Epidemiology of leptospirosis in Groote Schuur Hospital

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SUBMITTED TO THE UNIVERSITY OF CAPE TOWN

In fulfilment of the requirements of the degree

Master of Medicine (MMed) in Medicine

Faculty of Health Sciences

University of Cape Town

Date of submission: 16/09/20

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DECLARATION

I, *Dr* Ziningi Charity Mteshana , hereby declare that the work on which this dissertation/thesis is based is my original work (except where acknowledgements indicate otherwise) and that neither the whole work nor any part of it has been, is being, or is to be submitted for another degree in this or any other university.

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ABSTRACT

Background

The burden of leptospirosis in sub-Saharan Africa remains unclear although it is accepted that this infection is widely spread in this region. The global estimated number of cases is one million with 58 900 deaths attributable to leptospirosis annually.

Objective

To describe the profile of patients with suspected leptospirosis and to compare their in-hospital outcomes.

Methods & Material

We performed a retrospective study at a tertiary referral hospital in South Africa. All adults with suspected leptospirosis who had a laboratory request for leptospirosis ELISA IgM testing between 2005 and 2015 were included. Clinical and laboratory findings at presentation were correlated with the patient's subsequent clinical course and ELISA IgM status.

Results

During the study period 223 patients who had ELISA IgM test requests were enrolled. Leptospirosis ELISA IgM was positive in 45 (20%) patients. Enrolled patients had a median age of 38 (IQR 31 – 53) years, 147/223 (66%) were males and 80/223 (36%) were HIV positive. There were 12/45 (27%) HIV-positive patients in the IgM-positive group compared to 68/178 (38%) in the IgM-negative group, p=0.22. Compared to IgM-negative patients, patients with positive IgM were more likely to present with jaundice 37/45 (82%) vs. 82/178 (46%), p<0.01, and acute kidney injury (AKI) 34/45 (76%) vs.102/178(57%), p=0.06.

The median length of hospital stay was 13 days (IQR 8-22 days) for IgM-positive compared to 10 days (IQR 6-21 days) in IgM-negative patients, p= 0.10. A total of 11/45 (24%) IgM-positive patients required ICU admission compared to 41/178 (23%) of IgM-negative patients, p=0.84 and the median length of ICU stay was 7 days (IQR 4-11) for IgM-positive compared to 6 days (IQR 3-9.5) for IgM-negative patients, p=0.51. There were 13/45 (29%) IgM-positive patients who needed dialysis compared to 42/178 (24%) of IgM-negative patients, p= 0.46. The mortality rate was 7/45 (16%) in IgM-positive compared to 52/178 (29%) in IgM-negative patients, p=0.07.

Conclusion

Patients with positive IgM presented predominantly with jaundice and AKI. There was no statistically significant difference in HIV status and outcomes between the two groups of patients.

DEDICATION

This thesis is dedicated to my mother Nombuso Mteshana for constantly encouraging me to accomplish the thesis work.

ACKNOWLEDGEMENTS

First and foremost, all praises to God, the almighty for his protection and strength throughout my research work.

I would like to express my deep and sincere gratitude to my supervisor Professor Sipho Dlamini and co-supervisors Professor Rudzani Muloiwa and Dr Preneshni Naicker for giving me the opportunity to do research and providing invaluable guidance and support throughout this project. A big thank you also goes to my family and friends for their love and support. I also acknowledge the medical superintendent of Groote Schuur Hospital for granting access to patients' medical records.

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LIST OF ABBREVIATIONS

AKI	Acute kidney injury
ELISA	Enzyme-linked immunosorbent assay
GSH	Groote Schuur Hospital
HIV	Human immunodeficiency virus
ICU	Intensive care unit
IgM	Immunoglobulin M
MAT	Microscopic agglutination test
NHLS	National Health Laboratory Service
NICD	National Institute for Communicable Diseases
PCR	Polymerase chain reaction
RDT	Rapid diagnostic test
TB	Tuberculosis
WHO	Word Health Organization

CHAPTER 1: BACKGROUND AND LITERATURE REVIEW

BACKGROUND

Introduction

Leptospirosis is a systemic bacterial infection caused by pathogenic spirochete bacteria from the genus *Leptospira*. It is primarily a zoonosis and humans serve as accidental hosts (1). Leptospires may be saprophytic (free-living and considered not to cause disease), found in fresh water or pathogenic, which may cause acute or chronic infection in humans and animals. Saprophytic leptospires are susceptible to complement-mediated killing because they do not bind host complement regulatory proteins factor H (FH) and C4b binding protein (C4BP). By contrast pathogenic leptospires evade complement attack by acquiring soluble proteins of the alternative and classical pathways on their surfaces (2, 3).

Infections occur more in warm climates than in temperate regions with most cases occurring predominantly during the rainy season. This is most likely due to the longer survival of leptospires in warm, humid environments (4, 5). However, in 2011 an outbreak of leptospirosis occurred in a relatively dry district of Anuradhapura, Sri Lanka (6). This outbreak provided evidence that leptospirosis does not occur exclusively in tropical and subtropical areas.

Many wild and domestic animals such as cattle, pigs and dogs can serve as reservoir hosts but the brown rat has been shown to be the most important source of human infection (1, 7). Individuals living in urban environments characterized by overcrowding, inadequate sanitation and poor housing are at high risk of rat exposure and also therefore, of leptospirosis. The role of poor housing is supported by a study demonstrating a greatly increased risk of leptospirosis exposure among individuals who live in the same household as a leptospirosis patients (8). In developed countries infection arises mainly from occupational exposure, travel to endemic areas, recreational activities, or importation of domestic and wild animals (9). Individuals with occupations at risk for direct contact with potentially infected animals like livestock farmers, veterinary/meat inspectors and abattoir workers are considered at high risk of contracting this infection. This is highlighted by a study which showed seroprevalences of 19.4%, 18.1% and 17.1% in livestock farmers, veterinary/meat inspectors and abattoir workers respectively and subjects who reported milking cows were significantly more likely to be found seropositive for *Leptospira* spp infection than the other subjects (10).

Humans get infected through consumption of food or water contaminated with animal urine or through saliva and faeces of infected animals and penetration of tissue barriers by the bacteria. The portals of entry include the skin through a cut or abrasion and the mucous membranes of the oral cavity or conjunctivae. The oral route of infection is suggested by evidence from a study that highlighted an association of swallowing contaminated water whilst swimming as a risk factor for infection (11). Penetration of the skin through cuts or abrasion is supported by several studies mainly done after recreational activities (12, 13).

A study by Hochedez in 2011 during an outbreak of leptospirosis after a race in the tropical forest of Martinique proved that cutaneous cuts were associated with the outcome of leptospirosis; however, swallowing river water was not associated with illness (12). In

another study on triathletes, open wounds were identified as a risk factor for leptospirosis (13). Although rare, human to human transmission through sexual intercourse or transplacental transmission (which can result in more than 50% fetal death) or during lactation have been reported (14-16).

