Travel Medicine and Infectious Disease 32 (2019) 101461

Contents lists available at ScienceDirect

Travel Medicine and Infectious Disease

journal homepage: www.elsevier.com/locate/tmaid

Melioidosis in travelers: An analysis of Dutch melioidosis registry data 1985–2018

Emma Birnie[a,*], Jelmer Savelkoel[a], Frans Reubsaet[b], Joris J.T.H. Roelofs[c], Robin Soetekouw[d], Saskia Kolkman[e], Anne Lia Cremers[f], Martin P. Grobusch[f], Daan W. Notermans[b], W. Joost Wiersinga[a,g], for the Dutch Melioidosis Study Group (Wouter Rozemeijer[h], Annemieke Rijkeboer[i], Emma Birnie[j], Anne Lia Cremers[j], Martin P. Grobusch[j], Saskia Kolkman[j], Joris J.T.H. Roelofs[j], Jelmer Savelkoel[j], W. Joost Wiersinga[j], Maarten Scholing[k], Karin van Dijk[l], Ellen M. Mascini[m], Henk van der Veen[n], Wouter van den Bijllaardt[o], Daan W. Notermans[b], Frans Reubsaet[b], Maaike de Vries[b], Leonard C. Smeets[p], Alewijn Ott[q], Kees van Krimpen[r], Robin Soetekouw[r], Bjorn L. Herpers[s], G. Hanke Watttel-Louis[t], Karola Waar[u], Noortje L.Q. Schwandt[v], Ianthe Maat[w], Anthonius S.M. Dofferhoff[x], Joost N. Vermeulen[y], Mireille van Westreenen[z], Peter de Man[aa], Regina W. Hofland[ab], Joost van Gorp[ac], Steven Thijsen[ac], Lieven B. van der Velden[ad], Cornelis M. Verduin[ad])

[a] *Center for Experimental and Molecular Medicine and Melioidosis Expertise Center, Amsterdam UMC, Location Academic Medical Center (AMC), Amsterdam Infection & Immunity Institute, University of Amsterdam, Amsterdam, the Netherlands*
[b] *Center for Infectious Disease Control, National Institute for Public Health and the Environment (RIVM), Bilthoven, the Netherlands*
[c] *Department of Pathology, Amsterdam UMC, Location AMC, University of Amsterdam, Amsterdam, the Netherlands*
[d] *Spaarne Gasthuis, Haarlem, the Netherlands*
[e] *Department of Radiology, Amsterdam UMC, Location AMC, University of Amsterdam, Amsterdam, the Netherlands*
[f] *Center of Tropical Medicine and Travel Medicine, Division of Infectious Diseases, Amsterdam UMC, Location AMC, University of Amsterdam, Amsterdam, the Netherlands*
[g] *Division of Infectious Diseases, Amsterdam UMC, Location AMC, University of Amsterdam, Amsterdam, the Netherlands*
[b] *Center for Infectious Disease Control, National Institute for Public Health and the Environment (RIVM), Bilthoven, the Netherlands*
[h] *Department of Medical Microbiology, Northwest Clinics, Alkmaar, the Netherlands*
[i] *Department of Intensive Care, Flevo Hospital, Almere, the Netherlands*
[j] *Center for Experimental and Molecular Medicine, Center of Tropical Medicine and Travel Medicine, Division of Infectious Diseases, Department of Pathology, Department of Radiology, Amsterdam UMC, Location Academic Medical Center, University of Amsterdam, Amsterdam, the Netherlands*
[k] *Department of Medical Microbiology, OLVG General Hospital, Amsterdam, the Netherlands*
[l] *Department of Medical Microbiology, Amsterdam UMC, Location VUmc, Vrije Universiteit, Amsterdam, the Netherlands*
[m] *Laboratory for Medical Microbiology and Immunology, Rijnstate Hospital, Arnhem, the Netherlands*
[n] *Department of Surgical Oncology, Red Cross Hospital, Beverwijk, the Netherlands*
[o] *Microvida Laboratory for Microbiology, Amphia Hospital, Breda, the Netherlands*
[p] *Department of Medical Microbiology, Reinier de Graaf Group, Delft, the Netherlands*
[q] *Department of Medical Microbiology, Certe, Groningen, the Netherlands*
[r] *Department of Internal Medicine and Department of Pathology, Spaarne Gasthuis, Haarlem, the Netherlands*
[s] *Regional Public Health Laboratory Kennemerland, Haarlem, the Netherlands*
[t] *Department of Internal Medicine, Spaarne Gasthuis, Hoofddorp, the Netherlands*
[u] *Department of Medical Microbiology, Izore Center for Infectious Diseases Friesland, Leeuwarden, the Netherlands*
[v] *Department of Otorhinolaryngology-Head and Neck Surgery, Medical Center Leeuwarden (MCL), Leeuwarden, the Netherlands*
[w] *Department of Medical Microbiology, Radboud University Medical Center, Nijmegen, the Netherlands*
[x] *Department of Internal Medicine, Canisius-Wilhelmina Hospital, Nijmegen, the Netherlands*
[y] *Dijklander Hospital, Purmerend, the Netherlands*
[z] *Department of Medical Microbiology and Infectious Diseases, Erasmus Medical Center, Rotterdam, the Netherlands*
[aa] *Department of Medical Microbiology, Franciscus Gasthuis and Vlietland, Rotterdam, the Netherlands*

[*] Corresponding author. Center for Experimental and Molecular Medicine, Amsterdam UMC, location AMC, University of Amsterdam, Meibergdreef 9, 1105 AZ, Amsterdam, the Netherlands.
E-mail addresses: e.birnie@amc.uva.nl (E. Birnie), w.j.wiersinga@amc.uva.nl (W.J. Wiersinga).

https://doi.org/10.1016/j.tmaid.2019.07.017

Available online 29 July 2019

[ab] Department of Respiratory Medicine, University Medical Center Utrecht, Utrecht, the Netherlands
[ac] Department of Medical Microbiology and Department of Pathology, Diakonessenhuis, Utrecht, the Netherlands
[ad] Department of Medical Microbiology, Laboratory for Pathology and Medical Microbiology (PAMM), Veldhoven, the Netherlands

ARTICLE INFO

Keywords:
Burkholderia pseudomallei
Melioidosis
Fever in returned travelers
Travel medicine
Surveillance

ABSTRACT

Background: Melioidosis, caused by the Gram-negative bacterium *Burkholderia pseudomallei,* is an opportunistic infection across the tropics. Here, we provide a systematic overview of imported human cases in a non-endemic country over a 25-year period.
Methods: All 55 Dutch microbiology laboratories were contacted in order to identify all *B. pseudomallei* positive cultures from 1990 to 2018. A response rate of 100% was achieved. Additionally, a systematic literature search was performed, medical-charts reviewed, and tissue/autopsy specimens were re-assessed.
Results: Thirty-three travelers with melioidosis were identified: 70% male with a median-age of 54 years. Risk factors were present in most patients (n = 23, 70%), most notably diabetes (n = 8, 24%) and cystic fibrosis (n = 3, 9%). Countries of acquisition included Thailand, Brazil, Indonesia, Panama, and The Gambia. Disease manifestations included pneumonia, intra-abdominal abscesses, otitis externa, genitourinary, skin-, CNS-, and thyroid gland infections. Twelve (36%) patients developed sepsis and/or septic shock. Repeat episodes of active infection were observed in five (15%) and mortality in four (12%) patients. Post-mortem analysis showed extensive metastatic (micro)abscesses amongst other sites in the adrenal gland and bone marrow.
Conclusions: The number of imported melioidosis is likely to increase, given rising numbers of (immunocompromised) travelers, and increased vigilance of the condition. This first systematic retrospective surveillance study in a non-endemic melioidosis country shows that imported cases can serve as sentinels to provide information about disease activity in areas visited and inform pre-travel advice and post-travel clinical management.

