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Travel-related leptospirosis in the Netherlands 2009–2016: An epidemiological report and case series

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ABSTRACT

Background: Leptospirosis is a potentially fatal zoonotic disease that is prevalent in travellers. Here, we describe epidemiological and diagnostic characteristics of all returning travellers diagnosed with leptospirosis in the Netherlands between 2009 and 2016. Furthermore, we present a detailed clinical case series of all travellers with leptospirosis who presented at the Academic Medical Center (AMC) in the same period.

Method: We extracted data from the records of the Dutch Leptospirosis Reference Center (NRL) of all cases of leptospirosis in travellers in the Netherlands from 2009 to 2016. Patients who presented at the AMC were identified and clinical data were extracted from the hospital records.

Results: 224 cases of travel-related leptospirosis were included. An increase of cases was observed from 2014 onwards. The majority of cases were male (78.1%), and had travelled to South-East Asia (62.1%). Of 41 AMC cases, 53.7% were hospitalised, but most patients had a relatively mild disease course, with no fatalities. A longer delay in diagnosis and treatment initiation existed in hospitalised compared to non-hospitalised patients, suggesting a benefit of early recognition and treatment.

Conclusions: Leptospirosis was increasingly observed in returning travellers in the Netherlands, and is a diagnosis that should be considered in any returning febrile traveller.

1. Introduction

Leptospirosis is a zoonotic disease [1], caused by pathogenic *Leptospira* that are shed in the environment via the urine of host animals, such as rodents and livestock. In warm and wet conditions, they can survive for several months [2]. Transmission patterns are complex; risk factors largely encompass water-related exposures, such as flooding, heavy rain, and recreational water activities [3–5]. Other risk factors include open wounds or abrasions, animal contact, and contact with soil, for example through gardening or walking barefoot [3]. Outbreaks may become more common due to climate change [6].

Leptospirosis is responsible for over a million severe cases and 60,000 deaths worldwide [7]. These numbers are likely underestimated due vague clinical symptoms and difficult laboratory diagnostic technique. Classic severe disease (also known as Weil's disease) presents with acute renal failure, jaundice and (pulmonary) haemorrhages, but most commonly the disease presents as a mild acute febrile illness. The list of differential diagnostic considerations is long, including malaria, (arthropod borne) viral infections, rickettsial disease, and typhoid fever; these cannot be excluded on the grounds of clinical presentation only [1,8]. Early treatment is thought to prevent disease complications [8–10], and therefore establishing an early diagnosis is crucial. The present reference tests (Microscopic Agglutination Test (MAT) and culture) are cumbersome methods requiring sophisticated laboratories, and cannot provide early diagnosis. MAT is based on detection of antibodies (which appear in the blood after only 5–10 days of illness), and can determine a presumptive infecting serogroup [11]. Culturing *Leptospira* can take months [1,2]. Immunoglobulin M Enzyme-Linked Immunosorbent Assay (IgM ELISA) is more widely used but meets similar problems with establishing an early diagnosis, as it is based on antibody detection. Polymerase chain reaction (PCR) is based on DNA/RNA detection, and is therefore applicable in the first week of illness when the

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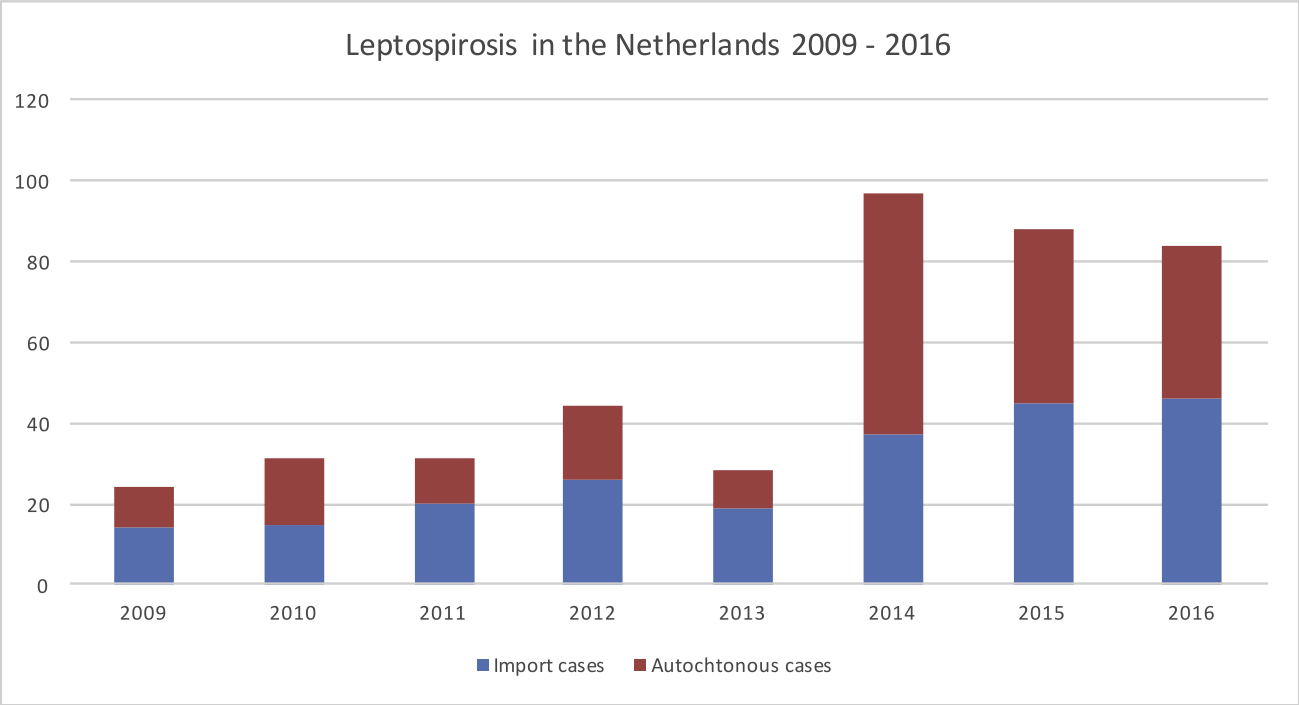

