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Challenges in the diagnosis of leptospirosis outwith endemic settings: a Scottish single centre experience

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Abstract

Background Leptospirosis is a zoonotic infection occurring worldwide but endemic in tropical countries. This study describes diagnostic testing for leptospirosis at our institution in Scotland over a 10-year period.

Method We identified patients with blood samples referred to the Public Health England reference laboratory for leptospirosis testing between 2006 and 2016.

Results A total of 480 samples were sent for IgM ELISA testing with 26 positive results from 14 patients. Two patients met criteria for 'confirmed' leptospirosis (microscopic agglutination test > 1:320 in one case and a positive PCR in the other) and the remaining 12 were 'probable' on the basis of IgM ELISA positivity, though 9 did not have microscopic agglutination testing performed. Nine infections were imported, mostly from Asia and with a history of fresh water exposure. Three co-infections (respiratory syncytial virus, influenza B and *Campylobacter* sp.) were identified.

Conclusions Practical issues with microscopic agglutination testing (insufficient blood sent to reference laboratory) and PCR (travellers returning > 7 days after illness onset) represent challenges to the laboratory confirmation of a clinical diagnosis of leptospirosis. Co-infection and infectious/auto-immune causes of false positive serology should be evaluated.

Keywords: ELISA, *Leptospira interrogans*, leptospirosis, microscopic agglutination test, Weil's disease

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Background

Leptospirosis is a zoonotic infection caused by pathogenic spirochetes of the *Leptospira interrogans* complex. *L. interrogans* can establish asymptomatic chronic colonisation of the renal tubules of carrier mammals. Bacteria are then excreted in urine and can remain viable in fresh water or moist soil.[1, 2] Human infection occurs when water or soil contaminated with infected animal urine (dogs and rats are the most important reservoirs) comes into contact with mucous membranes or broken skin. Such exposure may occur in the context of recreational activities (e.g. swimming, water sports or trekking through moist conditions in jungles) or occupational exposures (e.g. sewer workers). *L. interrogans* is present worldwide but is endemic in tropical countries where favourable environmental conditions encourage transmission.[1,2]

The outcome of human infection with *L. interrogans* is heterogeneous. Most patients will experience a mild, self-limiting undifferentiated febrile illness occurring after a 1–2 week incubation period.[1,2] A smaller number of patients develop severe manifestations of infection during a second phase of the disease, encompassing hepatitis, jaundice, acute kidney injury, aseptic meningitis and haemorrhagic complications (together termed Weil's disease). Mortality associated with critical illness due to Weil's disease has been reported at 24%, increasing to 50% if there is acute lung injury due to pulmonary haemorrhage.[3,4]

Culture-based isolation of *L. interrogans* from blood or urine has low sensitivity and is technically difficult, requiring special culture medium (Ellinghausen-McCullough-Johnson-Harris medium) and incubation for up to 13 weeks with regular