Once infection has been acquired, the next step in the pathogenesis is haematogenous dissemination resulting in single or multiple organ involvement. The kidneys are the primary targets of leptospirosis but other organs like liver, lungs, heart and central nervous system may also be infected (1). Multi-organ involvement and old age (>60 years) is associated with very high mortality (17, 18). Leptospirosis is associated with severe disease which is also associated with prolonged hospital stay (19).

Literature review

Objectives

1. To evaluate burden of leptospirosis in the world, Africa and in South Africa.
2. To describe the clinical presentation and outcomes of patients with leptospirosis.
3. To highlight different tests available for diagnosis.

Literature search strategy

An extensive search of existing literature for applicable articles was undertaken using PubMed and Google Scholar. All retrieved studies were evaluated for relevance. The following phrases were used either alone or in combination: “leptospirosis in humans”, “global burden, Africa and South Africa”, “transmission”,“ risk factors “, “outcome”, “clinical presentation”, “cardiovascular, neurological, pulmonary and renal manifestation”, “diagnosis”. Articles were saved directly to My Bibliography and citation manager using Endnote X9 for citation and reference management.

Results

From 1201 articles that were identified from both PubMed and Google Scholar, 392 were selected after excluding duplicates and studies done in animals. Seventy-nine articles were used after screening for relevance.

Burden of leptospirosis

The global burden of leptospirosis may be an underestimate for several reasons. These would include the lack of proper notification systems for leptospirosis in most countries, a low clinical index of suspicion, its broad spectrum of clinical presentation, often mimicking other infections, and lack of laboratory diagnostic support in resource-poor countries. Globally, 1.03 million (95% CI 434,000–1,750,000) cases are estimated to occur each year, while deaths attributable to leptospirosis are estimated to number 58 900 (95% CI 23,800–95,900) annually (20). A large proportion of cases 48% (95% CI 40–61%) and deaths 42% (95% CI 34–53%) are estimated to occur in adult males 20–49 years of age (20). Male gender predominance is possibly due to exposure-related bias, as reports of leptospirosis outbreaks related to athletic events where males and females have similar levels of exposure have found no significant effects of gender on the development of illness (21).

The highest morbidity and mortality is estimated to occur in resource-poor countries which include regions of South and Southeast Asia, Oceania, Caribbean, Andean, Central, and tropical Latin America, and east sub-Saharan Africa (20).

In sub-Sahara Africa, leptospirosis is recognised as a neglected tropical disease due to lack of epidemiological data in many countries, together with an absence of morbidity and mortality estimates (22). Most data available is from animals, this relates to the economic losses caused by leptospirosis in livestock (22). In an African survey, the prevalence of acute leptospirosis in hospitalised patients with fever from 26 countries ranged from 2.3 % in Mozambique to 19.8 % in Egypt (23). Data from Tanzania indicate that human disease incidence is also high (75 to 102 cases per 100,000 per year) (23).

A systemic review that included 32 surveys with most of the data from West Africa (Ghana and Nigeria) and East Africa (Kenya and Tanzania) with 12 studies each. The report was that in West Africa the prevalence ranges from 0.4 % to 33 % in Ghana and 13.5 % to 100 % in Nigeria however the sample size was too small (10 patients) in a group of patients that yielded 100 % result. In East Africa the prevalence ranges from 0.0 % to 11.0 % in Kenya and 0.3 to 36.4 % in Tanzania (22).

In South Africa a few human studies have been done, mainly in the form of case reports. There are about eight case reports describing the clinical and laboratory parameters of these patients, two case series and three surveys, therefore the true burden of the disease is unknown(24-35).

In a cross sectional survey on healthy people in Durban (2008), the seroprevalence was reported to be 18.9 %, with a significantly higher percentage among youth aged 18-22 years (60 % of the sample) and females (82 %) (34). In another cross-sectional survey that was done in Johannesburg, human samples were tested in febrile patients suspected to have leptospirosis, showing a seroprevalence of 7.8 % (33).

In a case series in Johannesburg of five patients in 1958, who showed symptoms of meningo-encephalitis, antibodies to leptospira canicola were detected in their sera (32). Another case series describing clinical and laboratory features of fourteen patients with leptospirosis was done in Cape Town (GSH) in 1981 (31). A study done in Mpumalanga Province (2018) on the prevalence of nine zoonotic diseases. Two groups of participants were included: malaria-negative acute febrile illness patients, called febrilers at three clinics (n=74) and a second group of farmers, herders, and veterinary staff found at five government cattle dip-tanks, called dip-tanksters (n=69). Lepotosprosis was more prevalent in deep tanksters (21,9 %) compared to 6,8 % in febrilers (35).

Clinical presentation

The disease may manifest with a broad spectrum of signs and symptoms, ranging from a mild febrile illness to severe life-threatening illness with multi-system organ involvement. It is often mistaken for other causes of febrile illnesses (1, 36). Flu-like symptoms spontaneously resolve in 90% of cases with 10% of patients develop severe form of the disease that is characterised by multi-organ failure (37). This is associated with a dramatic increase in mortality (37). It is therefore crucial for clinicians to consider leptospirosis as a differential

diagnosis in all patients with febrile illness, more especially if risk factors for infection are present.

Non-specific signs and symptoms such as headache, malaise, myalgia, jaundice and conjunctival suffusion have been reported in multiple studies (38-41). Patients may present with the severe form (Weil's disease) of the disease, which is characterised by shock, jaundice, acute kidney injury (AKI) and haemorrhage, particularly in the lungs (42). This form of the disease is associated with a high mortality rate (17).

Cardiac manifestations

Involvement of the cardiovascular system is reported to be high in two autopsy studies. The observation was that the common manifestation being interstitial myocarditis (observed in 50% and 100% of cases respectively), followed by acute coronary arteritis, affecting the main branches of the coronary arteries in 70% of the cases and aortitis in 57.8% of the cases (43, 44). However, the clinical presentation of patients with cardiovascular involvement is not well described as these patients present with multiple nonspecific symptoms that may resemble clinical presentation of other organ systems.

Cardiac involvement in leptospirosis may resemble that of sepsis syndrome, with myocardial systolic dysfunction being common (45). However, left ventricular dysfunction as assessed by echocardiogram and doppler examination is not observed in other studies (46, 47). Sinus tachycardia, bradycardia, atrial fibrillation and myocarditis have been shown to be complications of leptospirosis with no residual complications on repeat echocardiogram post-recovery (48, 49).

Neurological manifestations

It is uncommon for leptospirosis to present as a primary neurological disease but both central and peripheral neurological manifestations may be seen (50). Aseptic meningitis has been shown to be the commonest manifestation of neuroleptospirosis with patients presenting with headache, fever and neck stiffness (32, 50, 51). Other presentations include: myeloradiculopathy, myelopathy, Guillain-Barré syndrome-like presentation, meningoencephalitis, intracerebral bleed, cerebellar dysfunction, iridocyclitis, and tremor/rigidity (50). The prognosis is generally good but altered level of consciousness and seizures at presentation are poor prognostic signs (50, 52).