Fig. 1. Total number of patients diagnosed with leptospirosis in the Netherlands, autochthonous and imported, 1 January 2009–31 December 2016.

bacteria are circulating in the blood. There is an increase in the routine use of this method; however, diagnostic accuracy may vary among settings and laboratories [12,13].

International travel is a major independent risk factor for leptospirosis [14]. Numbers of international tourists are increasing annually [15], and tourists are increasingly visiting high-endemic regions and engaging in high-risk activities involving leisure freshwater exposure such as river rafting, canoeing, and other adventure sports [5,16]. Consequently, reported proportions of travel-related leptospirosis worldwide are increasing [14,16–18]. A recent systematic review showed an association between leptospirosis and whitewater sports, spelunking (exploring caves), and adventure races and trekking, and recommends that prophylactic doxycycline should be considered in those participating in such activities in endemic areas [5].

In 2015, there were 18.1 million holidays abroad among Dutch people (with a total population of 16.9 million in 2015) [19]. In the Netherlands, in the period from 1924 to 2008, a gradual increase of imported leptospirosis was observed, along with an increase of imported infections in general: in the period 2005–2008, 53% of all infections in the Netherlands were imported; 80% of these cases were associated with water-related activities [16]. In 2014, a four-fold increase in autochthonous leptospirosis, and a 1.6-fold increase in cases of imported leptospirosis were observed in the Netherlands [20].

Here, we describe the epidemiological and demographic characteristics of confirmed acute leptospirosis in returned travellers in the Netherlands in the period from 2009 to 2016. Furthermore, we describe detailed clinical presentations of all leptospirosis cases presenting in the Academic Medical Center (AMC) of the University of Amsterdam (UvA), the Netherlands. Finally, we demonstrate the clinical spectrum of acute leptospirosis, by detailing four cases of returned travellers who presented at the AMC.

2. Methods

Leptospirosis is a notifiable disease in the Netherlands since 1928 [21], and ~99% of cases are confirmed by the World Organisation for Animal Health and the National Collaborating Centre for Reference and Research on Leptospirosis (NRL). For definitions of cases, national guidelines are followed [22]. When clinicians and general practitioners across the Netherlands suspect leptospirosis, clinical samples are submitted to the NRL, where serology (MAT and IgM ELISA) is done for the detection of antibodies. If blood is collected before the 11th day of illness, culture is performed as well, and, from September 2012 onwards, also PCR; PCR is performed on urine in all disease stages). MAT can determine the presumptive infecting serogroup. A confirmed case of leptospirosis is defined by: a positive culture and/or PCR and/or serology (MAT or IgM ELISA) and fever or at least two of the following signs and symptoms: rigors, headache, myalgia, conjunctival injection, skin or mucosal bleeding, rash, jaundice, myocarditis, meningitis, renal failure or pulmonary haemorrhages [11,16].

Confirmed imported cases of leptospirosis diagnosed at the NRL from the 1st of January 2009 to the 31st of December 2016 were selected, as all cases of leptospirosis in the Netherlands up to 2008 have been described elsewhere [16]. Epidemiological and diagnostic data were extracted, including presumptive infecting serogroups. For all patients who had presented at the AMC, clinical data were extracted from patient files. From those, four case vignettes were selected, representing the broad spectrum of clinical presentation and disease course. Diagnostic delay was defined as the number of days between the first visit to any healthcare professional in the Netherlands, and the first request for leptospirosis diagnostics. Data were organized and analysed using Microsoft Excel (Microsoft Corporation, 2010). The vector map was created using an open source vector map (https://commons.wikimedia.org/wiki/Atlas_of_the_world), and further edited using Adobe® Illustrator® CS6 (Adobe Systems Incorporated).