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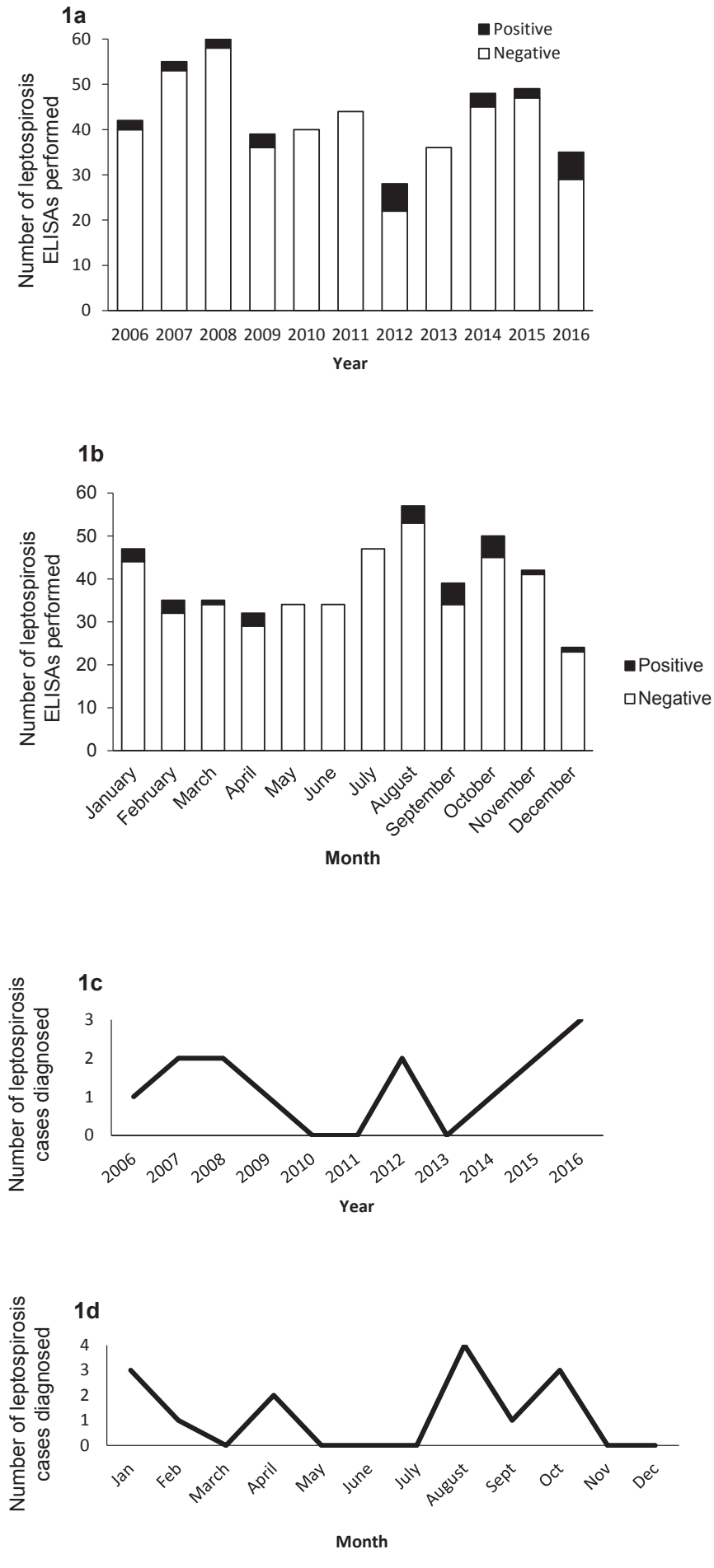

Figure 1 Temporal trends in leptospirosis testing and diagnosis

a) There was no clear temporal trend to testing across the study period, with the number of tests per year ranging from 60 (2008) to 28 (2012); b) Most tests were sent between July and November with a second peak in January; c) The number of cases diagnosed each year varied from 0–3, with the possibility of a consistent presence of, and increase in, cases from 2014 onwards; d) There were peaks in diagnosis of new cases in January, August and October.

examination by dark field microscopy. Due to poor survival of *L. interrogans* in urine, the sample should be placed in culture medium within 2 h of voiding.[5] Serological testing is the normal diagnostic modality used to confirm infection, utilising IgM ELISA and microscopic agglutination testing (MAT) on blood samples. In addition, there is an increasing role for molecular testing of blood and urine. The Public Health England (PHE) Leptospirosis Enhanced Surveillance Protocol considers a case of leptospirosis to be 'confirmed' if one of the following criteria is met: single MAT titre ≥ 1:320, a MAT titre that when repeated has increased to 1:320; a four-fold increase in MAT titre between acute and convalescent samples; or a positive PCR assay. A case can be considered 'probable' on the basis of a positive IgM ELISA alone or a MAT titre < 1:320.

We describe diagnostic testing for leptospirosis at our institution, in a non-endemic setting, over a 10-year period, and highlight the challenges in achieving laboratory confirmation of a clinical diagnosis of leptospirosis.

Methods

Data collection

We retrospectively identified patients managed in two tertiary care hospitals in Edinburgh, UK, who had samples sent to a PHE reference laboratory for leptospirosis testing between April 2006 and February 2016. Documentation of the indications for leptospirosis testing in these patients was not available. Clinical and laboratory information on the leptospirosis cases was collected retrospectively from patient records. Complete details were unavailable for four cases due to destruction of archived case notes.