Pulmonary manifestations

Pulmonary manifestations include: cough, haemoptysis, dyspnoea and chest pain (53, 54). Delayed antibiotic treatment and thrombocytopenia have been shown to be risk factors for the development of pulmonary complications (55). Pulmonary involvement at presentation is a strong predictor of mortality among patients with severe leptospirosis (56). Severe pulmonary involvement is characterized by alveolar haemorrhage and acute respiratory failure, which may progress rapidly resulting in death in less than forty-eight hours after onset of respiratory signs (57). Pulmonary manifestations are often associated with multi-organ involvement, intensive care unit (ICU) admission, dialysis, mechanical ventilation, and the development of nosocomial infection (53). This high mortality rate is most likely

secondary to asphyxiation due to massive haemoptysis from pulmonary haemorrhage and acute respiratory failure (53, 58).

Renal manifestations

The kidney is one of the principal target organs of Leptospires. The incidence of acute kidney injury (AKI) varies from 44% to 67%, depending on the severity of the disease, age and definition of AKI (59). Renal impairment is a common complication in patients with severe forms of leptospirosis, frequently leading to multi-organ involvement (60) . It is thought that AKI is due to multitude of causes including, direct invasion of Leptospires, inflammation, hemodynamic changes, high bilirubin levels that may facilitate reduction in glomerular filtration rate and the ability to concentrate the urine (61-64). Recovery of renal function may take several months. Complete recovery is described in multiple studies but a small proportion of patients may progress to early chronic kidney disease (65-67).

Diagnosis of leptospirosis

Leptospirosis is a treatable infection with early diagnosis and prompt treatment important for good outcomes. The clinical presentation, which often is non-specific, makes the early recognition of this infection challenging. This makes the diagnosis difficult requiring sophisticated serological and molecular tests. Definitive diagnosis is suggested by isolation of the organism by culture or a positive result on the microscopic agglutination test (MAT) or polymerase chain reaction (PCR) tests. However, leptospiral culture becomes positive only late into the illness, and antibodies become detectable in the second week. Hence these tests do not contribute significantly in the initial diagnosis of leptospirosis (68). It is therefore crucial for clinicians to be familiar with clinical presentation of these patients.

In endemic or resource-poor areas the diagnostic laboratory capacity is extremely limited. Early diagnosis depends on the knowledge of epidemiological factors, presenting features and use of supportive laboratory tests (69, 70). As a result in 1982 the World Health Organization (WHO) introduced Faine's criteria to assist in making a presumptive diagnosis of leptospirosis based on clinical history and epidemiological history supported by laboratory parameters (71). This was subsequently amended in 2012 to increase the sensitivity and specificity of diagnosis (Table 1) (70). This has been shown to be useful in making a presumptive diagnosis of leptospirosis in resource-poor areas where diagnostic tests are not available. Despite this, leptospirosis cases are still missed, most likely due to lack of awareness, the presence of high-profile diseases with similar presentation like tuberculosis (TB), human immunodeficiency virus (HIV), and malaria.

In Groote Schuur Hospital - a tertiary teaching hospital in South Africa, ELISA IgM is used in conjunction with epidemiological data and clinical presentation to make presumptive diagnosis. PCR has only recently been introduced but is done only by the National Institute for Communicable Diseases (NICD) in Johannesburg and certain private laboratories; therefore, results are not readily available to guide management of these patients.

Rapid Diagnostic Tests (RDTs)

Rapid diagnostic tests (RDTs) are useful to enable health care workers, particularly in low resource settings, to diagnose leptospirosis early and give timely targeted treatment. In a study done between 2001 and 2012 evaluating three rapid diagnostic tests, none of the investigated RDTs reached a sensitivity of >80% for detecting *Leptospira* spp infections in admitted patients. Therefore, confirmation of RDT results by a reference test is recommended (72).

Molecular diagnosis

Polymerase chain reaction (PCR) assays confirm the diagnosis in the early phase of the disease prior to antibody titres becoming detectable (73). Detection of leptospiraemia by quantitative PCR has been shown to have wider window of positivity ranging from 2 days to 15 days and its sensitivity is not affected by the length of the interval between the onset of symptoms and sample collection (74).

Serological diagnosis

Enzyme-linked immunosorbent assay (ELISA)

Enzyme-linked immunosorbent assay (ELISA) is the most widely used test. It is limited by the lack of antibodies at the acute stage of the disease. It has the highest sensitivity during the second, third and fourth weeks of illness (75). If done earlier in the course of illness it may be negative. The presence of antibodies due to previous exposure (in endemic regions) affects the performance of this test; therefore a positive ELISA result should be confirmed by the microscopic agglutination test (MAT) (76).

Microscopic agglutination test (MAT)

MAT is the gold standard test but is less sensitive in early phase of disease (77). It allows for the detection of group specific antibodies; however, its performance is restricted to laboratories that can maintain strains for the preparation of live antigen (78). In South Africa MAT is not available for human samples.

Conclusion

The burden of leptospirosis remains unknown possibly due to a number of reasons, lack of reliable estimates, recognition by clinicians, presence of other febrile diseases like malaria and other febrile diseases that may present with similar symptoms (20, 36). This is further impacted by a lack of diagnostic tests in low and middle income countries (79). Future human leptospirosis studies should focus on development of a rapid diagnostic test with high specificity and sensitivity. This will assist with early diagnosis and treatment of patients in order to reduce mortality, which is unacceptably high especially in the severe form of the disease (17). This will also shorten the length of hospital stay, which will subsequently limit the risk of hospital-acquired infections and psychological effects of hospitalisation.

Table 1 (70)

System of scoring using the modified Faine's Criteria (with amendment) 2012 for the diagnosis of leptospirosis.

Part A: Clinical data	Score
Headache	2
Fever	2
Fever >39 °C	2
Conjunctival suffusion	4
Meningism	4
Myalgia	4
Conjunctival suffusion +meningism + myalgia	10
Jaundice	1
Albuminuria / nitrogen retention	2
Hemoptysis / dyspnea	2
Part B: Epidemiological factors	**Score**
Rainfall	5
Contact with contaminated environment	4
Animal contact	1
Part C: Bacteriological and Laboratory Findings	**Score**
Isolation of Leptospira in culture- Diagnosis certain	
PCR[a]	25
Positive serology	
ELISA[b] IgM positive[f]	15
SAT[c] positive[f]	15
Other rapid tests[df]	15
MAT[e] – single positive in high titer[f]	15
MAT[e] – Rising titer / seroconversion (paired sera)	25

Presumptive diagnosis of leptospirosis is made of:

Part A or Part A & Part B score: 26 or more

Part A, B, C (Total) : 25 or more

A score between 20 and 25 suggests leptospirosis as a possible diagnosis.

