Outbreak of Melioidosis and Leptospirosis Co-infection Following a Rescue Operation

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SUMMARY

We analyzed the epidemiological data of all people who were involved in the search and rescue operation in Lubuk Yu, a natural recreational forest with waterfall and stream. The hospital admission records of the cases who fulfilled the case definition and the environmental samples result taken at Lubuk Yu recreational area were studied. 153 people were exposed to this outbreak, 85 (55.5%) were professional rescuers from various government agencies and 68 (44.5%) were villagers. 21 fulfilled the case definition. Ten cases were confirmed melioidosis, six melioidosis alone and four co-infected with leptospirosis. There were eight deaths in this outbreak, seven were villagers and one professional rescuer. Overall case fatality was 70%. All confirmed melioidosis cases and seven who died had diabetes mellitus. The morbidity rate were higher among the villagers, 23.5% compared to professional rescuers, 5.9%. The case fatality rate were also higher in this group which was 100% compared to 33.3% in professional rescuers. The soil and water samples in Lubuk Yu recreational area were positive for leptospira and *Burkholderia pseudomallei.* The presence of co-infection and co-morbidities especially diabetes mellitus among the exposed led to the high mortality in this outbreak hence a high index of suspicion is important among the healthcare professionals in the management of melioidosis cases. To avoid similar incident in future, search and rescue operation should be only conducted by professional rescuers with appropriate personal protective equipment. A register of rescuers should be maintained for surveillance and follow up if necessary.

KEY WORDS:
Co-infection, Leptospirosis, Melioidosis, Outbreak, Rescue

INTRODUCTION

Melioidosis is caused by the gram-negative bacillus, *Burkholderia pseudomallei*, a free living saprophyte in the soil commonly found in tropical and subtropical regions. It is endemic in Southeast Asia countries and Northern Australia[1-5]. A study in Pahang[3], one of the state in Malaysia has shown that the incidence of this infection is comparable with northern Thailand[4] which is 6.1 per 100,000 populations per year . The mortality rate for melioidosis ranged from 19 to 72% [1-6]. Melioidosis commonly occurs in people between the ages of 40 to 60 years and is related to farming[3]. The mode of transmission is inhalation of contaminated dust or direct entry of the organism into the blood stream via very minor wounds or skin abrasions. Person with underlying diseases especially diabetes mellitus are more likely to develop melioidosis (20-74% of cases)[1-5,7,8].

Leptospirosis is an infectious disease that affects humans and animals. It is considered the most common zoonotic disease in the world [9,10]. Leptospirosis is caused by pathogenic spiral bacteria that belong to the genus *Leptospira*[11,12]. The organism enters the body when mucous membranes or abraded skin come in contact with contaminated environmental sources[13]. The infection may cause systemic illness that sometimes leads to renal and hepatic dysfunction. Occupational exposure probably accounts for 30-50% of human cases. The main occupational groups at risk include farm workers, veterinarians, pet shop owners, field agricultural workers, abattoir workers, plumbers, meat handlers, coal miners, workers in the fishing industry, military troops, milkers, and sewer workers. Leptospirosis has also increasingly been recognized as a disease of recreation. Recreational activities that present some risk include canoeing, hiking, kayaking, fishing, windsurfing, swimming, waterskiing, wading, riding trail-bikes through puddles, white-water rafting, and other outdoor sports played in contaminated water[12]. Supramaniam in 1979 reviewed the status of leptospirosis among the Malaysian army personnel and found that serological studies revealed a 12-22% prevalence of antibodies indicating past infection. Study of febrile cases showed that only 4.6% of fever in Malaysian soldiers were due to leptospirosis [14]. Brown *et al* in 1984 studied 1629 patients with febrile illness from Pahang in Central Malaysia. Leptospirosis was accounted for 6.8% of five diseases diagnosed[15]. El Jalii *et al* in 2002 concluded that the overall prevalence of leptospiral infection from the study was 12.6%[16]. Most leptospiral serovars have their primary reservoir in wild mammals, which continually reinfect domestic populations. The most important reservoirs are rodents, and rats are the most common. Urinary shedding of organisms from infected animals is the most important source of these bacterial pathogens. Contact with the organism via infected urine or urine-contaminated media results in human infection. Humans are incidental hosts. The resulting leptospiraemia can spread to any part of the body but particularly affects the liver and kidney. Disseminated intravascular coagulopathy, haemolytic uremic syndrome, thrombotic thrombocytopenic purpura and overwhelming vasculitis are not uncommon complications[17,18]. Immunosuppressed patients may develop a fulminant course

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of leptospirosis, including transplanted patients[19]. Despite the possibility of severe complications, the disease is most often self-limited and non-fatal[20].