1. Introduction

Worldwide, the number of travelers is increasing substantially. 1.3 billion trips were made in 2017, an increase of 5% from the previous year [1]. Of those travelers, 323 million went to Asia and the Pacific regions with Southeast Asia facing the highest growth [1]. In addition, there is a continuous increase in older travelers with clinically significant co-existing conditions, such as diabetes, chronic lung, liver- and kidney disease, and cystic fibrosis as well as patients on immune-suppressing medications [2–4]. Any traveler exposed to contaminated soil and/or water due to outdoor activities can be infected with the Gram-negative environmental saprophyte *Burkholderia pseudomallei* and may consequently acquire melioidosis [5,6].

Melioidosis is an emerging tropical infectious disease and endemic in Southeast Asia and Northern Australia [5–7]. New evidence suggests that *B. pseudomallei* is more widely present than previously thought; predicted worldwide human melioidosis cases are 169,000 each year with a mortality of up to 50% [8]. The incubation period of melioidosis is generally 1–21 days [9]. However, the disease can also remain latent for up to 29 years [5]. Disease presentation can vary from skin abscesses to pneumonia and fulminant sepsis and can be acute (85%) or chronic (11%) [5]. Melioidosis often mimics other diseases (e.g. tuberculosis or cancer), has an uncommon presentation in temperate countries, and can be missed with standard microbiology techniques [5]. As a result, the disease is frequently misdiagnosed [5,7]. Up to 80% of patients with melioidosis have an underlying illness that makes them susceptible to acquire this life-threatening infection [5]. Individuals with diabetes mellitus, the most common risk factor, which is present in more than 50% of all patients with melioidosis, have a 12-fold higher risk to acquire melioidosis compared to non-diabetics [5,10].

Front-line clinicians in non-endemic countries need to be aware that fever in returning travelers may signal melioidosis [2]. Microbiologists should be aware of this condition, recognize the characteristics of *B. pseudomallei*, and be familiar with the work-up of any oxidase-positive Gram-negative rod isolated from samples from anyone who has spent time in an melioidosis-endemic area [11]. This is further underscored by the classification of *B. pseudomallei* by the Centers for Disease Control and Prevention as a Tier 1 Select Agent. Melioidosis therapy consists of a prolonged antibiotic treatment course; an intravenous phase of two weeks (carbapenem or ceftazidime) followed by an oral eradication phase of three to six months (trimethoprim–sulfamethoxazole (TMP-SMX), or amoxicillin-clavulanic acid) depending on disease presentation [5]. No vaccine for melioidosis is currently available [12]. Therefore, preventive measures are key to avoiding infection. In Thailand, Limmathurotsakul et al. attempted to produce evidence-based guidelines for the prevention of melioidosis [13]. However, at present, no advice is generally given to tourists, but this could include measures targeted at risk groups, such as the use of waterproof shoes when walking in wet soil, or the advice to stay indoors during periods of heavy wind and rain [7,14]. Here, we provide the first retrospective surveillance study of known imported melioidosis cases in a non-endemic country, The Netherlands, for a 25-year period.

2. Material and methods

2.1. Identification of cases

A melioidosis case was defined as the presence of an infection as determined by the primary physician and a confirmed positive *B. pseudomallei* culture [7]. Together with the Dutch National Institute for Public Health and the Environment (RIVM), we identified melioidosis cases from 1990. The RIVM offers laboratory identification and/or confirmation of cultured bacteria to all medical microbiological laboratories in the country [15]. This procedure consists of multiple phenotypical tests, fatty acid analysis, 16S rDNA sequence analysis, matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF), and a species-specific test based on three PCRs [15]. Furthermore, some melioidosis cases were reported in the weekly reports of The Netherlands Early Warning Committee of the RIVM.

In The Netherlands disease notification of melioidosis is not mandatory, in contrast to some other countries such as the United Kingdom. Therefore, we contacted all microbiology laboratories known by the Dutch Society for Medical Microbiology (NVVM) and the RIVM. We asked all Dutch laboratories to perform a systematic search in their system for any *B. pseudomallei* positive culture between 2003 and 2018. Several cases identified by laboratories before 2003 were also accepted into the analysis. If laboratories were unable to perform a search of the last 15 years due to data availability or altered laboratory systems, a

search period shorter than 15 years was accepted. Laboratories that identified melioidosis cases were asked to complete the standard case report form of The Netherlands Melioidosis Registry which includes questions on signs and symptoms, travel history, microbiology, histopathology, radiology, treatment, etc. (Supplementary Table S1). In addition, we searched Medline, Embase, and Google (Scholar) for reports of human melioidosis cases in The Netherlands published between January 1985 and December 2018. The following search terms were applied: 'melioidosis' or '*pseudomallei*' in combination with 'traveler*', 'tourist*', 'Dutch' or 'The Netherlands'. The literature search included articles in all languages. Literature references of relevant articles were examined to identify additional cases. Medical charts were reviewed and available histology specimens (of eight patients) were re-assessed by a dedicated clinical pathologist with a long track record in melioidosis research (J.J.T.H.R.) in order to collect complete epidemiologic, clinical, radiological, and histopathological characteristics of all identified cases.

2.2. Ethics and statistical analysis

Since the study was retrospective in nature and only involved data derived from medical records, an exemption from the need for consent was provided by the Medical Ethics Review Committee of the Amsterdam UMC, location Academic Medical Center (reference number W17_470 # 17.543). Under Dutch law, the secondary use of anonymized human tissue specimens is not subject to ethical review or patient consent, following the "Code of conduct for responsible use of human tissue" designed by the Federation of Dutch Medical Scientific Societies in 2011 [16]. Patient's written consent was obtained for radiological images presented. Statistical analysis was performed using Prism/R (version 3.5.1). Non-parametric quantitative variables are presented as median and range.

3. Results

3.1. Case identification

The response rate for the 55 Dutch microbiology laboratories was 100%. Seventeen laboratories identified 25 cases between 2003 and 2018 by tracking positive *B. pseudomallei* culture results. Seven laboratories were unable to retrieve information prior to 2007. Four laboratories were able to retrieve cases from more than 15 years ago (1990, 1994, 1999, and 2002, respectively). Another 15 cases from the period 1990 to 2018 were identified through the existing informal registry and network of the RIVM. An extensive literature search from 1985 to 2018 identified another ten cases. Duplicates (n = 20) were removed. In total, 33 melioidosis patients were included in the study (Fig. 1).

3.2. Epidemiology and clinical features

Characteristics of returned travelers recorded with a confirmed *B. pseudomallei* infection (n = 33) are listed in Table 1. A complete list of every patient is provided in Supplementary Table S2. The median age of patients was 54 years (range 21–83). Twenty-three patients (70%) were male. All patients resided in The Netherlands, except one woman who was born and lived in Goa, India, and was on holidays in The Netherlands when the diagnosis was established (case no. 2 Supplementary Table S2). Twenty-three patients (70%) had one or more risk factors, namely diabetes (n = 8), smoking (n = 7), cystic fibrosis (n = 3), excessive alcohol consumption (n = 3), chronic kidney disease (n = 2), chronic liver disease (n = 2), chronic obstructive pulmonary disease (COPD) (n = 1), non-Hodgkin lymphoma (n = 1), and methotrexate and prednisone use (n = 1).