2.1. Ethical issues

This study was exempted from further ethical review of human subject research by the Medical Ethical Review Committee of the Academic Medical Centre, University of Amsterdam (protocol W16_311#16.366). All data were de-identified and not attributable to individual patients. For the individuals described in the case reports, consent was obtained.

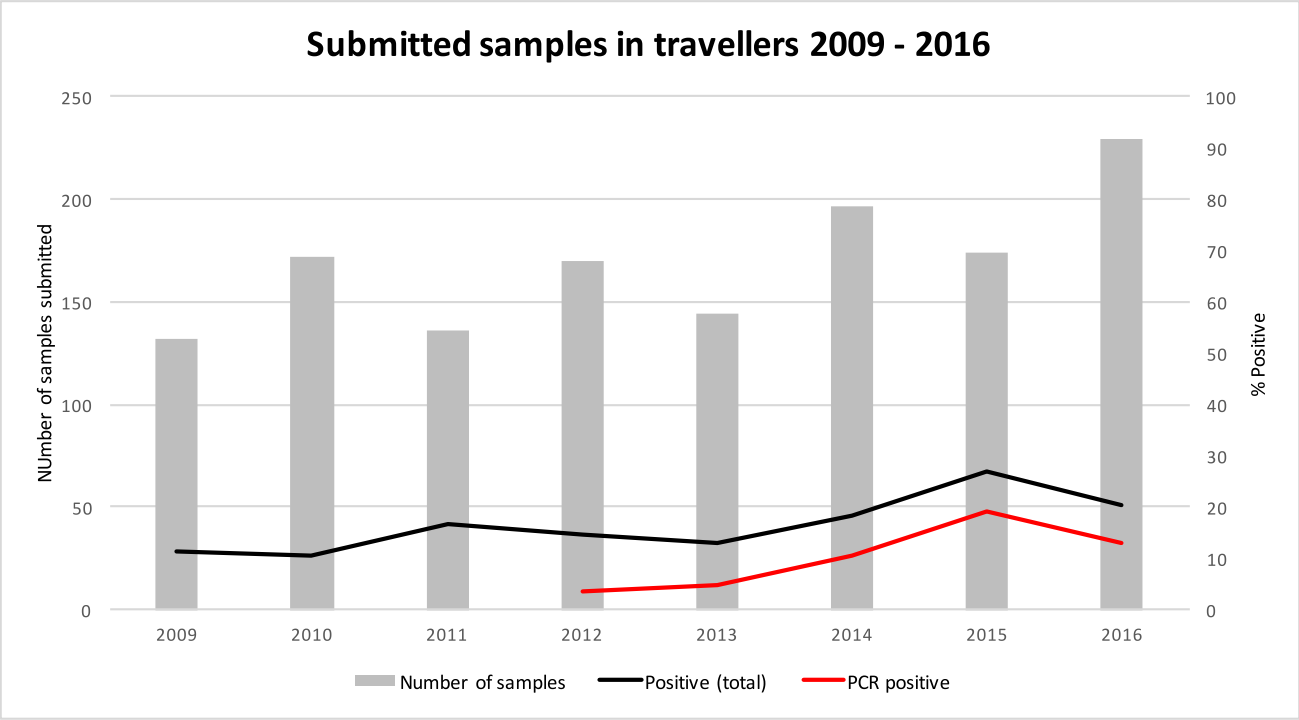

Fig. 2. Total number of samples of cases suspected for imported leptospirosis submitted from 1 January 2009–31 December 2016, and the percentage of positive samples, and PCR positive samples.

3. Results

3.1. Imported leptospirosis in the Netherlands

During 2009–2016, patient materials from 4642 Dutch patients suspected for leptospirosis were submitted to NRL for diagnostic testing, of which 428 were confirmed as leptospirosis (positivity rate 9.2%), comprising 224 (52.3%) imported cases. From 2014 onwards, an increase in the total number of confirmed cases was observed, with the proportion of imported cases remaining more or less the same (see Fig. 1). The increase of autochthonous cases in 2014 has been described elsewhere [20]. Fig. 2 displays the total number of traveller-samples submitted per year, the percentage of positive samples, and the percentage of PCR positive samples (the latter from 2012 onwards, when PCR was used routinely).

The majority of imported cases concerned male patients (175/224; 78.1%), who had mainly travelled to South-East Asia (139/224; 62.1%). Thailand was the most frequently reported country of infection (92/224; 41.1%). Exposure to fresh water was common (90/224; 40.2%) and the Sejroe/Hebdomadis/Mini complex was the most commonly found infecting serogroup (38/224; 17.0%). Characteristics of the imported leptospirosis cases are described in Table 1; exposure countries are shown in Fig. 3.

3.2. Cases of imported leptospirosis in the Academic Medical Center

From 1 January 2009 to 31 December 2016, 41 cases of leptospirosis were diagnosed and/or treated at the AMC. Details of four cases of leptospirosis meningitis, and a case of pulmonary haemorrhage have been described elsewhere [23,24]. Data of five of those 41 patients were also included in a recent GeoSentinel report on global data on leptospirosis in travellers (submitted for publication). Here, data of all 41 patients in our database from 2009 to 2016 are described.