At the end of June 2010 an outbreak of melioidosis and leptospirosis co-infection was reported in Lubuk Yu, Pahang, Malaysia following a search and rescue operation of a drowned victim. Lubuk Yu is a natural recreational forest with waterfall and stream. It is situated about 130 km from Kuantan, the capital state of Pahang. This recreation area is managed by Forestry Department which is freely accessible to general public. There are only basic facilities available which include toilets, changing rooms and prayer room without treated water supply. The visitors use river water during their visit to the area. There were few dilapidated food stalls that showed signs of rat infestation as evidenced by the presence of dead rats and rat droppings. The aim of this paper is to describe the outbreak, determine the causal factors and the cause of high fatality.

MATERIALS AND METHODS

The outbreak and case investigation

On 26th June 2010 a youth was reported missing and suspected drowned at Lubuk Yu. A rescue operation was immediately initiated by both relevant government agencies and nearby villager from 26th June to 30th June 2010. Government agencies involved were Fire and Rescue Department, Royal Malaysian Police, Royal Malaysian Navy and Civil Defence Department. A temporary operation centre was setup by the riverside throughout the five days operation. During this period, the rescuers used water from the river for daily chores. The body was found on the 30th June 2010 about 10 km downstream. The first case was admitted to the district hospital on July 1, 2010. Another eight cases were admitted to three different hospitals from July 3, 2010 to July 7, 2010 and four deaths were reported during the same period including two deaths at home. Only on July 8, 2010, the health department was notified about the incidents. Investigation revealed that all cases were involved in the search and rescue operation.

The case definition was anyone who had involved in the search and rescue operation and came down with fever (temperature ≥38^{0}C) within 30 days from the last date of search and rescue operation. This is based on the incubation period of Leptospirosis which is 2 -30 days. Those who were involved in the search and rescue operation were considered as exposed person. The exposed persons were identified through 'snow balling' method from interviews. Investigation was done at the hospital, Lubuk Yu recreational area and nearby villages to determine the cause of the illness.

Specimen collection

Specimens from the patients, animals and environment samples were collected. For each patient 10 mL of blood was cultured on blood agar in the respective hospital while 5 mL of blood was sent to Institute for Medical Research (IMR) for melioidosis and leptospira serology and Polymerase Chain Reaction (PCR) for detection of leptospira.

Soil and water were sampled at different locations at the outbreak site on three separate occasions according to standard protocol. 100 gm of surface soils and soils at depth of 30 cm were sampled using spade or small gardening shovel which was cleaned thoroughly between samples and sprayed with 70% alcohol and let dry. One hundred mL of stagnant water found along the river bank were collected in a sterile whirl packs. All soil and water samples were labelled and sent to IMR on the same day for culture and leptospira PCR. Animal reservoir for leptospirosis such as rodents and civet captured at the sites were tested for presence of leptospira.

Laboratory method

Serological diagnosis of melioidosis and leptospirosis

Serological diagnosis of melioidosis was carried out using indirect immunofluorescent antibody test for detection of IgM antibodies against *Burkholderia pseudomallei* following the method outlined by Ashdown[21]. Positive serology was interpreted as having a four-fold rise in antibody.

Enzyme-linked immunosorbent assay (ELISA) was carried out for detection of IgM anti-leptospiral antibody following WHO guideline[22]. Leptospira biflexa serovar Patoc was used as antigen and fixed on a microtitre plates. Serial serum dilutions were carried out starting from 1:80 until 1:640 and added to the microtitre plates. ELISA test was interpreted as positive if there was a four-fold rise in antibody titre if paired serum sample was not available, the titre of ≥ 1:160 was considered as positive. For all positive ELISA results, the sera were then subjected to microscopic agglutination test (MAT). The MAT procedure was carried out following the WHO guideline[22]. A total of 20 leptospira serovars were included in the panel of antigens. The test was interpreted as positive if there was a four-fold rise in antibody titre or if paired serum sample is not available, the titre of ≥ 1:400 was considered positive.