[a] Polymerase chain reaction

[b] Enzyme linked immunosorbant assay

[c] Slide agglutination test

[d] Other rapid tests– latex agglutination test / Leptodipstick / LeptoTek lateral flow / Lepto Tek Dri Dot test

[e] Microscopic agglutination test

[f] Any one of the tests only should be scored

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CHAPTER 2: JOURNAL READY MANUSCRIPT

Epidemiology of leptospirosis in Groote Schuur Hospital.

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Key words: Leptospirosis burden, symptoms, laboratory profile, outcomes and HIV.

Abstract

Background

The burden of leptospirosis in sub-Saharan Africa remains unclear although it is accepted that this infection is widely spread in this region. The global estimated number of cases is one million with 58 900 deaths attributable to leptospirosis annually.

Objective

To describe the profile of patients with suspected leptospirosis and to compare their in-hospital outcomes.

Methods & Material

We performed a retrospective study at a tertiary referral hospital in South Africa. All adults with suspected leptospirosis who had a laboratory request for leptospirosis ELISA IgM testing between 2005 and 2015 were included. Clinical and laboratory findings at presentation were correlated with the patient's subsequent clinical course and ELISA IgM status.

Results

During the study period 223 patients who had ELISA IgM test requests were enrolled. Leptospirosis ELISA IgM was positive in 45 (20%) patients. Enrolled patients had a median age of 38 (IQR 31 – 53) years, 147/223 (66%) were males and 80/223 (36%) were HIV positive. There were 12/45 (27%) HIV-positive patients in the IgM-positive group compared to 68/178 (38%) in the IgM-negative group, p=0.22. Compared to IgM-negative patients, patients with positive IgM were more likely to present with jaundice 37/45 (82%) vs. 82/178 (46%), p<0.01, and acute kidney injury (AKI) 34/45 (76%) vs. 102/178(57%), p=0.06.

The median length of hospital stay was 13 days (IQR 8-22 days) for IgM-Positive compared to 10 days (IQR 6-21 days) in IgM-negative patients, p= 0.10. A total of 11/45 (24%) IgM-positive patients required ICU admission compared to 41/178 (23%) of IgM-negative patients, p=0.84 and the median length of ICU stay was 7 days (IQR 4-11) for IgM-positive compared to 6 days (IQR 3-9.5) for IgM-negative patients, p=0.51. There were 13/45 (29%) IgM-positive patients who needed dialysis compared to 42/178 (24%) of IgM-negative patients, p= 0.46. The mortality rate was 7/45 (16%) in IgM-positive compared to 52/178 (29%) in IgM-negative patients, p=0.07.

Conclusion

Patients with positive IgM presented predominantly with jaundice and AKI. There was no statistically significant difference in HIV status and outcomes between the two groups of patients.

Introduction

Leptospirosis is a systemic bacterial infection caused by pathogenic spirochete bacteria from the genus *Leptospira* (1). It is primarily a zoonosis and humans serve as accidental hosts (1). Infections occur commonly in warm-climate countries than in temperate regions, and most cases occur predominantly during rainy season, most likely due to longer survival of Leptospires in warm, humid environments (2-4).

Many wild and domestic animals serve as reservoir hosts, but the brown rat has been shown to be the most important source of human infections (1, 5). Individuals living in urban environments characterized by overcrowding, inadequate sanitation and poor housing are at a high risk of rat exposure and also therefore, of leptospirosis (6). Growth of informal settlements together with an increasing population growth in South Africa including in Cape Town, may produce favourable conditions for rat borne transmission.

The most frequent modes of transmission to humans are consumption of food and water contaminated with urine, faeces and saliva of infected animals and leptospire penetration of mucous membranes or broken skin (1, 7, 8). Though rare, human-to- human transmission through sexual intercourse, transplacental transmission and during lactation, have been reported (9-11) .

The global burden of leptospirosis may be grossly underestimated for several reasons. These include the lack of proper notification systems for leptospirosis in most countries, low clinical index of suspicion, its broad spectrum of clinical presentation often mimicking other infections and lack of laboratory diagnostic support. Globally, 1.03 million cases are estimated to occur each year, while deaths attributable to leptospirosis are estimated to number 58 900 annually (12).

In an African survey, the prevalence of leptospirosis in patients with fever ranged from 2.3 % in Mozambique to 19.8 % in Egypt. Data from Tanzania indicate human disease incidence of 75 to 102 cases per 100,000 per year (13). A few studies have been done in South Africa, mainly in the form of case reports and surveys. A survey in Durban among healthy volunteers showed a seroprevalence of 18.9 % (14). In a cross sectional survey done in Johannesburg in febrile patients, 7. 8 % of patients were found to be seropositive for leptospirosis (15).

The disease manifests with a broad spectrum of symptoms and signs, ranging from mild febrile illness which spontaneously resolves in 90% of cases. It is estimated that 10% of patients develop the severe form of the disease that is characterised by multi-organ involvement and associated with a dramatic increase in mortality (1, 16). Non-specific signs and symptoms such as fever, headache, malaise, myalgia, rash, jaundice and conjunctival suffusion have been reported in multiple studies (17-20).

Definitive diagnosis is suggested by isolation of the organism by culture or a positive result on the microscopic agglutination test (MAT) or by polymerase chain reaction (PCR) tests. However, leptospiral culture becomes positive only late into the illness while MAT and PCR are not available in resource-poor areas where they are needed the most. Therefore the World Health Organization (WHO) introduced Faine's criteria to make a presumptive diagnosis of leptospirosis based on clinical and epidemiological history supported by

laboratory parameters (21). This was subsequently amended in 2012 to increase the sensitivity of the diagnosis (22). However, leptospirosis cases are still missed due to lack of awareness. The presence of high-profile diseases such as tuberculosis (TB), human immunodeficiency virus (HIV), and malaria, with similar presentation confounds things further.

In South Africa there are few human studies done. In our hospital (GSH)- a tertiary teaching hospital, the last study (case series) that described the clinical presentation of fourteen patients with leptospirosis was done in 1981 (23).

In this study we aim to describe the clinical presentation, laboratory profile and outcomes of patients with suspected leptospirosis. Secondarily we aim to compare clinical presentation and outcome of patients with positive leptospirosis ELISA IgM to those with negative results and to investigate the relationship between HIV and leptospirosis.

Materials and methods

We conducted a retrospective cross-sectional study of patients who presented to Groote Schuur Hospital (GSH), an 893-bed tertiary teaching hospital in South Africa, between 2005 and 2015 and investigated for suspected leptospirosis. Potential study participants were identified from the National Health Laboratory Service (NHLS) data base. Patients older than 12 years of age who were admitted at GSH and had the Panbio® Leptospira IgM ELISA (Abbott, Illinois, USA) done were included in the study. Patients who did not require admission at Groote Schuur Hospital and those whose folders were not available for review were excluded from the study. To maintain patient's confidentiality alphanumeric codes were used to identify patients in the data base and only the principal investigator knew their identity. The University of Cape Town's Human Research Ethics Committee (HREC REF:006/2017) approved the study. As this is a retrospective study, waiver of consent was granted by the HREC as part of the ethics review process.