Provision of diagnostic testing

Clotted blood samples were referred to a PHE reference laboratory for serological and molecular testing. Testing was initially provided by the Leptospira Reference Unit at the Hereford PHE laboratories until April 2015 when the National Leptospirosis Service was created, with testing provided by the Porton and Colindale PHE laboratories. Currently, initial testing is performed at the Rare and Imported Pathogens Laboratory at PHE Porton with subsequent confirmatory testing performed at the Bacteriology Reference Department at PHE Colindale.

Results

Trends in serological testing

A total of 480 samples from 409 patients were sent for leptospirosis IgM ELISA testing from our institution between April 2006 and February 2016 (Figure 1a). Of these, 450 tests were negative, 26 were positive (from 14 patients) and 4 were equivocal (1 was repeated and was negative; the remaining 3 were not considered significant). There was no clear temporal trend in testing across the study period (Figure 1). The number of leptospirosis cases diagnosed each year varied from 0 to 3, with the possibility of a consistent presence of, and increase in, cases from 2014 onwards. There were peaks in diagnosis of new cases in January, August and October.

Diagnostic results and serological follow-up of cases

Patterns of testing performed by the reference laboratories changed over the course of the study period. From 2006 to 2014 the Leptospira Reference Unit at the Hereford PHE laboratories performed an IgM ELISA and MAT on blood samples. Since 2015, the Rare and Imported Pathogens Laboratory at PHE Porton performs an IgM ELISA along with *16S* and *Lep32* DNA PCR on blood (PCR for urine samples was not provided during the study period though is now tested if sample obtained within 7 days of symptom onset).[5] Positive samples are sent to the Bacteriology Reference Department at PHE Colindale for a confirmatory MAT. Currently 1.5 ml blood is preferred with a minimum of 0.5 ml required for testing. Blood cultures collected within 5 days of onset may be submitted, but are rarely referred (none during the study period).

Full diagnostic results are shown in Table 1. All 14 patients with a positive IgM ELISA were given a diagnosis of leptospirosis by their clinical team. In 9 cases the IgM ELISA was positive but the MAT was not performed, due to lack of sufficient sample. Follow-up samples were sent in 9 of the 14 cases, ranging from 1–3 subsequent samples per patient sent between 4 and 168 days from the initial sample. The infecting serogroup was determined in 1 case (Saxkoebing).

Exposure details

Sufficient history was available for 11 patients and a compatible exposure was identified in 10 of these (Table 2). The majority of cases (9/11) were imported, mostly acquired in Asia with a specific history of fresh water exposure or rat contact. Of the two autochthonous cases, there was a history of fresh water exposure for one (kayaking) but no attributable exposure was identified for the second case.

Clinical and laboratory features

The median age of patients was 26 (interquartile range 20.5–38.8); 12 of the 14 were male. Twelve were managed by Infectious Diseases, one by General Medicine (with subsequent outpatient review by Infectious Diseases) and one by Hepatology (at the Scottish Liver Transplantation Unit, due to acute liver failure), all in conjunction with Microbiology.

Patients usually presented with non-specific symptoms, most commonly fever (10/11), myalgia (8/11) and headache (7/11) (Table 3). Only one patient presented with clinically apparent jaundice. Biochemical evidence of hepatitis (8/10) and acute kidney injury (5/10) was common (Table 4). Lymphopaenia (n = 8/10) and monocytosis (n = 5/10) were common, with a normal total white cell and neutrophil count. C-reactive protein was elevated in 8/10 patients (median 115 mg/L).