Culture for Bulkholderia pseudomallei

For soil culture for *Burkholderia pseudomallei*, sterile water (100 mL) was added to the soil sample, mixed well and left overnight at room temperature to sediment. 10 µL of the upper layer of water was then plated onto Ashdown's selective agar plate and another 1 mL of the upper layer of water was added to 9 mL of CV-C50 selective enrichment broth. The plates and broth medium were incubated at 42°C and visually inspected for 4 days. While for water culture, 15 mL of water were centrifuged and the sediment inoculated onto Ashdown media and CV-C50 and incubated accordingly. Colonies suspected to be *Burkholderia pseudomallei* were picked and identified using biochemical tests.

Polymerase Chain Reaction (PCR) for leptospira

Two mls of blood was centrifuged at 3000 rpm for five minutes and the tube was left upright for one hour. Two hundred µl of the supernatant was then extracted for DNA using QIAamp DNA Blood Kits (QIAGEN). For water samples, 15 mls of water was centrifuged for 15 minutes and the supernatant removed. The pellet was resuspended with one ml sterile water and subjected to DNA extraction using QIAamp DNA Blood Mini Kits (QIAGEN). Polymerase chain reaction was carried out using G1/G2 primers for amplification of Leptospira DNA[23]. DNA from Escherichia coli was used. A285 bp PCR product will be obtained for the specimen is positive for leptospirosis.

Laboratory confirmed melioidosis was defined as positive culture or titre ≥ 1:80 or four-fold rise in IgM against *Burkholderia pseudomallei* [24]. The titre ≥ 1:80 was used to differentiate between true infections and background titres

due to basal antibody levels in endemic areas[25]. Laboratory confirmed leptospirosis was defined as a positive PCR or MAT titre > 1:400 or a four-fold rise in antibody titre.

Outbreak control measure

Upon confirmation of outbreak a special task force comprising of Health, Veterinary Services and Forestry Departments as well as local authority and other related agencies was established to investigate and control the outbreak. Lubuk Yu recreational park was closed to the public and the rubbish from the area was disposed. Dilapidated food stalls were cleared. A signboard to warn visitors regarding the hazards and health risk of contracting disease was erected. Health awareness campaign on melioidosis and leptospirosis to the public and health care providers were carried out. Health alert to all government and private medical practitioners in the affected and the neighbouring districts were also issued.

RESULTS

One hundred and fifty-three (153) people were involved in the search and rescue operation thus considered exposed in the outbreak. Median duration of exposure at the incident site was 11.5 hours ranging from 1 to 96 hours. They comprise of 85 (55.5%) professional rescuers from various government agencies namely Royal Malaysian Police, 32 (37.6%), Fire and Rescue Department, 28 (32.9%), Civil Defence Department, 13 (15.3%), Royal Malaysian Navy, 12 (14.1%) and the rest were local villagers. Most of the rescuers, 147 (96.1%) were male.

Socio-demographic characteristics of the exposed are shown in Table I.

Table I: Socio demographic characteristics of the exposed

Socio Demographic	N	%
Age group (years)		
Less than 20	3	2.0
20 - 34	75	49.0
35 - 59	71	46.4
60 and above	4	2.6
Search and rescue team		
Professional	85	55.6
Villagers	68	44.4

Table II: Lubuk Yu melioidosis and leptospirosis outbreak case listing and clinical outcome