Most patients acquired the infection in Thailand (52%, n = 17) followed by Brazil (6%, n = 2), Australia, Cambodia, Indonesia, Myanmar, India, and Vietnam (all n = 1; Table 2). Some patients (18%, n = 6) visited multiple countries and therefore the exact country of

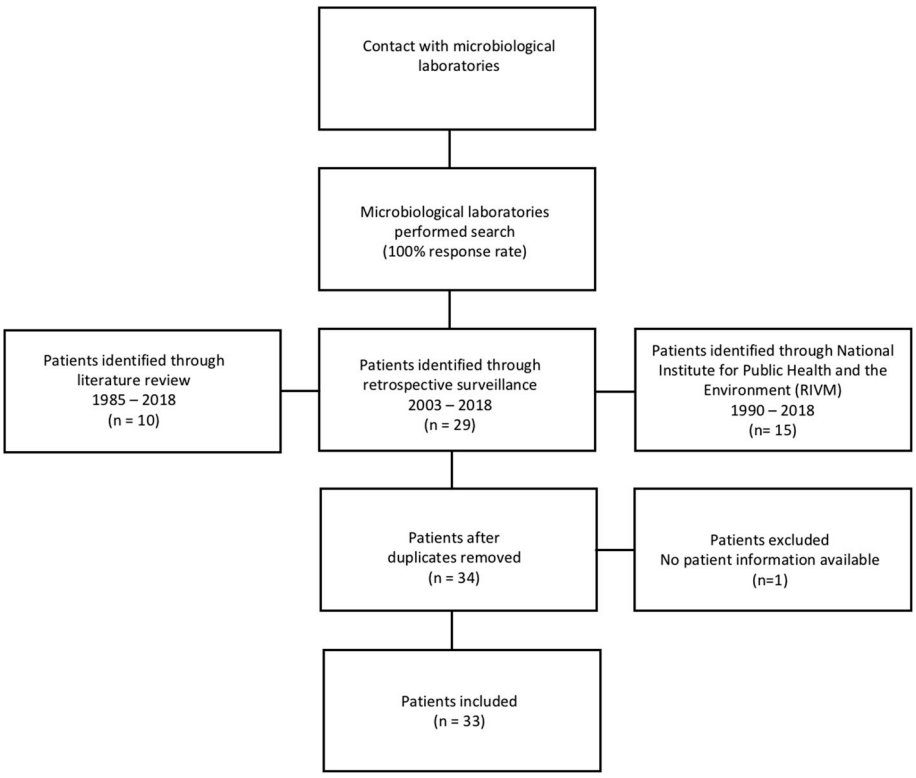

Fig. 1. Flow diagram of patient inclusion.

Table 1
Clinical characteristics of 33 returned travelers with confirmed *Burkholderia pseudomallei* infection.

Variable[a]	No. (%) of patients
Baseline characteristics	
Male sex	23 (70)
Age yr - median [range]	54 [21–83]
Risk factors	23 (70)
Diabetes	8 (24)
Smoking	7 (21)
Cystic fibrosis	3 (9)
Excessive alcohol consumption	3 (9)
Chronic kidney disease	2 (6)
Chronic liver disease	2 (6)
COPD	1 (3)
Malignancy	1 (3)
Use of immunosuppressive drugs[b]	1 (3)
Exposure	
Environmental exposure[c]	18 (55)
Traumatic injury (fall, fracture)[d]	2 (6)
Unknown	15 (45)
Presenting symptoms	
Fever	20 (61)
Coughing/dyspnea	15 (45)
Malaise/fatigue	13 (39)
Gastrointestinal symptoms	6 (18)
Myalgia/joint pain	6 (18)
Thoracic pain	6 (18)
Weight loss	6 (18)
Painful urination	5 (15)
Headache	3 (9)
Dysarthria, diminished consciousness, ataxia or double vision	3 (9)
Days from onset of symptoms to presentation - median [range]	8 [1–168]
Hospital admission	29 (88)
Foci of infection	
Pneumonia	19 (58)
Genitourinary infection[e]	6 (18)
Skin and soft-tissue infection	6 (18)
Intra-abdominal abscess (liver, spleen, kidney)	5 (15)
Lymphadenitis	4 (12)
Brain infection (abscess, meningitis, encephalitis)	3 (9)
Other[f]	6 (18)
Bacteremia	17 (52)
Sepsis and/or septic shock	12 (36)
Treatment[g]	
Intervention (e.g. drainage of abscess, bronchoscopy)	21 (64)
Days of antibiotic treatment total – median [range]	99 [26–308]
Days of antibiotic treatment IV - median [range]	18 [10–266]
Days of antibiotic treatment oral - median [range]	84 [14–252]
Outcome	
Days of hospital stay – median [range][h]	21 [1–335]
ICU admission	12 (36)
Days of ICU admission	4 [1–24]
Post-infectious sequelae[i]	10 (30)
Repeat episodes of infection	5 (15)
Mortality	4 (12)

[a] Patients could have multiple risk factors, exposures, presenting symptoms and foci of infection. For patients who had repeat episodes of infection all variables were summed. For a complete overview see Supplementary Table S2.
[b] One patient used both methotrexate and prednisone.
[c] Environmental exposure pertains water and soil exposure.
[d] Two patients had environmental exposure and traumatic injury.
[e] Genitourinary infection contained three patients with prostatitis (9%).
[f] Other foci of infection in patients were bone marrow and adrenal glands (n = 1), bilateral otitis externa (n = 1), mycotic aneurysm (n = 1), polyarthritis (n = 1), and the thyroid gland (n = 1).
[g] Initial intensive intravenous therapy consisted of ceftazidime (n = 22), meropenem (n = 9) or imipenem (n = 1) followed by subsequent eradication therapy with oral amoxicillin/clavulanic acid (n = 8), TMP-SMX (n = 21), and/or doxycycline (n = 8) to prevent recrudescence or relapse of the disease.
[h] One patient was admitted 60 days in the hospital and 275 days in a rehabilitation centre.
[i] Several patients developed severe post-infectious sequelae, including acute kidney failure requiring dialysis, intravenous catheter-associated candidemia, critical illness neuropathy requiring prolonged rehabilitation, and purpura fulminans following severe necrosis of fingers and toes requiring amputation and prolonged rehabilitation (all n = 1).

Table 2
Country of acquisition of melioidosis by 33 returned travelers.

Country of acquisition*	No. (%)
Thailand	17 (52)
Brazil (Ceara state)[a]	2 (6)
Australia (North and South)	1 (3)
Cambodia	1 (3)
The Gambia	1 (3)
India (Goa)	1 (3)
Indonesia	1 (3)
Myanmar	1 (3)
Panama	1 (3)
Vietnam	1 (3)
Multiple countries visited on trip[b]	6 (18)

For a complete overview see Supplementary Table S2.
[a] One patient, earlier reported in the literature [19], acquired the infection two days after an eight-day travel in Brazil (Ceara state), fifteen years before the patient traveled to Vietnam.
[b] China, Indonesia, Malaysia, Nepal, Thailand, Vietnam.

melioidosis acquisition cannot be determined. Of interest, a 63-year old male patient developed the disease two weeks after a trip to The Gambia which is the second known case from this country (case no. 12 Supplementary Table S2) [17,18]. Another patient, an 83-year old female, developed clinical evidence of melioidosis two years after a trip to Panama (case no. 29 Supplementary Table S2). Eighteen patients reported contact with soil or water e.g. one patient showered with unchlorinated water (case no. 6 Supplementary Table S2). In addition, two of those eighteen patients reported traumatic injuries; one patient exhibited hematomas after a collapse and another suffered from a distal radius fracture after a motorcycle accident. Two patients reported leech bites before the occurrence of disease symptoms. For full details see Supplementary Table S2.