A detailed folder review of all included participants was undertaken. We extracted data including demographics (age and gender), HIV status, clinical presentation (headache, confusion, fever (temperature ≥38˚C), jaundice, cough, myalgia, haemorrhage (as defined by one of the following: haemoptysis, hematemesis or epistaxis) and conjunctival suffusion, laboratory results including renal function tests (sodium, potassium and creatinine), liver function tests (total bilirubin, AST and ALT), within 24 hours of admission. Acute kidney injury (AKI) was assessed using modified acute kidney injury network (AKIN) criteria, which use an abrupt (within 48 hours) reduction in kidney function, defined as absolute increase in serum creatinine of more than or equal to 26.4 µmol/l (24). However, due to the retrospective nature of our study, patients were given similar baseline creatinine of 110 µmol/l, and creatinine rise of more than 26.4 µmol/l (i.e., >136.4 µmol/l) within 24 hours of admission was used to define AKI.

Patients who tested positive with the ELISA IgM at a ratio of more than 1:1 were classified as possible cases of leptospirosis. Outcomes included length of hospital stay, number of patients admitted to intensive care unit (ICU), length of stay in ICU, number of patients who needed dialysis and mortality rate.

Data analysis

Descriptive statistics for baseline demographics, clinical findings, and laboratory results at presentation were used. All data were analysed using Stata version 16 (StataCorp, College Street, Texas). Percentages and their 95% confidence intervals (CI) were used to depict proportions of categorical variables while medians with interquartile ranges (IQR) were used to summarise all continuous variables. The χ^2 test or Fisher's exact test were used to assess the strength of association between two categorical variables as appropriate. Continuous variables were tested for normality and the appropriate statistical test used to test for association.

Results

A total of 391 patients were tested for leptospirosis between January 2005 and December 2015. Of these, 223 met the inclusion criteria and had sufficient data for analysis, while 168 were excluded. A presumptive diagnosis of leptospirosis was made in 45 (20%) patients who tested positive for leptospirosis serology (ELISA IgM) with any symptoms (Figure 1).

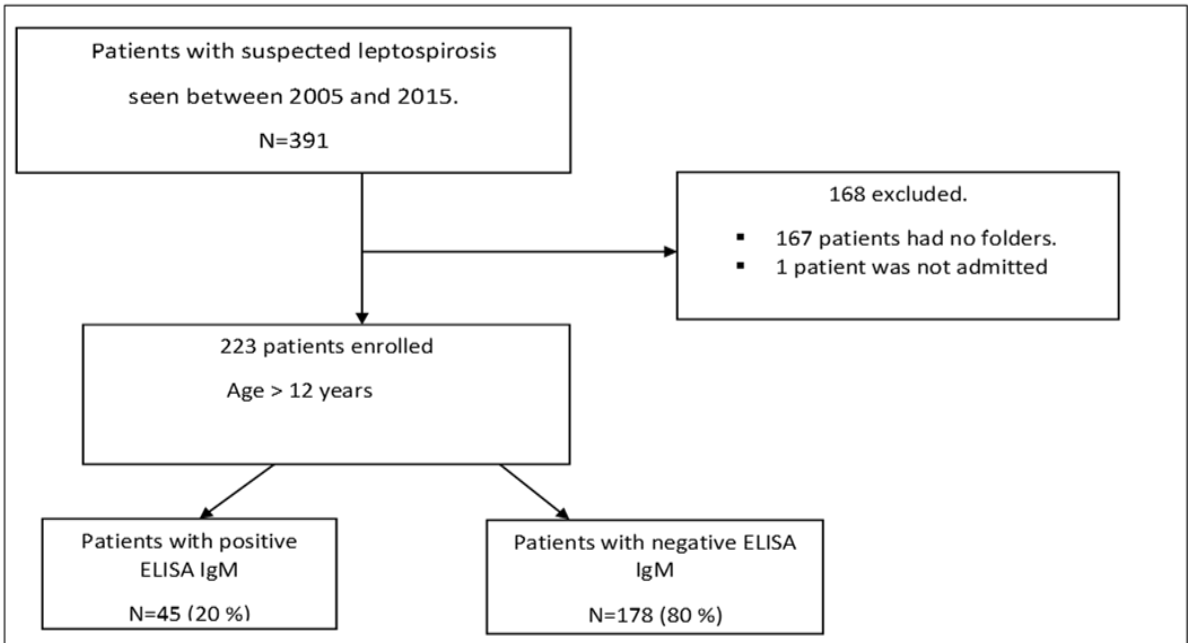

Figure .1 Flow chart showing study population and selection criteria

Enrolled patients had a median age of 38 (IQR 31 – 53) years, of which 147/223 (66%) were males, and 80/223 (36%) were HIV positive. The main symptoms and signs at hospital admission were jaundice in 119/223 (53%) and confusion 87/223 (39 %). The patients' demographics and their presenting symptoms are presented in Table 1.

Table 1. Baseline characteristics of 223 patients with suspected leptospirosis.

Characteristics	N=223 (%)
Gender	
Males	147 (66)
Comorbidity	
HIV	80 (36)
Symptoms	
Jaundice	119 (53)
Confusion	*87 (39)*
Cough	67 (30)
Headache	56 (25)
Fever	48 (22)
Haemorrhage	33 (15)
Conjunctival suffusion	24 (11)
Myalgia	24 (11)

HIV= Human immunodeficiency virus

Laboratory findings included hyponatraemia (Na<135mmol/L) in 109/223 (49%), AKI in 136/223 (61%), total bilirubin >25umol/L in 124/223 (56%) and hepatitis (ALT >80 U/L) in 99/223 (44%).

Out of 45 (20%) IgM-positive patients, 31/45 (69%) were males, 12/45 (27%) were HIV infected. The most common symptoms were jaundice 37/45 (82%), confusion 23/45 (51%), cough 13/45 (29 %), haemorrhage 11/45 (24 %), headache 9/45 (20%), fever 8/45 (18 %), conjunctival suffusion 7/45 (16 %) and myalgia 5/45 (11%).

There were 31/45 (69 %) males in the IgM-positive group compared to 116/178 (65 %) in IgM-negative group. A total of 12/45 (27%) patients with positive IgM were HIV positive compared to 68/178 (38%) patients with negative IgM, p=0.22. Compared to IgM-negative patients, patients with positive IgM were more likely to present with jaundice 37/45 (82%) vs. 82/178 (46%), p<0.01, and AKI 34/45 (76%) vs. 102/178 (57%), p= 0.06. The comparison of the frequency of documented symptoms and laboratory profile between IgM-positive and negative patients is shown in Figure 2 and Table 3 respectively.