Age (years)/Sex/ Catogery of Rescuer	Date of Onset	Symptoms	Co-morbidity	Laboratory Diagnosis	Patient Outcome
58/Male/Villager	27/6/2010	Fever, Chill, Headache, Myalgia, Shortness of breath, Vomiting, Diarrhoea	Diabetes Mellitus, Hypertension, Congestive Cardiac Failure, Chronic Obstructive Pulmonary Disease, Thyrotoxicosis	Melioidosis	Died
52/ Male/ Villager	28/6/2010	Fever, Myalgia, Cough, Shortness of breath, Vomiting, Diarrhoea, Nausea	Diabetes Mellitus, Hypertension	Melioidosis	Died
40/ Male/ Villager	30/6/2010	Fever, Headache, Myalgia, Cough, Shortness of breath, Vomiting, Diarrhoea	Diabetes Mellitus	Melioidosis	Died
50/ Male/Professional	1/7/2010	Fever, Chill, Headache, Myalgia, Arthralgia, Cough, Shortness of breath, Vomiting, Diarrhoea, Nausea, Jaundice	Diabetes Mellitus	Melioidosis and Leptospirosis	Died
60/ Male/Villager	2/7/2010	Fever, Chill, Myalgia, Shortness of breath, Diarrhoea	Diabetes Mellitus	Melioidosis and Leptospirosis	Alive
29/ Male/Villager	3/7/2010	Fever, Myalgia, Cough, Shortness of breath, Vomiting, Diarrhoea	Diabetes Mellitus, Hypertension	Melioidosis and Leptospirosis	Died
55/ Male/Villager	3/7/2010	Fever, Chill, Myalgia, Arthralgia, Cough, Shortness of breath, Vomiting, Diarrhoea, Nausea	Diabetes Mellitus /Ischemic Heart Disease	Melioidosis and Leptospirosis	Died
23/ Male/Professional	9/7/2010	Fever, Headache, Cough	Impaired Glucose Tolerance Test	Melioidosis	Alive
47/ Male/Villager	9/7/2010	Fever, Chill, Headache, Myalgia, Arthralgia, Cough, Vomiting, Diarrhoea, Nausea	Diabetes Mellitus, Hypertension	Melioidosis	Alive
62/ Male/Villager	4/7/2010	Fever, Headache, Myalgia, Arthralgia, Cough, Shortness of breath, Vomiting, Diarrhoea, Nausea	Diabetes Mellitus, Hypertension	Melioidosis	Died
56/ Male/Villager	29/6/2010	Fever, Chill, Myalgia, Arthralgia, Nausea	Diabetes Mellitus, Hypertension	Died at home.	Died
45/ Male/Villager	30/6/2010	Fever, Chill, Headache, Vomiting, Nausea	None	Not Melioidosis or Leptospirosis	Alive
42/ Male/Professional	29/6/2010	Fever, Headache, Myalgia, Arthralgia, Shortness of breath	None	Not Melioidosis or Leptospirosis	Alive
58/ Male/Villager	6/7/2010	Fever, Myalgia, Arthralgia, Cough, Nausea	Diabetes Mellitus	Not Melioidosis or Leptospirosis	Alive
42/ Male/Villager	28/6/2010	Fever, Headache, Arthralgia	None	Not Melioidosis or Leptospirosis	Alive
59/ Male/Villager	7/7/2010	Fever, Headache, Myalgia, Arthralgia,	Diabetes Mellitus, Hypertension	Not Melioidosis or Leptospirosis	Alive
42/ Male/Villager	8/7/2010	Fever, Chill, Headache	None	Not Melioidosis or Leptospirosis	Alive
27/ Male/Professional	12/7/2010	Fever, Myalgia, Arthralgia, Cough, Nausea	None	Not Melioidosis or Leptospirosis	Alive
30/ Male/Professional	24/7/2010	Fever, Chill, Myalgia, Arthralgia, Cough, Shortness of breath, Vomiting, Diarrhoea, Nausea	Obese	Not Melioidosis or Leptospirosis	Alive
56/ Male/Villager	18/7/2010	Fever, Headache, Myalgia, Arthralgia, Vomiting	Diabetes Mellitus, Hypertension	Not Melioidosis or Leptospirosis	Alive
64/ Male/Villager	28/6/2010	Fever, Chill, Cough	Diabetes Mellitus, Hypertension	Not Melioidosis or Leptospirosis	Alive