The time between onset of symptoms and presentation to the hospital ranged from 1 to 168 days (median 8). Most patients exhibited fever (n = 20), cough/dyspnea (n = 15), and malaise/fatigue (n = 13). Thoracic pain or weight loss was reported in six patients. In total, 29 patients (88%) were admitted to the hospital. Pneumonia, skin and soft-tissue infection (SSTI), and genitourinary infections (including prostatitis) were the most common manifestations of disease occurring in respectively 58% (n = 19), 18% (n = 6), and 18% (n = 6) of travelers. Interestingly, several patients had notable isolated infections; one with a prostate abscess, two patients with lymphadenitis, and one patient with an abscess of the chest wall (respectively case no 7, 10, 13, 21 Supplementary Table S2).

Infection was often multi-focal. Twelve (36%) of the patients presented with or progressed to sepsis and/or septic shock (Table 1, Fig. 2). Rare disease presentations included melioidosis of the ear, thyroid gland, adrenal gland, encephalitis and meningitis, and mycotic aneurysm (respectively case no. 6, 33, 23, 31 and 30 Supplementary Table S2).

Fig. 3 provides characteristic computed tomography (CT), positron emission tomography (PET-CT), and magnetic resonance imaging (MRI) images, as well as a chest X-ray and an angiography of different foci of infection in selected patients with melioidosis.

3.3. Microbiology

All 33 identified patients had a positive *B. pseudomallei* culture. Specimens most frequently positive were blood (n = 17, 52%) and sputum (n = 14, 42%; Table 3). *B. pseudomallei* was also isolated from

Fig. 2. Clinical presentation of returned travelers with melioidosis. Pneumonia (n = 19, 58%); Genitourinary infection (n = 6, 18%)[a], Skin and soft-tissue infection (n = 6, 18%), Intra-abdominal abscess (liver, spleen, kidney) (n = 5, 15%), Lymphadenitis (n = 4, 12%), Brain infection (abscess, meningitis, encephalitis) (n = 3, 19%), polyarthritis (n = 1, 3%), Other (n = 5, 15%)[b], Sepsis and/or septic shock (n = 12, 36%)

[a] Prostate biopsy (n = 3) and urine culture (n = 4). Two patients had both a positive prostate biopsy and a positive urine culture.

[b] Other foci of infection in patients were bone marrow and adrenal glands, bilateral otitis externa, mycotic aneurysm, and the thyroid gland (all n = 1). Please refer to Supplementary Table S2 for details of cases.

bronchoalveolar lavage fluid (BALF; n = 7), genitourinary samples including prostate biopsies (n = 6), SSTI derived pus (n = 6), lymph node aspirates or biopsies (n = 4), intra-abdominal abscesses (n = 2), cerebrospinal fluid (CSF) (n = 1), ear swab (n = 1), pleural fluid (n = 1), and biopsies from the thyroid gland (n = 1).

Thirteen cases were confirmed with 16S rDNA sequencing in combination with classical phenotypical tests, (including API20 NE) and fatty acid analysis [15]. After 2003, species specific PCRs [15] were added (n = 12). Two cases before 1996 were only confirmed by classical phenotypical tests (see Table 3 and Supplementary Table S2 for details). *B. pseudomallei* is generally resistant to gentamicin and colistin (polymyxin E) and susceptible to amoxicillin/clavulanic acid [5]. All isolates followed these antibiotic patterns except five which showed resistance to amoxicillin/clavulanic-acid (n = 3, case no. 3, 19 and 27 Supplementary Table S2) and trimethoprim/sulfamethoxazole (TMP-SMX, n = 2, case no. 2 and 17 Supplementary Table S2).

3.4. Treatment and hospitalization

Initial intensive intravenous therapy consisted of ceftazidime (n = 22), meropenem (n = 9) or imipenem (n = 1) followed by subsequent eradication therapy with oral amoxicillin/clavulanic acid (n = 8), TMP-SMX (n = 21) and/or doxycycline (n = 8) to prevent recrudescence or relapse of the disease. Median length of antibiotic therapy was 99 days (26–308), divided by a first period of 18 days (10–266) of intravenous treatment followed by 84 days (14–252) of oral treatment (Table 1). Of note, three patients were not empirically treated with melioidosis-covering antibiotics as initial regimen on presentation, as these patients died between 2 and 6 days after admission before melioidosis was diagnosed.

Seven patients experienced severe adverse effects of antimicrobial agents (TMP-SMX (n = 5, respectively case no. 7, 13, 16, 18, and 19 Supplementary Table S2) doxycycline (n = 1, case no. 22 Supplementary Table S2), imipenem and meropenem (n = 1, case no. 31 Supplementary Table S2)) that warranted using an agent from another antibiotic class. These side effects included severe skin rash, ataxia, leucopenia/thrombocytopenia, proteinuria, and impaired liver function (see Supplementary Table S2). The median length of hospital stay was 21 days (1–335). Twelve patients required intensive care admission (median 4, 1–24 days) due to multi-organ failure including need for vasopressors and mechanical ventilation.