Table 1 Results of diagnostic testing in leptospirosis cases

Case	IgM ELISA titre	MAT	PCR	Follow-up samples (n)	Time from first sample (days)	Follow-up results
1	1:320	Not done	Not done	Yes (1)	67	Reduced ELISA titre (1:160)
2	1:640	Not done	Not done	No		
3	1:2560	1:320	Not done	No		
4	1:2560	Not done	Not done	No		
5	1:320	1:160	Not done	Yes (1)	71	ELISA and MAT negative
6	1:160	Not done		Yes (2)	21 and 168	ELISA titre static (1:160) then reduced (1:80)
7	1:640	1:320	Not done	Yes (1)	14	ELISA titre static (1:640); MAT titre reduced (1:160)
8	1:80	Negative	Negative	Yes (3)	4, 7 and 29	ELISA titre increased (1:160), static then decreased (1:80); MAT consistently negative
9	1:1280	1:1280	Not done	Yes (2)	16 and 44	ELISA titre static (1:1280) then decreased (1:160); MAT titre decreasing (1:640 then 1:320)
10	Positive*	Not done	16S DNA positive Lip32 DNA negative	No		
11	Positive*	Not done	16S DNA negative Lip32 DNA negative	No		
12	Positive*	Not done	16S DNA negative Lip32 DNA negative	Yes (1)	29	ELISA positive; insufficient sample for MAT
13	Positive*	Not done	16S DNA negative Lip32 DNA negative	Yes (1)	43	ELISA positive; insufficient sample for MAT
14	Positive*	Not done	16S DNA negative Lip32 DNA negative	Yes (1)	11	ELISA positive; insufficient sample for MAT

ELISA, MAT and PCR were all performed on blood. *IgM ELISA 'positive'; titre not reported. PCR testing comprised 16S DNA and Lip32 DNA testing. The infecting serogroup was determined in one case (Saxkoebing).
MAT, microscopic agglutination test

One patient developed Weil's disease with multi-organ failure (including liver and kidney failure) and required intensive care unit admission, renal replacement therapy, mechanical ventilation and vasopressor support (peak creatinine 741 µmol/L, bilirubin 707 µmol/L, ALT 3062 units/L and prothrombin time 25 s). This patient developed a retroperitoneal haemorrhage with CT angiography demonstrating angiopathy of hepatic, renal and external iliac arteries (and associated renal infarcts) suggestive of vasculitis. The patient was treated with intravenous ceftriaxone and recovered, with normal renal function and much improved liver function on discharge from the intensive care unit.

Antimicrobial therapy and outcomes

In the cases where antimicrobials were prescribed (7/11), doxycycline was used most frequently (5 cases). Most patients (8/11) were hospitalised though with a median length of stay of 4 days. There were no deaths at 30 days and only one patient had abnormal renal function at follow-up.

Additional microbiology and virology investigations

An extensive and diverse infection evaluation was performed for the 14 patients ultimately diagnosed with leptospirosis. Most patients had blood cultures drawn (n = 9/11, no cases of bacteraemia identified) and malaria excluded by three blood smears (n = 7/11). Serology was performed for the following:

- HIV (antigen/antibody combination, n = 8/11)
- Hepatitis A (IgM, n = 4/11)
- Hepatitis B (surface antigen, n = 7/11; core antibody, n = 2/11)
- Hepatitis C (IgG, n = 6/11)
- Hepatitis E (IgM, n = 3/11)
- Cytomegalovirus (IgM, n = 5/11)
- Epstein-Barr virus (IgM, n = 5/11)
- Parvovirus B19 (IgM, n = 1/11)
- Rickettsia (IgG and IgM, n = 5/11)
- Q fever (IgG, n = 2/11)
- *Borrelia burgdorferi* (IgG, n = 1/11)

Table 2 Exposure details of leptospirosis cases

Case	Location of acquisition	Relevant exposure history
3	Thailand	Mountain trek
5	Thailand & Laos	Swam in river Rat contact
6	Rural Java (Indonesia)	Not recorded
7	Cambodia, Laos & Thailand	Not recorded
8	Thailand & Cambodia	Jungle trek
9	Scotland	Fresh water kayaking
10	Kitulgala (Sri Lanka)	White water rafting
11	Borneo	White water rafting Fresh water swimming
12	Scotland	Unknown
13	Costa Rica	Swam in hot springs Jungle hike (dirty water)
14	Luanda (Angola)	Rat contact (infested accommodation)

Details unavailable for cases 1, 2 and 4

and was negative in all cases. An Arbovirus serology panel was performed by the reference laboratory in 7/11 cases as part of a geographical screen, based on submitted travel details. One patient had a positive dengue virus IgM, though this was attributed to cross-reactivity to the Leptospira IgM by the reference laboratory and the dengue IgG was negative. Stool cultures were performed in 4 patients and this identified 1 case of *Campylobacter* sp. co-infection. Respiratory virus throat swabs were performed in 8 patients, identifying one respiratory syncytial virus and one influenza B virus co-infection.