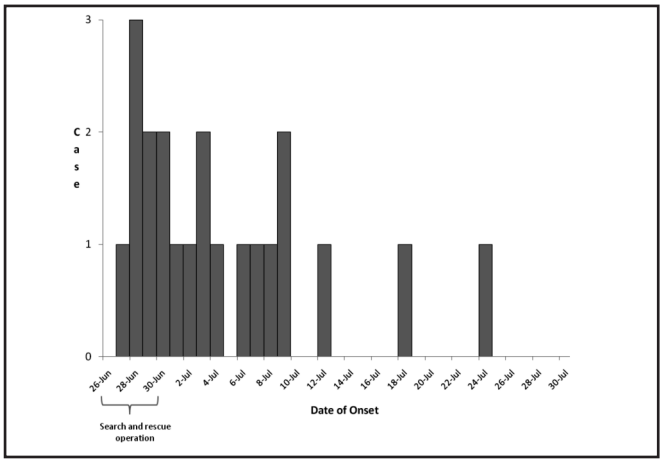

Fig. 1: Epidemic Curve For Lubuk Yu Melioidosis and Leptospirosis Outbreak.

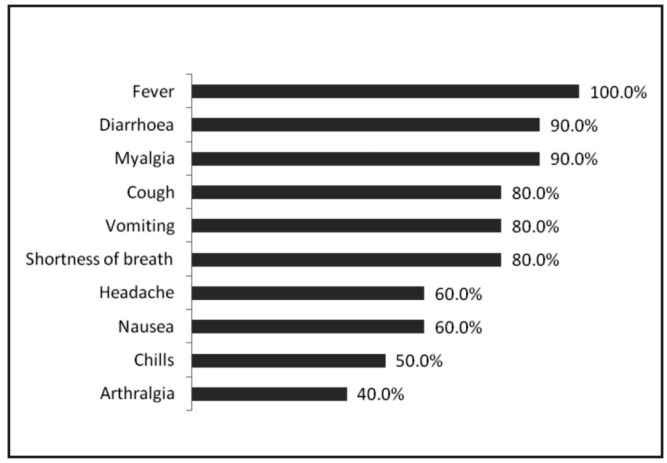

Fig. 2: Symptomatology of confirmed cases in Lubuk Yu outbreak.

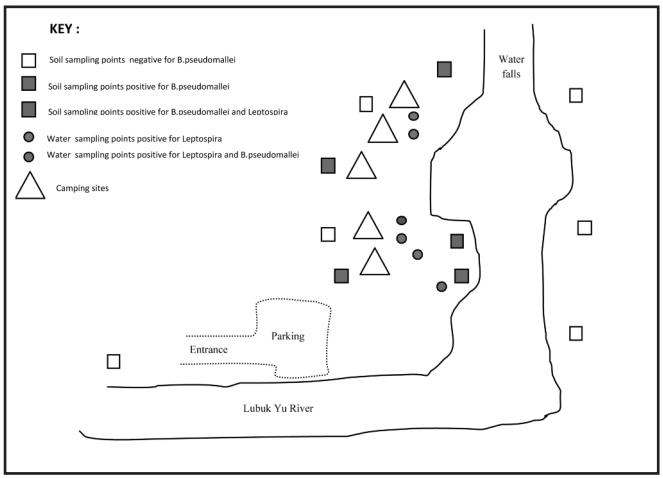

Fig. 3: Sampling points for water and soil.

From 153 people exposed, 21 fulfilled the case definition. There were 3 cases (14.3%) aged 60 and above, 14 cases (66.7%) aged 40-50 and 4 cases (19.0%) were below 40 years old. The median incubation period of the cases was 7 days (IQR 1-13 days). The epidemic curve of the cases is shown in the figure 1.

Clinical samples were taken from all the cases except one who died at home. Ten were laboratory confirmed melioidosis (all cultured positive for *Burkholderia pseudomallei*), out of which four had co-infection with leptospirosis while another ten were negative for both melioidosis and leptospirosis. The case listing and clinical outcome are as in Table II. Among those confirmed melioidosis, 90% had diarrhoea and myalgia. The symptomatology is shown in Figure 2.

80% of confirmed melioidosis cases were aged 40 and above. There were eight deaths in this outbreak, four had melioidosis alone and three had co-infection of melioidosis and leptospirosis. The case who died at home with no clinical samples taken had clinical description of melioidosis infection. Overall case fatality of this outbreak is 38.1% (8 out of 21). Case fatality for co-infection cases of melioidosis and leptospirosis and melioidosis alone were 75% (3 out of 4) and 66.7% (4 out of 6) respectively. From eight cases that died, seven were villagers and only one professional rescuer. All confirmed melioidosis cases and seven who died had diabetes mellitus.