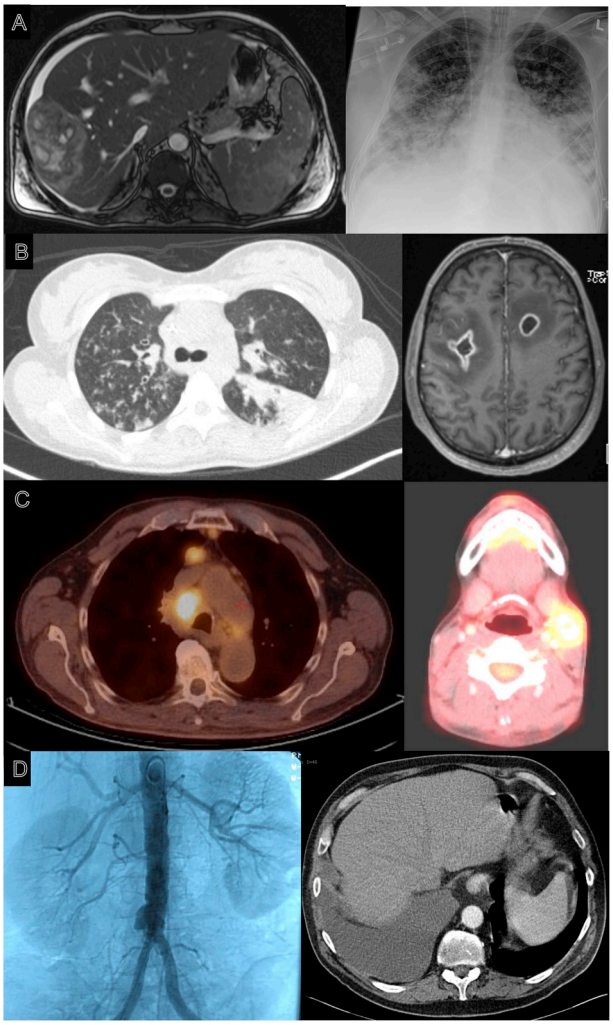

Fig. 3. Radiological manifestations of melioidosis in returned travelers. A (left): Characteristic magnetic resonance imaging (MRI) image of a 46-year old male showing an area of partly hyper-intense signal intensity in the liver suspected for a liver abscess (case no. 14). **A (right):** Chest X-ray of the same 46-year old patient showing extensive bilateral consolidations suspect of bilateral pneumonia. **B (left):** Computed tomography (CT) scan of a 21-year old female with cystic fibrosis showing multiple pulmonary consolidations suspect of lobar pneumonia and bronchial tree-in-bud opacities (case no. 11). **B (right):** Characteristic MRI image of a 49-year old male depicting two brain abscesses with edema (case no. 9). **C (left):** ^{18}F-fluorodeoxyglucose (18F-FDG) positron emission tomography-CT (PET-CT) scan of a 69-year old male; note the mediastinal/hilar lymphadenopathy (images of first episode; case no. 3). **C (right):** PET-CT image of a 30-year old female revealing intense uptake in the neck on the left side suspected for a neck abscess with a “cold” area, highly suspicious for central necrosis (case no. 13). **D (left):** Aortic angiography of a 61-year old male showing a mycotic aneurysm of the distal abdominal aorta (case no. 30). **D (right):** Characteristic CT scan image with intravenous contrast of a 57-year old male showing extensive pleural fluid on the right side (case no. 19). See Supplementary Table S2 for details of cases.

Table 3
Microbiology: identification of *Burkholderia pseudomallei* in 33 returned travelers.

Culture[a]	No (%)	
Positive identification by culture[b]	33 (100)	
Blood	17 (52)	
Sputum	14 (42)	
BALF	7 (21)	
Genitourinary[c]	6 (18)	
Skin and soft-tissue derived pus	6 (18)	
Lymph node aspirate or biopsy	4 (12)	
Intra-abdominal abscess[d]	2 (6)	
Other[e]	6 (18)	
Identification methodology by culture[f]	33 (100)	Confirmed by RIVM[g]
Methodology not specified	2 (6)	0
API20 NE	10 (30)	15 (45)[h]
VITEK/Phoenix	8 (24)	4 (12)
MALDI-TOF *B. pseudomallei* in database	9 (27)	4 (12)
MALDI-TOF *B. pseudomallei* not in database	5 (15)	2 (6)[i]
16S rDNA sequencing	8 (24)	13 (39)
Species specific PCR on isolate	3 (9)	12 (36)
Other	0	14 (42)[j]
Identification methodology non-culture	4 (12)	0
Serology (ELISA)	2 (6)[k]	1 (3)
Specific PCR on patient tissue (relapse case)	2 (6)	0

Abbreviations: BALF = bronchoalveolar lavage fluid, ELISA = enzyme-linked immunosorbent assay, MALDI-TOF = matrix assisted laser desorption/ionization time-of-flight, RIVM = Centre for Infectious Disease Control, National Institute for Public Health and the Environment (RIVM), Bilthoven, The Netherlands.

[a] Patients could have multiple positive cultures from different sites. For a complete overview see Supplementary Table S2.

[b] One patient had a positive culture in Thailand during travels and had a repeat episode of infection in The Netherlands.

[c] Prostate biopsy (n = 3) and urine culture (n = 4). Two patients had both a positive prostate biopsy and a positive urine culture.

[d] One patient had intra-abdominal abscesses in liver, spleen and kidneys.

[e] Other locations included cerebro-spinal fluid, ear swab, pleural fluid, bone marrow biopsy, adrenal gland biopsy and thyroid gland biopsy (all n = 1).

[f] Identification was based on a single method in six cases.

[g] The RIVM confirmed in total 15 strains by making use of multiple phenotypical tests, fatty acid analysis, 16S rDNA sequence analysis, matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF), and PCR based species-specific test [15].

[h] Phenotypical tests included API20 NE (n = 15).

[i] Two confirmed by a second method [15].

[j] Other Identification methods included fatty acid analysis (n = 12), and antiserum agglutination (n = 2).

[k] Culture confirmed by RIVM (n = 1), culture identification by API20 NE not confirmed by RIVM (n = 1).

3.5. Outcome

Overall mortality in this cohort of returning travelers with melioidosis was 12% (n = 4); these patients all suffered from therapy refractory sepsis and died within 4–112 days (median 10) after the start of symptoms and between 2 and 26 days (median 2) after hospital admission. Several patients developed severe post-infectious sequelae, including acute kidney failure requiring dialysis, intravenous catheter-associated candidemia, critical illness neuropathy requiring prolonged rehabilitation, and purpura fulminans leading to severe necrosis of fingers and toes requiring amputation and prolonged rehabilitation.

Repeat episodes of active melioidosis infection were observed in five patients (15%). One was possibly due to reinfection as this patient traveled again to Thailand, the country where he had contacted the disease just four months before (case no. 30 Supplementary Table S2). The other four cases were due to relapse as no recurrent travel was reported. Most repeat of active melioidosis infection occurred within the first year after primary disease display with a median duration of 252 days (28–465).

Three patients underwent autopsy. Fig. 4A–C shows lung, spleen, liver and bone marrow tissues obtained through autopsy of a 73-year old female who died after cardiopulmonary resuscitation after seven days of illness caused by bilateral pneumonia, which was complicated by septic shock and respiratory insufficiency requiring intubation and mechanical ventilation (case no. 23 Supplementary Table S2). Autopsy findings included severe bronchopneumonia with extensive tissue necrosis in both lungs (Fig. 4A), accompanied by metastatic (micro)abscesses in the spleen (Fig. 4B left), liver (Fig. 4B right), bone marrow (Fig. 4C left), and adrenal glands (not shown). Post-mortem lung tissue cultures were positive for *B. pseudomallei*.

Several cases stand out because of their uniqueness or illustrative history; some of these are highlighted in Textbox 1 (see Supplementary Table S2).

4. Discussion

This retrospective surveillance study provides a systematic overview of culture confirmed imported melioidosis cases in a non-endemic country for a 25-year period. In total, 33 cases were identified, although this number is probably an underreporting. Clinicians may not be aware of melioidosis, particularly, if first seen in non-specialized units after a long latency period and not clearly connected to previous travels. Moreover, disease manifestations are often nonspecific, especially at an early stage. This is underscored by our finding that three patients died before the correct diagnosis was made and thus before the start of administration of correct antibiotic regimens. Overall the burden of melioidosis in terms of mortality and morbidity of individual patients affected was substantial, with a case fatality rate of 12%. Moreover, in 30% of the cases, both short- and long-term sequelae were observed ranging from renal replacement therapy requiring dialysis to long-term rehabilitation due to amputation of fingers and toes.

In this study, 70% of the patients had one or multiple risk factors for melioidosis, such as diabetes and cystic fibrosis. This corresponds with changing traveler patterns wherein individuals with such risk factors are often able to travel (longer) and to more adventurous holiday destinations because of improved clinical management [2]. For example, the life expectancy of patients with cystic fibrosis is rising and due to an aging population more senior tourists are traveling. None of the travelers in this study were HIV-1 positive, which is in line with the notion that HIV is not a risk factor for the acquisition of melioidosis [5]. Additionally, excessive alcohol consumption (9%) and smoking (21%) were reported. Smoking has been earlier identified by some as an independent risk factor for melioidosis [20] and could potentially impair the host response to invading pathogens and enhance the risk of infection by inhalation [13]. Thirty percent of patients were not known or diagnosed with a risk factor, indicating that healthy people can also develop a severe *B. pseudomallei* infection, which is in line with earlier reports in both autochthonous cases and travelers [9,21, 22].