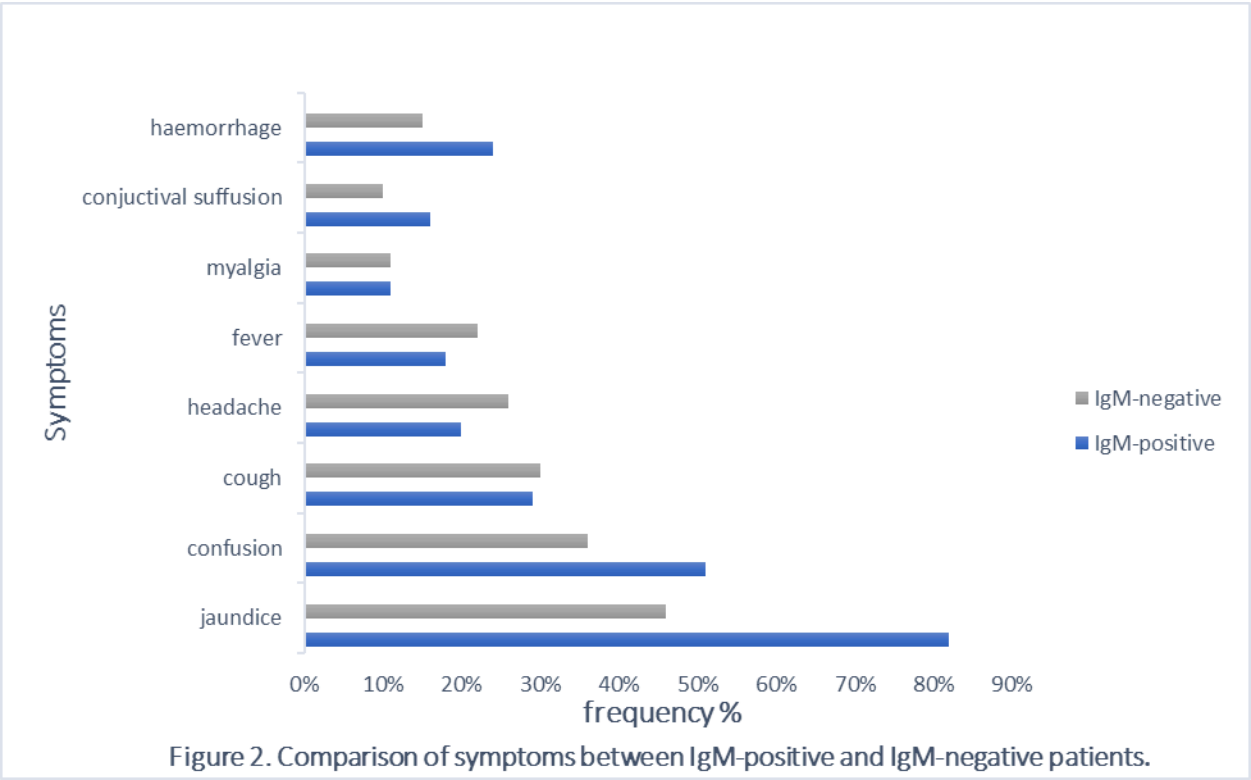

Figure 2. Comparison of symptoms between IgM-positive and IgM-negative patients.

Table 2. Comparison of laboratory profile between IgM-positive and IgM-negative patients.

Results	IgM-positive 45 (%)	IgM-negative 178 (%)	P value
Na <135	24 (53)	85 (48)	0.59
K <3.5	9 (20)	6 (3)	0.87
Creat >136.4	34 (76)	102 (57)	0.06
Tbil >25	36 (80)	88 (49)	0.001
Alt >80	16 (36)	83 (47)	0.24
Ast >80	*21 (47)*	91 (51)	0.46

Na= sodium, K= potassium, Creat= creatinine, Tbil= total bilirubin, Alt= alanine aminotransferase, Ast= aspartate aminotransferase, IgM = immunoglobulin M.

Outcomes

Overall median length of hospital stay was 11 days (IQR 6-21 days). A total of 52/223 (23%) patients needed ICU admission with a median ICU stay of 7 days (IQR 3-10). A total of 55/223 (25%) patients needed dialysis and the overall mortality was 58/223 (26%).

The median length of hospital stay was 13 days (IQR 8-22 days) for IgM-positive compared to 10 days (IQR 6-20 days) in IgM-negative patients, p=0.10. A total of 11/45 (24%) IgM-positive patients required ICU admission compared to 41/178 (23 %) of IgM-negative patients, p=0.84 and the median length of ICU stay was 7 days (IQR 4-11) for IgM-positive compared to 6 days (IQR 3-9.5) of IgM-negative patients, p=0.51. There were 13/45 (29%) IgM-positive patients who required dialysis compared to 42/178 (24%) of IgM-negative group, p=0.46. Mortality was reported in 7/45 (16%) patients with positive IgM compared to 52/178 (29%) in IgM-negative patients, p=0.07. The comparison of outcomes between IgM-positive and negative patients is shown in Table 3.

Table 3. Comparison of outcomes between IgM-positive and IgM-negative patients.

Outcomes	IgM-positive 45 (20%)	IgM-negative 178 (80%)	P value
Length of hospital stay in days, median (IQR)	13 (8-22)	10 (6-20)	0.10
ICU admissions	11 (24)	41 (23)	0.84
Length of ICU stay in days, median (IQR)	7 (4-11)	6 (3-10)	0.51
Dialysis	13 (29)	42 (24)	0.46
Mortality	7 (16)	52 (29)	0.07

ICU= intensive care unit, IQR interquartile range.

Discussion

This study describes the prevalence of laboratory-confirmed leptospirosis in hospitalised patients, in addition to describing the clinical, laboratory profile and outcomes of patients with suspected leptospirosis. This is the largest reported cohort of leptospirosis patients to date in our hospital (GSH). Apart from the significantly higher prevalence of jaundice in confirmed cases, there was no difference between IgM-positive and IgM-negative cases with respect to demographic characteristics, clinical presentation and outcomes.

In our study there were slightly more IgM-positive male cases than in the IgM-negative group although this difference was not statistically significant. The incidence of leptospirosis has been reported to be higher in males than females in most studies and this is thought to be due to high level of exposure to risk factors in males compared to females (25, 26). Unfortunately, in our study a history of risk factors could not be obtained due to the retrospective nature of the study.

Similarly, there was no statistically significant difference in the prevalence of HIV infection between IgM-negative patients and IgM-positive patients. This is consistent with the findings reported in a study done in northern Tanzania (27). However, more studies are needed in order to address this question properly as the prevalence of HIV in patients with leptospirosis is not well studied.

Jaundice was the most common symptom amongst patients with positive IgM (37/45 (82%) vs. 82/178 (46%), p<0.01). The finding of jaundice in patients with confirmed leptospirosis is consistent with other studies that have reported similar findings (19, 28).

