Analyzing the linear correlation between the *X. cheopis* index and the seropositive rates of murine typhus, there was no significant in Taichung Harbor or Kaohsiung Harbor (Taichung Harbor, t=1.964, r=0.304, p=0.057; Kaohsiung Harbor, t=0.034, r =0.004, p =0.973).

Discussion

The purpose of this study is to evaluate the seropositive rates of murine typhus of murine-like animals in important international ports in Taiwan, and to analyze the linear correlation between *X. cheopis* index and the aforementioned seropositive rates. In the rat-flea-rat cycle of *R. typhi*, murine-like animals are important reservoirs of murine typhus. The most common murine-like animals found in this study were *R. norvegicus* and *S. murinus*; the results were compatible with previous investigations [12]. There was no significant difference between airports and seaports, except in Taoyuan International Airport, where more *B. indica* and *R. losea* could be found, and in Kinmen, where more *R. losea* were trapped because the hinterland of the seaports was smaller.

Table 4 . Numbers of tested animals, numbers and seropositive rates of animals for murine typhus between November 2004 and December 2011

	Keelung Harbor	Taoyuan International Airport	Taichung Harbor	Mailiao Harbor	Kaohsiung Harbor	Kaohsiung International Airport	Suao Harbor	Hualien Harbor	Kinmen	Matsu	Total
2004 (Nov and Dec)	0/9 (0)*	0/23 (0)	0/18 (0)	0/11 (0)	0/21 (0)	0/7 (0)	0/9 (0)	0/6 (0)	0/8 (0)	0/6 (0)	0/118 (0)
2005	0/50 (0)	0/31 (0)	0/42 (0)	6/89 (6.74)	7/94 (7.45)	1/63 (1.59)	0/50 (0)	0/12 (0)	0/82 (0)	0/18 (0)	14/531 (2.64)
2006	0/44 (0)	3/71 (4.23)	7/56 (12.5)	2/75 (2.67)	13/72 (18.06)	1/33 (3.03)	0/57 (0)	0/8 (0)	2/100 (2.00)	0/52 (0)	28/573 (4.89)
2007	0/28 (0)	6/81 (7.41)	9/32 (28.13)	4/51 (7.84)	33/87 (37.93)	10/14 (71.43)	2/54 (3.7)	0/22 (0)	4/85 (4.71)	0/42 (0)	68/496 (13.71)
2008	0/38 (0)	0/75 (0)	5/31 (16.13)	0/26 (0)	44/98 (44.9)	7/12 (58.33)	0/51 (0)	1/26 (3.85)	2/138 (1.45)	0/37 (0)	59/531 (11.11)
2009	2/52 (3.85)	0/80 (0)	2/16 (12.5)	8/25 (32)	27/84 (32.14)	0/8 (0)	1/37 (2.7)	1/27 (3.7)	3/152 (1.97)	0/35 (0)	44/516 (8.53)
2010	0/47 (0)	1/77 (1.3)	16/45 (35.56)	0/30 (0)	32/93 (34.41)	0/47 (0)	0/25 (0)	1/59 (1.69)	1/186 (0.54)	0/10 (0)	51/619 (8.24)
2011	0/34 (0)	1/152 (0.66)	18/63 (22.22)	1/22 (4.55)	66/301 (19.27)	0/60 (0)	0/51 (0)	0/15 (0)	2/174 (1.15)	0/7 (0)	86/876 (9.82)
Total	2/302 (0.66)	11/590 (1.86)	55/304 (18.09)	21/329 (6.38)	222/850 (26.12)	19/244 (7.79)	3/334 (0.9)	3/175 (1.71)	14/925 (1.51)	0/207 (0)	350/4,260(8.22)

* The number of animals seropositive for murine typhus / the number of tested animals (positive rate, %)