Our study further underscores the ability of *B. pseudomallei* to cause infection in virtually any organ. To our knowledge, we are the first to describe an adult patient with a bilateral otitis externa caused by *B. pseudomallei*. Additionally, *B. pseudomallei* infection was identified post-mortem amongst other sites in the adrenal glands and the bone marrow (case no. 23 Supplementary Table S2) and in the thyroid gland (case no. 33 Supplementary Table S2). Infections of these organs are rarely reported [10,23–25] and no clinical findings were reported to suggest involvement of the adrenal and thyroid gland in those patients.

The number of travelers to melioidosis endemic areas is substantially increasing. Half of the patients in our study had traveled to Thailand, resonating with Thailand being a popular travel destination welcoming 236,300 Dutch tourists in the year [26]. It remains challenging however to determine if the relative high number of melioidosis cases associated with travel to Thailand is a reflection of a high risk in that country or simply the number of people who holiday there. Moreover, we identified patients who traveled to Australia, Brazil [19],

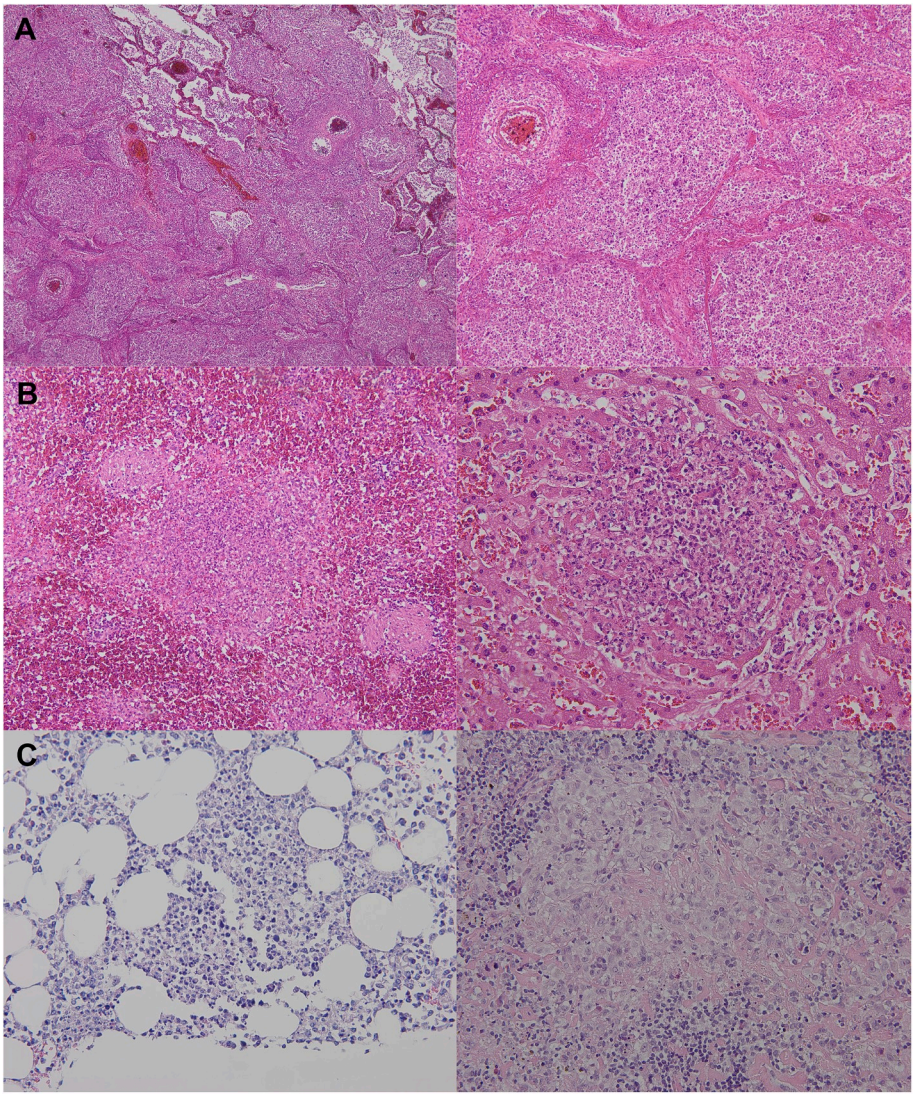

Fig. 4. A selection of histopathological findings of returned travelers with melioidosis. A-C: Representative photomicrophotographs (all H&E stainings) of autopsy specimens from a 73-year old female patient (case no. 23). **A (left):** Lung tissue sample (left lower lobe; original magnification ×4) showing alveolar parenchyma with signs of severe suppurative bronchopneumonia. **A (right):** Higher magnification (×10) of the same sample, showing damaged pulmonary architecture due to necrosis of alveolar septa. **B (left):** Spleen tissue sample (original magnification ×10) with presence of metastatic micro abscess and necrosis. **B (right):** Liver tissue sample (original magnification ×10) with presence of intra parenchymatous metastatic micro abscess. **C (left):** Bone marrow tissue sample (original magnification ×20) with presence of inflammation. **C (right):** Lymphadenectomy specimen ((original magnification ×20) of the 69-year old male patient presented in Textbox 1 (case no. 3). Image shows lymph node tissue with extensive granulomatous inflammation with areas of confluent tissue necrosis. See Supplementary Table S2 for details of cases.

Textbox 1
Two case vignettes

A 55-year old male (case no. 6 Supplementary Table S2) presented with a one-month history of fatigue, subfebrile temperatures not exceeding 38°, and bilateral ear pain after traveling to Thailand (case no. 6 Supplementary Table S2). He was diagnosed with a bilateral otitis externa. His medical history revealed bilateral otosclerosis and an ear bone chain prosthesis on the right side. Bilateral ear swab cultures grew *B. pseudomallei,* which was confirmed by PCR. CT scan showed no signs of mastoiditis nor any foci in other body parts but did show wall thickening of the external ear canals. Daily showers with unchlorinated water on the countryside in Thailand might have been the source of infection. The patient was treated on an outpatient basis and fully recovered on ten days of meropenem via outpatient parenteral antibiotic therapy followed by three months of oral TMP-SMX.

Another patient, a 69-year old male (case no. 3 Supplementary Table S2) with an unremarkable medical history, was admitted to the hospital with complaints of coughing, sub-febrile temperatures not exceeding 38°, and 12 kg weight loss after traveling to Thailand seven months earlier. A PET-CT showed an extensive confluent and abscess like mediastinal lymphadenopathy (Fig. 3C left). Biopsies taken during mediastinoscopy showed lymph node tissue with extensive granulomatous inflammation with areas of confluent tissue necrosis (Fig. 4C right). Culture of the pus yielded Gram-negative rods that were initially misidentified as *B. thailandensis* by MALDI-TOF because the Security Database was not used. PCR, however, confirmed the microorganism to be *B. pseudomallei.* He was treated with ceftazidime for two weeks followed by ten weeks of TMP-SMX. Soon thereafter, the CRP value increased again, and PET/CT-imaging still showed 18F-fludeoxyglucose (FDG) avid mediastinal lymphadenopathy, despite the patient being asymptomatic. At this time, treatment consisted of meropenem for six weeks followed by six months of TMP-SMX. Four months after completion of this second prolonged course of antibiotics the CRP levels rose again; meanwhile the patient remained asymptomatic. Consequently, a transbronchial ultrasound-guided fine-needle aspiration was performed. The cytology specimen again showed signs of granulomatous inflammation. Whilst cultures remained negative, PCR was positive for *B. pseudomallei*. CRP levels normalized spontaneously and it was decided to not treat the patient again but adopt a wait and see policy instead. Currently, 1.5 years after the second antibiotic course, the patient is in good health.