The majority of patients presenting at the AMC was young (median age 27.8 years), male (33/41; 80.5%), and had visited South-East Asia (29/41; 70.7%) (Table 1). Thailand was the most frequently reported

Table 1
Characteristics of imported cases of leptospirosis in the Netherlands, 1 January 2009–31 December 2016.

Characteristic	Total imported cases, n = 224	AMC cases, n = 41
Male sex (%)	175 (78.1)	33 (80.5)
Median age (range)	30 (8–75)	27.8 (10–63)
Mean age (SD)	34.3 (14.4)	32.2 (11.9)
Region of exposure (%)		
South-Eastern Asia	139 (62.1)	29 (70.7)
Europe	22 (9.8)	0 (0.0)
Caribbean	19 (8.5)	3 (7.3)
Central America	18 (8.0)	3 (7.3)
South America	11 (4.9)	3 (7.3)
Southern Asia	8 (3.6)	1 (2.4)
Sub-Saharan Africa	7 (3.1)	2 (4.9)
Likely route of infection (%)		
Water	90 (40.2)	30 (73.2)
Water and animals	6 (2.7)	7 (17.1)
Animals	3 (1.3)	1 (2.4)
Unknown	125 (55.8)	3 (7.3)
Serogroup		
Sejroe-Hebdomadis-Mini Complex	38 (17.0)	8 (19.5)
Icterohaemorrhagiae	21 (9.4)	4 (9.8)
Australis	10 (4.5)	4 (9.8)
Grippotyphosa	8 (3.6)	1 (2.4)
Celledoni	6 (2.7)	5 (12.2)
Bataviae	5 (2.2)	1 (2.4)
Pyrogenes	5 (2.2)	–
Autumnalis	2 (0.9)	2 (4.9)
Canicola	2 (0.9)	–
Cynopteri	2 (0.9)	2 (4.9)
Tarassovi	1 (0.4)	2 (4.9)
Ballum	1 (0.4)	–
Javanica	1 (0.4)	1 (2.4)
Shermani	1 (0.4)	–
Not identified	121 (54.0)	9 (22.0)