Conjunctival suffusion is considered as a hallmark clinical sign of leptospirosis when present (29). In our study it was more frequently observed in IgM-positive patients, however there was no statistically significant difference between two groups of patients 7/45 (16%) vs. 17/178 (10%), p=0.11.

Fever is considered the most common symptom of leptospirosis in multiple studies (3, 19, 30, 31). Surprisingly, fever was less frequently observed, as it was only reported in 22% of enrolled patients with no statistically significant difference between IgM-positive and IgM-negative patients. This may be explained by the fact that fever in patients with leptospirosis may be biphasic and may recur after remission (32). It is therefore possible that these patients presented when fever was in remission and therefore their temperatures were found to be normal, or leptospirosis was not the primary cause of their illness.

In this study AKI and need for dialysis were reported more in IgM-positive patients, although there was no statistically significant difference between the two groups. The kidney has been shown to be one of the principal target organs of Leptospira spp. The incidence of AKI varies from 44% to 67 %, depending on the severity of the disease, age and definition of AKI (33). This is thought to be due to multifactorial causes including, direct invasion of leptospires, inflammation, hemodynamic changes, high bilirubin levels that may facilitate reduction in glomerular filtration rate and the ability to concentrate the urine, and rhabdomyolysis, which may induce vasoconstriction, tubular obstruction, and direct toxicity of myoglobin (34-38).

Hyperbilirubinemia was observed more in IgM-positive patients (36/45 (80%) vs. 88/178 (49%) p=0. 001. This may have contributed to AKI that is observed more in this group of patients. Recovery of renal function may take several months; however, complete recovery is described in multiple cases, but a small proportion of patients may progress to early chronic kidney disease (39-41). In our cohort, the baseline renal function and history of comorbidities such as hypertension and diabetes that may contribute to abnormal baseline renal function were not known, making it difficult to draw clear correlation.

Leptospirosis is associated with high mortality, with mortality rates of >50 % in severe cases (42). However, access to critical care service with early initiation of treatment in the form of early antibiotic administration, protective ventilation strategies, conservative fluid resuscitation, traditional thresholds for renal replacement therapy initiation and corticosteroid therapy, is associated with a reduced case-fatality rate (43).

In our study the mortality rate was higher in IgM-negative patients [52/178 (29%)] compared to IgM-positive patients [7/45 (16%)]; however, there was no statistically significant difference between the two groups of patients. This was an unexpected finding, not in keeping with what is reported in the literature. However, the disease profile of IgM-negative patients was unknown, and this may have contributed to high mortality rate in this group of patients. Mortality rate is reported to be higher in adults than children. Among adults, age above 60 years is independently associated with higher risk of death (20, 44, 45). Most of the patients in our study were young adults with a median age of 38 (IQR 31 – 53), which may have contributed to the observed lower mortality rate.

Our study is limited by a small sample. As a result, some of the findings that suggested a difference could not reach statistical significance. Some of the data of enrolled patients were poorly documented or not available due to the retrospective nature of the study. We wanted to report epidemiological data, but this was not documented. The documentation of occupational history was poorly done, hence we were unable to establish the role of occupation as a risk factor for infection. It may be in this setting clinicians are not aware that occupation may play a role in the risk of acquisition of infection. The duration of comorbidities was not reported or documented in the folders, therefore would be difficult to interpret the role they contributed or play with the current clinical presentation. We had planned to do a univariate analysis for any variables with $p<0.1$ however both univariate and multivariate analysis were not done for this paper.

Although the ELISA test used has high sensitivity (96.5%) and specificity (98.5%), it detects the presence of IgM antibodies, which are usually positive during the second stage or immune stage of the disease. This generally occurs during the second week after onset of symptoms (32). It is therefore possible that some cases that were tested in the acute phase, before formation of antibodies, were missed hence the low positivity rate of the results.

Conclusion

Our study shows that patients with leptospirosis may present with nonspecific symptoms and signs, therefore clinicians should have high index of suspicion, especially if patients have risk factors. This will reduce mortality, which is unacceptably high in these patients, by facilitating early therapy and where indicated, supportive care, including critical care. The study highlights the importance of knowing the clinical presentation, laboratory parameters and outcomes of patients with suspected leptospirosis. Leptospirosis should be considered as a differential diagnosis in patients presenting with nonspecific symptoms, more especially those with risk factors for the disease. A single negative ELISA IgM test does not exclude the presence of the disease; it is therefore necessary to initiate treatment when indicated regardless of the serological results.

Epidemiology of leptospirosis remains unknown in South Africa due to lack of diagnostics and low index of suspicion by clinicians; therefore, future studies should focus on developing sensitive, cost-effective and reliable frontline tests that are easy to perform at the bedside of patients. These may produce results within a short space of time at sites where treatment or care-related decisions are made. This will help shorten the length of hospital stay, limit the risk of hospital-acquired infection and psychological effects of hospitalisation.

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CHAPTER 3: APPENDICES

Research Protocol

Epidemiology of leptospirosis in Groote Schuur Hospital.

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Key words: Leptospirosis burden, symptoms, laboratory profile, outcomes and HIV.

Title: The Epidemiology of Leptospirosis in Groote Schuur Hospital

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Background

Leptospirosis is an infectious disease of both humans and animals that is caused by pathogenic spirochetes of genus *Leptospira*.

The genus *Leptospira* comprises in total 20 pathogenic, intermediate and saprophytic species with more than 250 pathogenic serovars (1).

It is one of the most important zoonoses in the world (2).

The disease was first described by Adolf Weil in 1886, but leptospires were identified as the causative organisms of Weil's disease in Japan in 1908, where it affected coal miners (3).

It is estimated that there are 1.03 million cases of leptospirosis worldwide. The estimated number of deaths attributable to leptospirosis is 58 900 deaths annually. As a zoonotic disease leptospirosis is one of the leading causes of morbidity and mortality among the zoonotic diseases (4). It constitutes a neglected tropical disease in sub-Saharan Africa due to the lack of epidemiological data in many countries together with absence of morbidity and mortality estimates (5).

Climate change, increasing flooding risk, population growth, and urbanization could increase the disease burden in the region (5).

It is transmitted via direct or indirect exposure to leptospire-contaminated animal urine.

Although many wild and domestic animals can serve as the reservoir host, the brown rat is the most important source of human infections.

The most frequent modes of transmission to humans are consumption of food and water contaminated with urine, saliva and faeces of infected animals and penetration of mucous membranes or broken skin.

Human to human transmission through sexual intercourse has been reported (6).

The disease may manifest as a broad spectrum of signs and symptoms, with a sudden onset of headache, high degree fever, malaise, myalgia, conjunctival suffusion and transient rash (7).

The severe form is characterized by jaundice, acute kidney injury (AKI) and haemorrhage, particularly in the lungs, known as Weil's disease (1, 7).

Early identification of severe or potentially severe cases of leptospirosis, and then possible ICU admission need, is of crucial importance to enhance more aggressive treatment and decrease mortality, which is still unacceptably high with this disease (8).