Discussion

Leptospirosis is an uncommon infectious disease in the south-east of Scotland; 409 patients at our institutions were tested over a 10-year period (2006–2016) resulting in 14 confirmed or probable diagnoses (~ 3% of tests referred to reference laboratory were positive); a median of 1 per year. Based on voluntary laboratory reporting for the whole of Scotland over an overlapping 9-year period (2006-2015), Health Protection Scotland reported a total of 34 cases with a median of 3 per year.[6] Due to the non-specific, mild and self-limiting nature of the majority of cases, it is likely that a large number of infections are not investigated and are thus undiagnosed.

Most infections in our Scottish cohort were imported; acquired in either Asia or South America. This contrasts with the findings of the UK-wide 2006–2010 study where only 25.9% of cases were imported, and a French study where 2/34 cases were imported.[7,8] In the UK-wide study, cases from England significantly outnumbered the total number of cases from the rest of the UK, so the higher incidence

Table 3 Clinical features, complications, outcomes and treatment of patients with leptospirosis

	No. of cases (n = 11*)
Presentation	
Fever	10
Myalgia	8
Headache	7
Nausea or vomiting	6
Abdominal pain	5
Arthralgia	4
Diarrhoea	4
Cough	2
Jaundice	1
Altered mentation	1
Haemoptysis	0
Bleeding	0
Complications	
Hepatitis	8/10*
Acute kidney injury	5/10*
Renal replacement therapy	1
Liver failure	1
Haemorrhage	1
Vasculitis	1
Intensive care unit admission	1
Intubation and ventilation	1
Vasopressor requirement	1
Chest X-ray infiltrates	0
Outcomes	
Total 30-day mortality	0
Inpatient admission	8
Inpatient length of stay (median, range)	4 (2–10)
Chronic kidney disease	1
Antimicrobial	
None	4
Doxycycline	5
Cefalexin	1
Ceftriaxone	1

*Full clinical details were available for 11 patients and laboratory results for 10 patients

of autochthonous infection may be related to the warmer climate in England (and similarly France) and thus increased pursuit of fresh water activities.

The 14 cases retrospectively reviewed in this report serve to highlight the challenges in achieving a laboratory diagnosis of leptospirosis. IgM ELISA is considered a useful screening test for acute infection with MAT considered confirmatory. Studies evaluating IgM ELISA testing in comparison to MAT as the reference standard have reported sensitivities of 80–97%

Table 4 Laboratory features of patients with leptospirosis

	Patient values median (IQR)	Local reference interval
Admission		
Haemoglobin, g/L	146 (127–155)	115–160
Total white cell count, x10^9/L	8.5 (5.9–12.8)	4.0–11.0
Neutrophils, x10^9/L	6.5 (4.6–10.0)	2.0–7.5
Lymphocytes, x10^9/L	**1.0** (0.5–1.3)	1.5–4.0
Monocytes, x10^9/L	**0.9** (0.5–1.2)	0.2–0.8
Platelets, x10^9/L	202 (164–247)	150–400
Creatinine, µmol/L	86 (69–293)	50–98
Bilirubin, µmol/L	15 (12–28)	3–21
ALT, units/L	40 (20–153)	10–50
Alk Phos, units/L	113 (81–121)	40–125
PT, s	12.0 (11.5–15.2)	10.5–13.5
CRP, mg/L	**115** (17–136)	0–5
Major laboratory abnormalities during illness		
Elevated creatinine (i.e. AKI)	**n = 5/10**	
Creatinine (µmol/L), in patients with AKI	**359** (160–653)	50–98
Elevated ALT (i.e. hepatitis)	**n = 8/10**	
ALT (units/L), in patients with hepatitis	**159** (86–278)	10–50
Lymphopaenia	**n = 8/10**	
Lymphocytes (x10^9/L), in lymphopaenic patients	**0.85** (0.4–1.0)	1.5–4.0
Elevated CRP	**n = 8/10**	
CRP (mg/L), peak value	**124** (24–160)	0–5
At discharge		
Creatinine, µmol/L	71 (64–76)	50–98
Bilirubin, µmol/L	12 (9–24)	3–21
ALT, units/L	27 (17–50)	10–50
CRP, mg/L	2 (1–6)	0–5