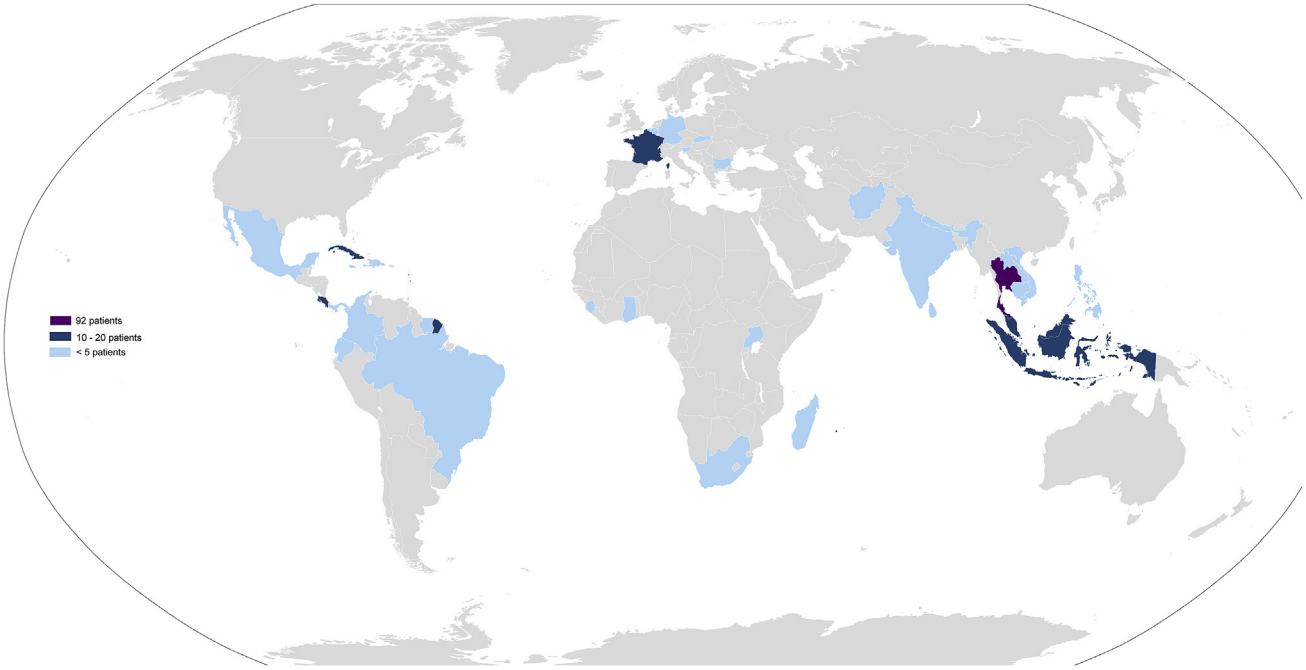

Fig. 3. Exposure countries among imported cases of confirmed leptospirosis in the Netherlands, 1 January 2009–31 December 2016 (n = 222) *†. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)
* Includes only patients for whom country of exposure data were available.
†Purple represents 92 patients exposed in Thailand. Dark blue represents countries with 10–20 exposed patients (19 for Malaysia; 16 for Indonesia; 13 for Cuba; 12 for Costa Rica; and 10 for France). Light blue represents countries with < 5 patients (4 each for Belgium, Colombia, Laos, Suriname, and Vietnam; 3 each for Germany, Panama, and Sri Lanka; 2 each for Bulgaria, Cambodia, Dominican Republic, Jamaica, Philippines, India, Nepal, South Africa; and 1 each for Afghanistan, Brazil, Ecuador, French Guiana, Ghana, Haiti, Luxembourg, Madagascar, Mauritius, Mexico, Sierra Leone, Slovakia, Slovenia, Trinidad & Tobago, and Uganda).

country of exposure (n = 19; 46.3%), followed by Indonesia (n = 5; 12.2%), Costa Rica (n = 4; 9.8%), Colombia, Laos, Malaysia (n = 2; 4.9% for all three countries), and the Dominican Republic, French Guiana, Ghana, Haiti, Sri Lanka, Trinidad and Tobago, Uganda (n = 1; 2.4% for all 7 countries). Exposure to fresh water was reported in 90.3% of all patients.

Table 2 summarizes general characteristics of the clinical disease courses of the patients. The majority of patients was hospitalised (22/41; 53.7%). One patient, with pulmonary haemorrhages [24], was admitted to the ICU; none required kidney dialysis. Of note is the observation that only one patient presented with jaundice, as part of the classic Weil's triad. The laboratory parameters of the 41 AMC patients are shown in Table 3. Renal impairment was observed in 17 (41.5%) patients, of which six (14.6%) had a glomerular filtration rate (GFR) of < 30 ml/min. Two patients presented with spontaneous bleeding, one with pulmonary haemorrhages [24] and one with rectal blood loss. Fifteen patients (36.6%) presented with low platelets.

Most patients were diagnosed by serological methods (MAT or IgM ELISA, n = 25; 61.0%). As expected, given the late occurrence of antibodies in the blood, in most of these cases the diagnosis was only made after a convalescent sample had been tested, with a median duration of illness of 12 days (range 6–41 days). PCR provided the diagnosis for 16 (39.0%) patients, all on the first submitted sample, at a median disease duration of 3 days (range 1–17 days). Nine patients (22.0%) had a positive culture, of which seven also had a positive PCR; two (4.9%) had a negative PCR result (their cultures became positive after 4 and 6 weeks of incubation, respectively).

3.3. Clinical description of cases

3.3.1. Case 1

A 37-year old previously healthy male presented to the emergency department, 12 days after his return from a two-week trip to Thailand, where he had visited Chiang Mai and the surrounding forests. He was exposed to fresh water during an elephant ride. Five days before presentation, he developed a severe headache (starting in the neck, later retro-orbital) with fever up to 40 °C, anorexia, and dark urine. The general practitioner had initially prescribed diazepam and oxycodone to treat his headache. From three days before presentation, he stopped urinating and did not pass any stools; one day later he started vomiting. On physical examination, his vital signs were normal, with modest enlargement of the liver. Laboratory investigation revealed a normal haemogram, creatinine 668 μmoL/l, C-reactive protein (CRP) 342 mg/L, and normal pH and oxygen saturation levels. Urine examination showed albuminuria and leukocyturia. A chest radiograph revealed no abnormalities; an ultrasound of the abdomen showed hepatomegaly, without signs of kidney abnormalities. Leptospirosis was clinically suspected, and he was admitted and treated with intravenous ceftriaxone and fluid resuscitation. During admission, because of the fluid resuscitation, he developed a cardiac decompensation with pulmonary oedema (without signs of pulmonary bleeding) and mild liver enzyme abnormalities. Urine production recovered rapidly, and from the fourth day after admission the creatinine levels started normalizing, and the patient was discharged 6 days after admission. The initial tests for leptospirosis (PCR, MAT and IgM ELISA) were negative, but the IgM ELISA turned positive on the 7th day of illness. Blood cultures and specific laboratory tests for malaria, dengue, chikungunya and typhoid fever were all negative.

3.3.2. Case 2

A 63-year old male, with a history of angina pectoris, for which he had a stent placed in the left anterior descending artery two years earlier, presented at the emergency department three days after returning from a 2-week trip to Malaysia, where he had been in contact with freshwater during a jungle trekking in Taman Negara National Park. He had presented to the general practitioner 11 days earlier with

Table 2
Clinical characteristics of patients with travel-related leptospirosis in the AMC.