A complete description of all cases is provided in Supplementary Table S2.

The Gambia [17], Indonesia, India [27], Myanmar, and Panama. Melioidosis can remain latent up to almost three decades [5] and therefore relevant travel may have occurred before most recent travel. Recent studies in sub-Saharan Africa [28,29] and Latin America [30], including modeling studies [8,31], indicated that melioidosis is more widespread across the tropics than previously thought. This illustrates that returning travelers with melioidosis serve as sentinels for detecting the emergence of this disease in tropical countries not yet considered as endemic and, more importantly, for understanding the global movement of melioidosis across borders [2]. It should be noted that cross-border movement is not a new phenomenon, as was shown by a recent sequencing study indicating that *B. pseudomallei* was already being spread via trade routes from Australia via Africa to (South) America in the 17th-19th century [32]. The only well documented example of transmission of melioidosis to a temperate climate occurred in the mid-1970s in France. An unexpected outbreak of melioidosis in a zoo in Paris resulted in the spread to other zoos and equestrians clubs. A number of animals were culled and at least two humans succumbed because of melioidosis [33].

Our study has several strengths. Multiple literature studies of melioidosis in travelers have been published [21,22,34,35]; however, to our knowledge this is the first systematic retrospective surveillance study in a non-endemic melioidosis country. This is reflected in the number of cases presented. A recent literature review of melioidosis cases imported into Europe included 77 patients [34], yet with only four Dutch cases [34]. Existing surveillance networks such as GeoSentinel and EuroTravNet are powerful tools to gain insights into travel-related diseases [36]. However, with respect to melioidosis, only 21 cases have been identified within the whole of Europe by the EuroTravNet in the period 1998–2018 [Grobusch MP. Personal communication EuroTravNet. 15 January 2019]. EuroTravNet includes traveler's information only derived from institutions whose clinics (often specialized academic centers) are already linked to the network. We believe our study can potentially contribute to the further improvement and continuous expansion of such networks. Moreover, in most countries, no advice on melioidosis is given to travelers with specific risk factors who are going to endemic areas [3,4]. Our study can contribute to the guidance of clinicians in the provision of such advice. The first evidence-based guidelines developed in Thailand propose that protection is required against inhalation, percutaneous inoculation, and contaminated food or water [13]. The Center for Disease Control in The Northern Territory of Australia has developed specific guidelines and advice regarding prevention measures, for example, avoiding swimming in fresh water during the rainy season in endemic areas for various risk groups (diabetes, excessive alcohol consumption, chronic lung, liver, and kidney disease) [14]. In addition, the Central Public Health Laboratory in London, United Kingdom, has formulated a melioidosis specific advice targeted at cystic fibrosis patients to avoid rural areas in endemic countries, especially during the rainy season and severe weather events [37]. Nonetheless, given the extent of global travel, the chance for any individual traveler to acquire melioidosis remains relatively small.

Several limitations to our efforts should be mentioned. Complete data capture was challenging because of the retrospective nature of our study. Furthermore, caution needs to be exercised in interpreting the rise in numbers over the years as long as denominators remain indeterminable, and the influence of factors improving the awareness of melioidosis remain unrecognized. There is a chance that melioidosis cases might have been missed as seven laboratories were unable to retrieve information before 2007. Moreover, as culture remains the gold standard to identify *B. pseudomallei* infection and has an estimated sensitivity of ~60% [5] melioidosis cases might have been passed undetected. Due to the extended study period, not all laboratory identification methods were standardized. Of interest, some bacterial isolates showed resistance against amoxicillin/clavulanic acid and TMP-SMX. However, results should be interpreted with caution as the European Committee on Antimicrobial Susceptibility Testing (EUCAST) was only founded in 1997 and at the moment, no EUCAST standards for the assessment of *B. pseudomallei* resistance to recommended antibiotic therapies have been developed [38]. The same holds true for the Clinical and Laboratory Standards Institute (CLSI) in the US. Acquired resistance to antibiotics is rare [39], indicating that the resistance rate we report in our study is likely an over estimation. More studies on *B. pseudomallei* antibiotic resistance together with the development of specific interpretive criteria are needed. Additionally, repeat episodes of active infection were observed in 15% of cases. Unfortunately, no sequence typing of bacterial isolates to enable distinction between relapse and reinfection was performed.

5. Conclusions

In conclusion, imported melioidosis is likely to increase in the light of rising numbers of travelers with a chronic condition (or who are otherwise immunocompromised), improvements in diagnostics, and increased vigilance towards the condition. This first systematic retrospective surveillance study in a non-endemic melioidosis country shows that imported cases can serve as sentinels to provide information about disease activity in areas visited and inform pre-travel advice and post-travel clinical management.

Funding

This work was supported by a Research Grant (2018) from the European Society of Clinical Microbiology and Infectious Diseases (ESCMID) and the Academic Medical Center PhD scholarship award [grant number: 22162] to EB; WJW received financial support through a VIDI-grant of The Netherlands Organization for Scientific Research [NWO; VIDI grant number: 9171647].

Authors' contributions

Designed the study: EB, DN and WJW. Executed the survey: EB supported by JS. Data collection: members of the melioidosis study group. Prepared and analyzed the data: EB, JS, ALC, JTHR, SK. Interpreted the results: EB, JS, FR, DN, MPG, WJW. Wrote the first draft: EB supported by ALC. Supervised the whole process: WJW. Reviewed, commented and approved the final manuscript: all authors.

Acknowledgments

We thank all clinicians and microbiology laboratories for responding and supplying us with clinical information, Abe Goedhart for drawing Fig. 2, and all patients.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.tmaid.2019.07.017.

Conflicts of interest

The authors state no conflict of interest.

References

[1] UNTWO tourism highlights 2018 edition Available at: https://www.e-unwto.org/doi/pdf/10.18111/9789284419876, Accessed date: 2 January 2019.
[2] Thwaites GE, Day NP. Approach to fever in the returning traveler. N Engl J Med 2017;376(6):548–60https://doi.org/10.1056/NEJMra1508435.
[3] van Aalst M, van Ruissen MCE, Verhoeven R, de Bree GJ, Goorhuis A, Grobusch MP. Travel-related health problems in the immunocompromised traveller: an exploratory study. Trav Med Infect Dis 2018;25:50–7https://doi.org/10.1016/j.tmaid.2018.05.005.

[4] Wieten RW, Leenstra T, Goorhuis A, van Vugt M, Grobusch MP. Health risks of travelers with medical conditions–a retrospective analysis. J Travel Med 2012;19(2):104–10https://doi.org/10.1111/j.1708-8305.2011.00594.x.

[5] Wiersinga WJ, Virk HS, Torres AG, et al. Melioidosis. Nat Rev Dis Primers 2018;4:17107https://doi.org/10.1038/nrdp.2017.107.

[6] Currie BJ. Melioidosis: evolving concepts in epidemiology, pathogenesis, and treatment. Semin Respir Crit Care Med 2015;36(1):111–25https://doi.org/10.1055/s-0034-1398389.

[7] Wiersinga WJ, Currie BJ, Peacock SJ. Melioidosis. N Engl J Med 2012;367(11):1035–44https://doi.org/10.1056/NEJMra1204699.

[8] Limmathurotsakul D, Golding N, Dance DA, et al. Predicted global distribution of Burkholderia pseudomallei and burden of melioidosis. Nat Microbiol 2016;1(1) https://doi.org/10.1038/nmicrobiol.2015.8.