Full laboratory results were available for 10 patients.
Creatine kinase was measured in one patient and was within the reference interval.
IQR, interquartile range; ALT, alanine aminotransferase; Alk Phos, alkaline phosphatase; CRP, C-reactive protein; AKI, acute kidney injury

and specificities of 55–97%.[9–13] Limitations of the MAT are that diagnosis will be retrospective if relying on serial samples to see a rising titre and the necessity for paired acute and convalescent samples to optimise sensitivity. The sensitivity of single measurement acute phase MAT when compared to paired samples is 55%. When applying the PHE Leptospirosis Enhanced Surveillance Protocol to our cohort, 2 patients would have met criteria for 'confirmed' leptospirosis (on the basis of a single MAT > 1:320 in one case and a positive PCR in the other) and the remaining 12 would have been 'probable' cases on the basis of IgM ELISA positivity alone, with or without a non-confirmatory MAT titre. Nine patients did not have MAT performed, usually due to insufficient sample for testing after the IgM ELISA, and in one case no convalescent MAT sample was obtained. False positive Leptospira IgM ELISA results have been demonstrated in *B. burgdorferi*, cytomegalovirus, Epstein-Barr virus, HIV, viral hepatitis and *Mycoplasma pneumoniae* infection and false positive MAT in *Chlamydophila pneumoniae*, cytomegalovirus, Epstein-Barr virus, viral hepatitis, *M. pneumoniae* and *Legionella pneumophila* infection.[12] Variable testing for these infections was performed, and negative, in our cohort, but this does emphasise the importance of interpreting serology results carefully in the clinical context. Further underscoring the importance of a thorough infection evaluation was the presence of co-infection in three of our 14 patients (respiratory syncytial virus, influenza B virus and *Campylobacter* sp.). Additional causes of false positive leptospirosis serology come from the presence of auto-antibodies: antinuclear antibody, anti-neutrophil cytoplasmic antibody and rheumatoid factor).[12]

PCR detecting Leptospiral DNA in blood is a sensitive and rapid test now available routinely with the caveat that sensitivity is highest in the first 7 days after illness onset.[9,14,15] The level of leptospiraemia, quantified by PCR, has been shown to correlate with disease severity.[16] Although until recently

not routinely available from the reference laboratory, PCR on urine within 7 days of illness onset is another attractive early test that may be more sensitive than PCR testing of blood, with the additional benefit that the organism is excreted and detectable in urine for longer.[17] PCR on blood was performed in 6 cases and was positive in 1. While we do not have the dates of travel/return, we infer from the fact that most cases were imported that samples were likely obtained more than 7 days after illness onset, perhaps explaining the multiple blood PCR-negative cases. The PCR-positive case was autochthonous, from the patient critically ill with Weil's disease, and was obtained within 7 days.

Conclusion

This 'real world' cohort of patients illustrates some of the practical challenges in achieving laboratory confirmation of a clinical diagnosis of leptospirosis outwith an endemic setting, where most cases are imported. The majority of diagnoses were 'probable', made on the basis of compatible clinical and epidemiological features combined with an initial positive IgM ELISA. The contribution of MAT was limited as the sample volume received by the reference laboratory was often insufficient for testing. The delay in testing related to patients returning from overseas likely means most PCR assays were performed on samples obtained more than 7 days from illness onset, limiting sensitivity. A combined approach of PCR and paired acute and convalescent MAT should offer the optimum sensitivity and clinicians should ensure a sufficient volume of blood is sent for MAT testing, and endeavour to obtain a convalescent sample. Causes of false positive Leptospira serology (IgM and MAT) due to infections that could mimic the clinical presentation should be considered and appropriate investigations performed, including HIV, cytomegalovirus, Epstein-Barr virus and viral hepatitis serology. False positive results due to auto-antibodies (antinuclear antibody, anti-neutrophil cytoplasmic antibody and rheumatoid factor) should also be considered. In addition, a thorough infection evaluation to identify possible co-infection (present in 3 patients in our cohort) should be undertaken.

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