	All (n = 41)
Male sex, n (%)	33 (80.5)
Median age (range)	27.8 (10–63)
Hospital admission, n (%)	22 (53.7)
ICU admission, n (%)	1 (2.4)
Dialysis, n (%)	0 (0.0)
Deaths, n (%)	0 (0)
Treatment with antibiotics, n (%)	
Yes	40 (97.6)
Oral	22 (53.7)
IV	2 (4.9)
IV then oral	16 (39.0)
Median number of days in the hospital, days (range)	5 (1–9)
Median time from return to presentation, days (range)	5 (0–13)
Median duration of illness at first visit to any clinic (range)	3 (0–32)
Median number of days between start of symptoms and start antibiotics (range)	4 (1–34)
Median day of illness at confirmed diagnosis (range)	8 (1–41)
Median diagnostic delay[a] (range)	1 (0–40)
Symptoms at presentation, n (%)	
Fever/sweats	41 (100.0)
Headache	39 (95.1)
Myalgia	30 (73.2)
Nausea	25 (61.0)
Arthralgia	20 (48.9)
Acute diarrhoea	18 (43.9)
Conjunctival injection	17 (41.5)
Skin rash	13 (31.7)
Vomiting	12 (29.3)
Cough	12 (29.3)
Abdominal pain	8 (19.5)
Tachycardia	7 (17.1)
Nuchal rigidity/meningism	5 (12.2)
Anuria/oliguria	4 (9.8)
Dyspnoea	4 (9.8)
Hepatosplenomegaly	4 (9.8)
Dysuria/haematuria	2 (4.9)
Spontaneous bleeding[b]	2 (4.9)
Weight loss	2 (4.9)
Lymphadenopathy	1 (2.4)
Jaundice	1 (2.4)
Petechial bleeding	1 (2.4)

ICU = Intensive Care Unit; IV = intravenous.

[a] Defined as: number of days between first visit to any clinic in the Netherlands and first leptospirosis diagnostics ordered.

[b] One case of pulmonary haemorrhage, and one case of rectal blood loss.

an itching confluent erythema rash on his arms, chest and lower legs, for which antihistaminic drugs and skin creams had been prescribed, with no effect. Two days later, he had developed a fever up to 40 °C with chills, and the next day a painful skin and arthralgia, mainly in the knees. He had nausea and vomiting, but no respiratory or urogenital complaints. On presentation at the emergency department, vital signs and physical examination were normal, besides erythema on his lower arms and lower legs. Laboratory tests revealed a thrombocytopenia ($135*10^9$/l, renal insufficiency (creatinine 285 μmoL/l) and increased liver enzymes (ASAT 58 U/l; ALAT 88 U/l; AF 120 U/l; Gamma-GT 87 U/l). Urinalysis showed proteinuria and leukocyturia. *Leptospira* DNA was detected in EDTA blood by PCR on the day of admission and intravenous ceftriaxone was started. Thick blood smear for malaria, dengue and rickettsia serology and cultures of urine and blood were negative. The fever and renal insufficiency subsided within two days after the start of antibiotics. The patient was released from the hospital and finished a seven days course of doxycycline at home. Two weeks after the initial presentation, a convalescent blood sample showed antibody titres in the MAT (highest titre 1:1280) in the Sejroe-Hebdomadis-Mini complex serogroup).

Table 3
Laboratory parameters of patients at presentation with travel-related leptospirosis in the AMC (n = 41).

Value	Number of cases (%)	Median value (range)	Reference ranges
Blood			
Elevated CRP	31 (75.6)	165 (26–368)	0–5 mg/L
Elevated creatinine	17 (41.5)	179 (111–668)	75–110 μmoL/L
Low platelets	15 (36.6)	131 (88–147)	150–400 $*10^9$/L
Elevated ALAT	15 (36.6)	88 (46–305)	0–45 U/L
Elevated ASAT	15 (36.6)	65 (43–354)	0–40 U/L
Leucocytosis	10 (24.4)	12.8 (11.0–18.9)	4–10.5$*10^9$/L
Elevated urea	9 (22.0)	9 (7.5–21.6)	2.1–7.1 mmoL/L
Elevated bilirubin	8 (19.5)	22 (18–75)	0–17 μmoL/L
Low haemoglobin	8 (19.5)	8 (7.3–8.4) (*all male*)	M 8,5–10,5/ F < 7,5–10 mmoL/L
Hypokalaemia	7 (17.1)	3.3 (3.0–3.4)	3.5–4.5 mmoL/L
Leukocytopenia	3 (7.3)	4 (0.4–4.4)	4–10.5 $*10^9$/L
Urine			
Haematuria	13 (31.7)	NR	0 - 17/μL
Proteinuria	11 (26.8)	NA	(yes/no)
Leukocyturia	3 (7.3)	NR	0 - 28/μL

CRP = C-reactive protein; ALAT = Alanine-aminotransferase; ASAT = Aspartate aminotransferase.