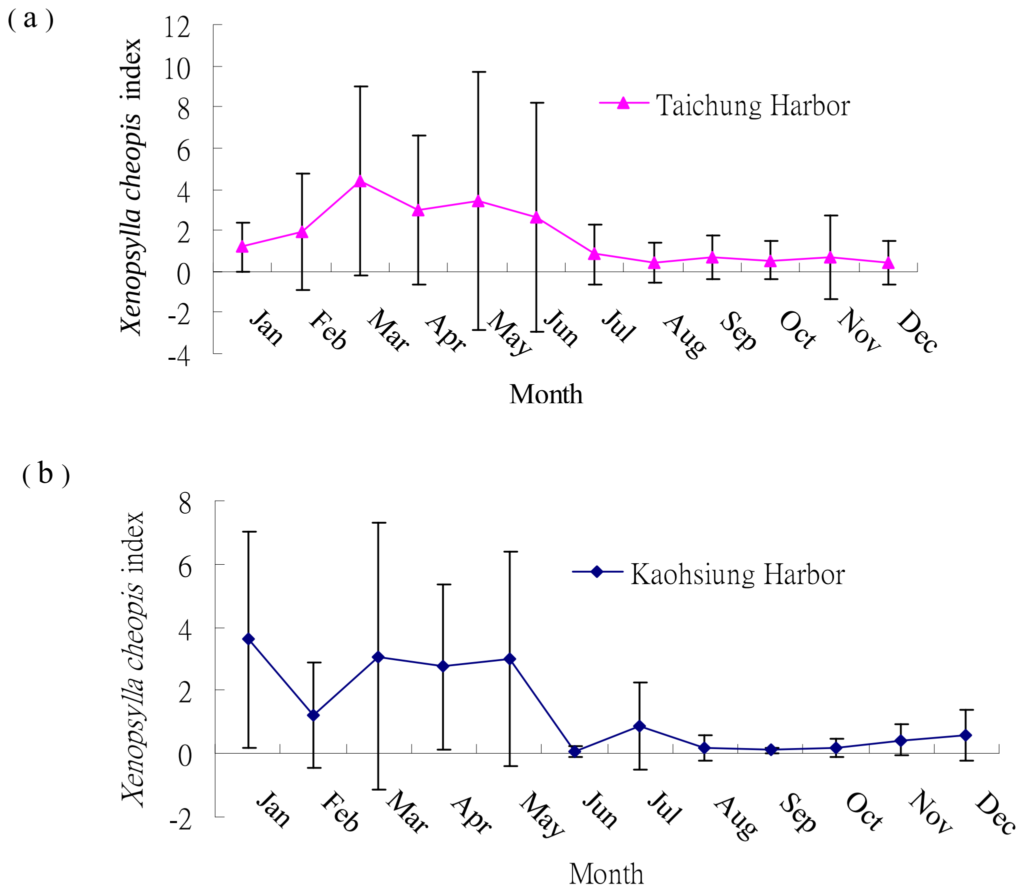

Figure. Monthly average of the *Xenopsylla cheopis* index in Taichung Harbor (a) and Kaohsiung Harbor (b), between November 2004 and 2011

In terms of the ectoparasites, *X. cheopis* was the most common species, which carrying *R. typhi* is the major transmission vector resulting in human murine typhus infection, and because of the ubiquitous distribution of *X. cheopis*, it is considered as the most important vector [1]. In Cyprus, a study that investigated the rats infected by *R. typhi* showed a significant correlation between the *X. cheopis* index and the seropositive rates of rats [13]. Similar results have been found in the study done in Yunnan, China [14]. All studies indicated that the higher the *X. cheopis* index, the higher the seropositive rates of murine typhus in animals and the higher the risk of human infection. Because the number of *X. cheopis* in Taichung Harbor and Kaohsiung Harbor peaked from January to June every year, control and preventive measures should be enhanced in these 6 months. Descents of *X. cheopis* index in February and June in Kaohsiung Harbor, as well as the significant annual variation of average *X. cheopis* index in both Taichung Harbor and Kaohsiung Harbor between January and June could be contributory to the regular anti-rodent and debugging campaign. In our study, *H. bandicota* was the second common ectoparasite, which had never been found to be associated with any vector-borne communicable diseases in human. Other ectoparasites captured from our ports could result in disease transmission among rats, but not in human [15]. Another investigation conducted in Iran, evaluating rats and their ectoparasites, found the most common rats were *R. norvegicus* and *R. rattus*; and the most common ectoparasites were *X. cheopis* and *X. astia* [16]. Similar study in Jayapura, Indonesia, a port city, also found *R. norvegicus* was the most common rat [17]. Therefore, *R. norvegicus* was probably the most common rat species in ports in Taiwan and other countries and could be the most common host of *X. cheopis*.

Reviewing the literature, there were quite a few studies focused on investigating the seropositive rates of murine typhus among murine-like animals. In Cyprus, using IFA method, the seropositive rates among *R. norvegicus* was 60.8%, which was significantly higher than that in *R. rattus* (21.1%), just like in our study [13]. In Malang Indonesia, the seropositive rates of murine typhus of human and murine-like animals in rural area, suburban areas, and big cities were compared using ELISA method. Although the higher seropositive rates of human and rats in city [18] could not prove the correlation between rat infection and human infection, it could still be considered as a reasonable assumption. In our study, the seropositive rate was significant higher in *R. norvegicus*, indicating that *R. norvegicus* was not only the most common murine-like animal, but also the most common reservoir of murine typhus, and should be considered a major target in disease control and prevention.

Previous studies showed a correlation between *X. cheopis* index and the rat seropositive rate of murine typhus [13, 14]. In our study, we failed to show a significant correlation between *X. cheopis* index of Taichung Harbor and Kaohsiung Harbor and the seropositive rates. The different result of our study could be due to the large hinterland of the seaports, change of sampling sites every month, and uneven geographic distribution of *X. cheopis* and *R. typhi*. The infection rate of *R. typhi* of *X. cheopis* could be more strongly correlated with seropositive rates of murine typhus, but we did not take samples from *X. cheopis*, further studies are needed to prove this assumption.

In conclusion, the seropositive rate of murine typhus of murine-like animals in Taichung Harbor and Kaohsiung Harbor were high, continuous monitoring may be necessary. Education to the working staff in ports, anti-rodent and debugging campaign are also important measures to prevent general public from getting murine typhus infection.

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Outbreak Investigation Express

An Outbreak of Meningoencephalitis Caused by *Angiostrongylus cantonensis* among Thai Workers in Changhua County, Taiwan, 2012 June

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Abstract

Angiostrongylus cantonensis is a parasite infesting rodents, mollusks, and incidentally, human hosts. *A. cantonensis* is the major pathogen of eosinophilic meningoencephalitis in Asia and the islands around pacific ocean. People contract *A. cantonensis* associated meningoencephalitis through taking raw or inappropriately cooked snails and slugs infested by the pathogen, or vegetables and water contaminated by the pathogen. We were notified of eight eosinophilic meningoencephalitis patients who were Thai factory workers in Changhua in June. All of the case patients had taken inappropriately cooked *Pomacea canaliculata* which is obtained from the pond in the factory. *A. cantonensis* was confirmed as the causative pathogen through the immunodiagnosis tests on the serum and cerebrospinal fluid specimens collected from the case patients. The larvae of *A. cantonensis* were identified in the *Pomacea canaliculata* sampled from the pond. This outbreak highlights the potential threat by taking inappropriately cooked *Pomacea canaliculata* in Taiwan.

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