All six water samples collected at the site of operation were positive for Leptospira including one sample positive for both Burkholderia pseudomallei and Leptospira. Two out of four surface soil samples were positive for Leptospira including one sample positive for both Burkholderia pseudomallei and Leptospira. Three out of 25 deep soil samples taken were also positive for *Burkholderia pseudomallei*. Animal samples were negative for leptospira. The sites from where the samples were taken are as shown in Figure 3.

DISCUSSION

This paper reports an outbreak of co-infection of melioidosis and leptospirosis among search and rescue workers in a case of drowning. To our knowledge there was no report of co-infection involving melioidosis and leptospirosis in Malaysia or elsewhere. Case fatality for laboratory confirmed melioidosis was 70%. It is higher than reported mortality in other studies in Malaysia and abroad which ranges from 33.8%-54%[1-5,26,27]. This may be because some of this patient has leptospirosis co-infection. They presented with viral-like symptoms such as myalgia, arthralgia, diarrhoea and only later developed rapidly progressive pneumonia. This had cause delay in diagnosis and antibiotic administration.

Among those ten cases that were cultured negative for leptospirosis and melioidosis, none of them died. This is probably they had a milder form of infection. Furthermore, most of these patients presented after the outbreak was reported to the health authority and the healthcare personnel were instructed to give antibiotic empirically.

In this particular search and rescue operation, seven (70%) confirmed melioidosis cases were villagers. The morbidity rate were higher in this group, 23.5% (16 out of 68) compared to professional rescuers, 5.9% (5 out of 85). The case fatality rate were also higher in this group which was 100% (7 out of 7) compared to 33.3% (1 out of 3) in professional rescuers . This high morbidity and mortality rate were probably due to they are relatively older, median age of 55.5 years (IQR 29-64) compared to professional group. (30 years , IQR 23-50).

Based on environmental sampling analysis, Lubuk Yu recreational area is endemic for melioidosis. Heavy downpour and flooding during the first two days of the search and rescue operation caused erosion of soils, exposing the pathogen and contaminating the water, especially stagnant water by the river bank. It was evidenced by the presence high water level mark at the river bank and accumulation of debris around the area. The soil is sandy, rich with humus and moist due to seepage of water from the surrounding hilly elevation. There were also a lot of movement by the rescuers causing aerosolization of dust and therefore predispose them to melioidosis infection through inhalation. The heavy rain also causes leaching of leptospira from surrounding area into the water thus increases risk of infection which was a known phenomenon in increasing leptospiral contamination [16,28-30]. The presence of the rescuers over four consecutive days resulted in accumulation of garbage and food waste which attracted rats that contaminated the water as evidenced by the detection of the leptospira in all water samples. The fact that the rescuers stayed for many days and used stagnant water for daily chores possibly contributed to the outbreak. The factors listed above could explain the mechanism of the co-infection in this outbreak.

To prevent similar incident in future, search and rescue operation should be only conducted by professional rescuers with appropriate personal protective equipment (PPE). A register of rescuers should be maintained for surveillance and follow up if necessary. For this type of rescue operation, personnel having chronic diseases especially diabetes mellitus should be excluded from the team since in melioidosis, underlying disease is the main contributor to epidemiological risk of established infection[31]. Personnel should monitor their health status for two weeks after the operation and they must seek treatment if they fall sick. Portable water should be made available for the search and rescue team for consumption, cleaning and daily chores. This is to lessen the risk of infection through direct contact with contaminated water.

All private and government medical practitioners should be made aware of melioidosis and leptospirosis infection and its management. This is to avoid delay in detecting melioidosis or leptospirosis and hence treatment will also be delayed. Guidelines on management of these diseases should be disseminated and made available to them.

In conclusion, the presence of co-infection and co-morbidities especially diabetes mellitus among the exposed led to the high mortality in this outbreak hence a high index of suspicion is important among the healthcare professionals in the management of melioidosis cases.

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