3.3.3. Case 3

A 27-year old male presented at the outpatient department, ten days after his return from a three-week trip to Colombia, where he had rafted and walked through mud with bare feet. Seven days before presentation, he developed fever up to 39.7 °C, with chills and arthralgia in the ankles, knees and lower back, and myalgia in the calves during the first two days of disease. Two days later, he became nauseated and vomited, and developed watery diarrhoea one day later. The next day, he developed pain in both testes. He finally presented at the AMC two days later. On physical examination, there was a conjunctival injection, and a light exanthema on the abdomen and lower back, and both testes were swollen and painful. Laboratory tests revealed no abnormalities other than an increased CRP (196 mg/L). The thick blood smear for malaria, stool and urine cultures, and serology for syphilis and mumps were all negative. As the patient started to feel better, no treatment was initiated. Two days later, at follow-up visit he had improved clinically. In the meantime, the PCR for leptospiral DNA on serum became positive. As complaints of orchitis continued, doxycyline was administered for seven days, with good result. Two weeks later, a follow-up sample showed a more than fourfold increase in MAT titre against strains in the Sejroe-Hebdomadis-Mini complex serogroup.

3.3.4. Case 4

A 31-year old male was admitted with fever, headache, nausea, and malaise. Symptoms had started the during a 2.5-week journey to Costa Rica, and at presentation, symptoms had existed for five days. In Costa Rica, there had been floods after heavy rainfall, and he had been exposed to prolonged water contact because the hotel was flooded. He presented with retro-orbital headache, photophobia, myalgia in the calves of his legs, nausea, vomiting, and rectal blood loss. Physical examination showed red, injected conjunctivae, mild jaundice, a tachycardia of 102 bpm, fever (39.9 °C), and a normal blood pressure. Laboratory investigations revealed a haemoglobin of 7.3 mmoL/l; thrombocytes of $107*10^9$/l, elevated liver enzymes (bilirubin 75 μmoL/l, ASAT 111 U/l; ALAT 104 U/l; AF 180 U/l; gamma-GT 98U/l), and a normal renal function. Leptospirosis, typhoid fever, or an arthropod borne viral infection were suspected, and the patient was admitted and treated with intravenous ceftriaxone. The PCR for *Leptospira* was positive, after which treatment was continued with oral doxycycline. The patient improved substantially, but after two days he developed a second-degree atrioventricular block, for which he was observed for

48 h in the cardiac observation unit. The atrioventricular block disappeared spontaneously, but an incomplete right bundle branch block remained, for which he still receives cardiologic follow-up. Despite initial slow resolution of general fatigue, he had recovered one month after discharge.

4. Discussion

During the time period 2009–2016, leptospirosis was increasingly observed in the Netherlands among returned travellers. In addition, a marked increase of the total number of leptospirosis cases, including autochthonous infections, was observed from 2014 onwards. The increase in autochthonous cases in 2014 was thought to be due to a warm winter, followed by the warmest year in centuries [20]. This trend continued over 2015 and 2016, possibly for the same reason. For the imported cases however, the explanation is less obvious. The number of Dutch tourists travelling abroad and their destinations have been more or less stable since 2008 [19,25]. Possibly, travellers are increasingly participating in high-risk activities, such as rafting and jungle trekking. Another explanation could be that physicians in the Netherlands have become more aware of leptospirosis, and thus request diagnostic tests more often. This is supported by the fact that the NRL has received increasing numbers of samples over the past years. Additionally, PCR was implemented in September 2012 in the NRL. PCR can identify leptospirosis cases in the early disease stages, which would have needed a convalescent sample for diagnosis in the period before September 2012, which is often not submitted to the laboratory. However, an increase of positivity rate was only observed from 2014 onwards. This increase was mainly attributable to PCR positive cases.

The majority of cases comprised relatively young males, who had travelled to Southeast Asia, consistent with other reports on leptospirosis in travellers [16,26,27]. Thailand, Malaysia, and Indonesia were the most frequently reported countries of exposure. Remarkably, Europe was the second most common region of exposure, with France contributing ten cases, and nine more cases from Belgium, Bulgaria, Luxembourg, Slovakia, and Slovenia. France is the number one destination for Dutch holidays, which could explain the relatively high number of cases. It does indicate however, that leptospirosis should be considered in all travellers presenting with a febrile illness, independent of the region they have visited, in particular also because delayed diagnosis leads to serious complications, shown in our cases.

Clinicians are usually well aware of the risk of leptospirosis when a typical exposure history is present, such as floods and contact with freshwater. In travellers, clear exposure histories have been reported [26,28], but in the general population, the mechanism of infection often remains uncertain [3]. In our data, exposure histories were not known in almost 56% of all travel-related leptospirosis cases in the Netherlands; among the patients that presented at the AMC, where leptospirosis is a frequently diagnosed travel related disease, a clear exposure history was registered in more than 90% of the patients. It is possible that the lower percentage in the national group is due to incompleteness of the data, or due to unfamiliarity with the disease among physicians who rarely encounter leptospirosis.