[9] Currie BJ, Ward L, Cheng AC. The epidemiology and clinical spectrum of melioidosis: 540 cases from the 20 year Darwin prospective study. PLoS Neglected Trop Dis 2010;4(11):e900https://doi.org/10.1371/journal.pntd.0000900.

[10] Currie BJ, Jacups SP, Cheng AC, et al. Melioidosis epidemiology and risk factors from a prospective whole-population study in northern Australia. Trop Med Int Health 2004;9(11):1167–74https://doi.org/10.1111/j.1365-3156.2004.01328.x.

[11] Dance DA, Smith MD, Aucken HM, Pitt TL. Imported melioidosis in england and wales. Lancet 1999;353(9148):208https://doi.org/10.1016/S0140-6736(05)77217-2.

[12] Titball RW, Burtnick MN, Bancroft GJ, Brett P. Burkholderia pseudomallei and Burkholderia mallei vaccines: are we close to clinical trials? Vaccine 2017;35(44):5981–9https://doi.org/10.1016/j.vaccine.2017.03.022.

[13] Limmathurotsakul D, Kanoksil M, Wuthiekanun V, et al. Activities of daily living associated with acquisition of melioidosis in northeast Thailand: a matched case-control study. PLoS Neglected Trop Dis 2013;7(2):e2072https://doi.org/10.1371/journal.pntd.0002072.

[14] Northern territoroy government. Centre for disease control Availbable at: http://www.melioidosis.info/download/melioidosis_factsheet_2016.pdf, Accessed date: 4 January 2019.

[15] van den Beld MJ, Reinders E, Notermans DW, Reubsaet FA. Possible misidentification of species in the Pseudomonas fluorescens lineage as Burkholderia pseudomallei and Francisella tularensis, and emended descriptions of Pseudomonas brenneri,Pseudomonas gessardii and Pseudomonas proteolytica. Int J Syst Evol Microbiol 2016;66(9):3420–5https://doi.org/10.1099/ijsem.0.001206.

[16] Human tissue and medical research: code of conduct for responsible use Available at: (https://www.federa.org/sites/default/files/digital_version_first_part_code_of_conduct_in_uk_2011_12092012.pdf 2011, Accessed date: 2 January 2019.

[17] Morelli F, Smeets L, Hobijn M, Boom H. Melioidosis and renal failure in a Dutch man after a trip to Gambia. Neth J Med 2015;73(6):296–8.

[18] Wall RA, Mabey DC, Corrah PT, Peters L. A case of melioidosis in West Africa. J Infect Dis 1985;152(2):424–5https://doi.org/10.1093/infdis/152.2.424a.

[19] Aardema H, Luijnenburg EM, Salm EF, Bijlmer HA, Visser CE, Van't Wout JW. Changing epidemiology of melioidosis? A case of acute pulmonary melioidosis with fatal outcome imported from Brazil. Epidemiol Infect 2005;133(5):871–5https://doi.org/10.1017/S0950268805004103.

[20] Meumann EM, Cheng AC, Ward L, Currie BJ. Clinical features and epidemiology of melioidosis pneumonia: results from a 21-year study and review of the literature. Clin Infect Dis 2012;54(3):362–9https://doi.org/10.1093/cid/cir808.

[21] Dan M. Melioidosis in travelers: review of the literature. J Travel Med 2015;22(6):410–4https://doi.org/10.1111/jtm.12236.

[22] Saidani N, Griffiths K, Million M, et al. Melioidosis as a travel-associated infection: case report and review of the literature. Trav Med Infect Dis 2015;13(5):367–81https://doi.org/10.1016/j.tmaid.2015.08.007.

[23] Lee SC, Ling TS, Chen JC, Huang BY, Sheih WB. Melioidosis with adrenal gland abscess. Am J Trop Med Hyg 1999;61(1):34–6https://doi.org/10.4269/ajtmh.1999.61.34.

[24] Currie BJ, Fisher DA, Howard DM, et al. Endemic melioidosis in tropical northern Australia: a 10-year prospective study and review of the literature. Clin Infect Dis 2000;31(4):981–6https://doi.org/10.1086/318116.

[25] Vlieghe E, Kruy L, De Smet B, et al. Melioidosis, phnom penh, Cambodia. Emerg Infect Dis 2011;17(7):1289–92https://doi.org/10.3201/eid1707.101069.

[26] Dutch Tourist. Available at: "international tourist arrivals to Thailand (2018)". Ministry of tourism & sports. Retrieved 28 january 2019.

[27] Kraaijnbrink BVC, Rozemeijer W, Frerichs FCP, Wagenaar JFP. Uw diagnose? Tijdschrift Infect 2018;13(6):212–3.

[28] Birnie E, Wiersinga WJ, Limmathurotsakul D, Grobusch MP. Melioidosis in Africa: should we be looking more closely? Future Microbiol 2015;10(2):273–81https://doi.org/10.2217/fmb.14.113.

[29] Wiersinga WJ, Birnie E, Weehuizen TA, et al. Clinical, environmental, and serologic surveillance studies of melioidosis in Gabon, 2012-2013. Emerg Infect Dis 2015;21(1):40–7https://doi.org/10.3201/eid2101.140762.

[30] Torres AG, Montufar FE, Gee JE, et al. Melioidosis is in the Americas: a call to action for diagnosing and treating the disease. Am J Trop Med Hyg 2018;99(3):563–4https://doi.org/10.4269/ajtmh.18-0418.

[31] Birnie E, Virk HS, Savelkoel J, et al. Global burden of melioidosis in 2015: a systematic review and data synthesis. Lancet Infect Dis 2019https://doi.org/10.1016/S1473-3099(19)30157-4.

[32] Chewapreecha C, Holden MT, Vehkala M, et al. Global and regional dissemination and evolution of Burkholderia pseudomallei. Nat Microbiol 2017;2:16263https://doi.org/10.1038/nmicrobiol.2016.263.

[33] Mollaret HH. "L'affaire du jardin des plantes" ou comment la mélioïdose fit son apparition en France. Med Maladies Infect 1988;18:643–54.

[34] Le Tohic S, Montana M, Koch L, Curti C, Vanelle P. A review of melioidosis cases imported into Europe. Eur J Clin Microbiol Infect Dis 2019. https://doi.org/10.1007/s10096-019-03548-5.

[35] Leder K, Torresi J, Libman MD, et al. GeoSentinel surveillance of illness in returned travelers, 2007-2011. Ann Intern Med 2013;158(6):456–68https://doi.org/10.7326/0003-4819-158-6-201303190-00005.

[36] Wilson ME, Weld LH, Boggild A, et al. Fever in returned travelers: results from the GeoSentinel surveillance network. Clin Infect Dis 2007;44(12):1560–8https://doi.org/10.1086/518173.

[37] Melioidosis and world-wide travel, factsheet Available at: https://www.cysticfibrosis.org.uk/the-work-we-do/publications/factsheets-and.information-packs; October 2016, Accessed date: 2 January 2019.

[38] Maloney S, Engler C, Norton R. Epidemiological cut-off value of clinical isolates of Burkholderia pseudomallei from Northern Queensland to meropenem, ceftazidime, trimethoprim/sulfamethoxazole and doxycycline by the microbroth dilution method. J Glob Antimicrob Resist 2017;10:291–4https://doi.org/10.1016/j.jgar.2017.04.012.

[39] Schweizer HP. Mechanisms of antibiotic resistance in Burkholderia pseudomallei: implications for treatment of melioidosis. Future Microbiol 2012;7(12):1389–99https://doi.org/10.2217/fmb.12.116.