It is likely that the cases reported here merely represent the more severe cases, as mild cases are more likely to remain unrecognized [1,29]. It has been described that the disease presentation in Dutch imported cases is less severe than in autochthonous cases, which has been postulated to be associated with a lower number of imported infections with serovars from the Icterohaemorrhagiae serogroup, linked to severe disease [16,20]. Indeed, the most common infecting serogroup in imported cases was the Sejroe-Hebdomadis-Mini complex (17% of cases), for which milder disease courses have been described [16], whereas only around 10% of the described cases were infected with the Icterohaemorrhagiae serogroup.

We did not collect detailed clinical data of the overall group of 224 travellers with leptospirosis in the Netherlands. We do report, however, detailed data on a subset of 41 cases (18.3% of all confirmed cases of leptospirosis in the Netherlands) who were diagnosed and treated in the AMC. Of note, no single patient of those 41 succumbed to leptospirosis. The diagnostic delay in this group was generally short, with most patients being tested for leptospirosis relatively quickly (median of one day).

The AMC is a tertiary hospital with a specialized travel clinic (the Center of Tropical Medicine and Travel Medicine), and the Leptospirosis Reference Center is located at the premises building, which likely explains the relatively short delay, and possibly the relatively high caseload at the AMC. Another explanation is that there is a low threshold for consideration of the disease. Diagnostic delay most frequently occurred before presentation at the AMC. In addition, several patients had received inadequate treatments before presentation, such as very short courses of oral antibiotics.

None of the patients presented with a classic Weil's syndrome. Hospital admission was required in only 22 cases (53.7%), which is lower than in the previous report [16]. There was only one ICU admission [24], and no need for renal replacement therapy in any patient. All patients survived. Further symptoms at presentation were similar to those described in other case series [16,30], except for lower rates of jaundice.

Case #4, a young male, developed a second-degree atrioventricular block during admission, which resolved spontaneously, but a right bundle-branch block remained. Electrocardiographic alterations have been described in case series on leptospirosis, with ventricular repolarization disorders, atrial fibrillation and first-degree atrioventricular blocks most common, also in younger patients [31,32]. Different theories on the aetiology of cardiac involvement in leptospirosis have been postulated [31–33]. In severe and fatal cases of leptospirosis, myocardial involvement has been described [33]. The more commonly observed electrocardiographic abnormalities could be an effect of the leptospiraemia, or a general occurrence in febrile disease, also through metabolic and electrolytic disturbances.

Case #3 developed an orchitis four days after the acute febrile episode started. Orchitis has been described as a complication of leptospirosis in the older literature [34–38]. Most of those cases had been infected with the Ballum serogroup, contracted from laboratory [34–36] or pet mice [37], and developed the orchitis at a later stage in the disease, after about 10–20 days. Our patient was PCR positive and later developed high MAT titres against serovars from the Sejroe-Hebdomadis-Mini complex serogroup. A similar case has been described in a 25-year old dairy farm worker who presented with a fever and epididymitis, who was also found to be infected with a serovar from the Sejroe-Hebdomadis-Mini complex serogroup [38].

In the group of 41 AMC patients, the diagnosis of leptospirosis was most often obtained through serology, and for most patients, only the follow up sample was positive (median 12 days after onset of symptoms). These patients were either tested too late to perform PCR, or the PCR was negative. In confirmed cases with a negative PCR, the test was performed later in the illness compared to the confirmed cases with a positive PCR, with a median of 5 days after the onset of symptoms in PCR negatives vs. a median of 2 days in PCR positive cases. The latter suggests that the PCR is more sensitive in the earliest stages of the disease, which is in line with previous studies [12,39]. These findings show that it is important to request diagnostic testing as early as possible in the disease course; when the time-frame for PCR is missed, it can take over a week for the serology to become positive. However, a negative PCR does not rule out the disease.

It is likely that leptospirosis is often missed, because many returning travellers with an acute febrile illness are treated with antibiotics empirically. If they improve, a diagnosis is not always sought. However, because of the potentially severe disease course when diagnosis is delayed or missed, there is an urgent need for an easy-to-use and simple diagnostic test in the acute phase of the disease.

Limitations to this study are that, even though ~99% of all

leptospirosis cases in the Netherlands are diagnosed in the NRL, the data presented here may be incomplete. Clinical data were only available in the subset of patients who had presented at the AMC. Data on the serogroups were mostly based on the MAT, which can only determine a presumptive infecting serogroup [11]. Furthermore, the cases presented in detail here, were diagnosed and treated at the AMC, which is a tertiary hospital with a specialized travel clinic, which possibly resulted in selection bias; this could may implicate that the clinical picture of diagnosed leptospirosis patients the total population is not accurately reflected.

We conclude that leptospirosis is an increasing and likely underestimated cause of febrile illness and hospitalisation in returned travellers in the Netherlands. The disease has different and often surprising clinical manifestations in travellers. Mild outcome is associated with early diagnosis after the start of symptoms. Therefore, diagnostic testing should therefore be performed with a low threshold of suspicion in any febrile returning traveller.

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Conflicts of interest

The authors declare that they have no competing interests.

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