As leptospirosis is endemic in many tropical and developing countries, a more adequate classification and approach to patients can probably impact on economic issues.

The concern is that leptospirosis often remains undetected in resource-limited settings due to a low clinical index of suspicion, its broad spectrum of clinical presentation (often mimicking other infections) and lack of availability of diagnostics.

Currently there is no confirmatory diagnostic test available in the public sector in South Africa, however serology can serve as a confirmatory test when appropriately applied and correctly interpreted.

It is also overshadowed in public and clinicians' awareness by high profile infections such as HIV/AIDS, tuberculosis, and malaria etc.

In case of presence of systemic inflammatory response syndrome (SIRS), clinical and laboratory findings can be mistaken for sepsis due to other causes, more especially in our setting where HIV, TB and other opportunistic infections due to immunosuppression are common.

A few studies have been conducted in South Africa; mainly in the form of case reports or series and two surveys documenting a prevalence of more than five percent.

The cross-sectional surveys done in Johannesburg and Durban estimate the disease incidence to be 7.8% and 18.9% respectively (5).

Of the three case reports on leptospirosis that have been reported in South Africa, two are from Cape Town. The last report from Cape Town was done in 1981, conducted in Groote Schuur Hospital (GSH), which described the clinical and laboratory features of 14 patients with leptospirosis, between 1969 and 1979 (9).

Since then no other reports or studies have been done. We thought it would be important to repeat this study and describe the clinical, laboratory, radiological features and complications of patients from 2005 to 2015.

Our study is going to be different from the previous one because it will be done after three decades and there are more infections in this era that can present in similar manner, i.e. TB, HIV and other opportunistic infections, and clinicians tend to focus more on these conditions.

Deficiencies in the sanitation infrastructure where slum inhabitants reside, were found to be environmental sources of leptospirosis transmission (10). Growth of slum settlements together with increased population growth might have produced conditions for rat-borne transmission of the disease in Cape Town as well. It is therefore important to describe the clinical presentation and complications of patients with leptospirosis in the current context.

This will allow us to gain insight into the burden of the disease and increase awareness of the disease to the clinicians and the public at large.

Methods

Aim

Our study aims to describe the clinical presentation and outcome of patients with suspected and confirmed leptospirosis seen at GSH between 2005 and 2015.

Objectives

To determine the proportion of patients with confirmed leptospirosis at GSH between 2005 and 2015.

To describe the clinical presentation and outcome of patients with confirmed leptospirosis.

To compare clinical presentation, outcome and comorbidities of patients with positive serology and negative serology results for leptospirosis.

To investigate the link between leptospirosis and poor housing/lack of sanitation and occupational exposure.

To investigate the relationship between leptospirosis and HIV infection.

To determine number of patients who required ICU admission.

Study design

We will conduct a retrospective cross-sectional study looking at patients who presented at Groote Schuur Hospital (GSH) between 2005 and 2015 investigated for suspected leptospirosis.

Potential study subjects will be identified from the National Health Laboratory Service (NHLS) database. All patients seen at GSH who had serological testing for suspected leptospirosis will be included.

The study will primarily describe the frequency of serologically-confirmed leptospirosis over the study period.

The clinical presentation and management of the cases will be described. Secondarily, we will investigate for possible associations between a positive finding and both clinical factors and laboratory findings.

Clinical description and case definition of leptospirosis.

Since there is no diagnostic test available in our country, clinical description and IgM ELISA was used to make the presumptive diagnosis.

Selection

All requests for Leptospirosis IgM ELISA will be identified from the NHLS Laboratory Information System between 2005 and 2015.

A detailed folder review of all included participants will be undertaken.

Data including demographics, co-morbidities, HIV/hepatitis/TB, clinical presentation, laboratory results, radiological results, primary diagnosis, treatment and complications will be extracted.

Criteria for entering the study

Inclusion

Patients of all age groups.

Patients tested with serology for possible leptospirosis.

Only patients seen or admitted at GSH during their illness.

Patients transferred from other facilities for further work up.

Exclusion

Patient who were not seen nor admitted at GSH.

Patients admitted outside our predefined period (2005-2015).

Sample size.

We estimate that at least 350 subjects will fulfil the inclusion criteria with a minimum confirmation rate of leptospirosis of 10%. The 95% confidence interval (CI) will range from 6.86% to 13.14%. Other possible precision values based on different estimates of proportions and sample sizes are shown in Table X.

Table X: Proportions of confirmed leptospirosis with upper and 95% confidence limits at varying sample sizes

Number enrolled	Proportion testing serologically positive for leptospirosis			
	2.5%	5%	10%	20%
250	0.56%; 4.44%	2.30%; 7.70%	6.28%; 13.72%	15.04%; 24.96%
300	0.73%; 4,27%	2.53%; 7.47%	6.61%; 13.39%	15.47%; 24.53%
350	0.86%; 4.14%	2.72%; 7.28%	6.86%;13.14%	15.81%; 24.19%
400	0.97%; 4.03%	2.86%; 7.14%	7.06%; 12.94%	16.08%; 23.92%
450	1.06%; 3.94%	2.99%; 7.01%	7.23%; 12.77%	16.30%; 23.70%
500	1.13%; 3.87%	3.09%; 6.91%	7.37%; 12.63%	16.49%; 23.51%

Data management

A case report form (CRF) will be designed containing six sections, which will record the patient's demographics, comorbidities, history, clinical findings, laboratory results within the first 48 hours of presentation and treatment.

Alphanumeric codes will be used to identify patients in order to protect their anonymity.

Only the principal investigator will have access to the patient's identity.

Data will be entered into password-protected database using tap forms, after which it will be exported to STATA version 13 to be checked and verified before analysis.

Statistical analysis

Demographic characteristics and baseline clinical findings at presentation will be tabulated to provide a background description of the study population.

Percentages and their 95% confidence intervals will be used to depict proportions of categorical variables, while medians with interquartile ranges will be used to summarise all continuous variables.

The χ^2 test or Fisher's exact test will be used to assess the strength of association between two categorical variables as appropriate. Continuous variables will be tested for normality and the appropriate statistical test used to test for association.

Factors that are associated with confirmed leptospirosis will be identified on a univariate analysis and any variables with $p<0.1$ will be further be analysed by multivariate analysis. Some of the variables that will be assessed include age of the patient, HIV status and source of referral.

In the final multivariable model, risk ratios with 95% confidence intervals will be used to quantify the measure of effect between groups controlled for relevant confounders.

A significance level of $P<0.05$ will be used for all analysis.

Risks and benefits

As this will be a retrospective study, patient care will not be affected. It is hoped that if found to be cost effective, future use of PCR will help shorten the length of hospital stay, limit the risk of hospital acquired infection and psychological effects of hospitalisation, as well as reduce unnecessary prolonged use of antibiotics and development of antibiotic resistance. We hope that this will have the effect of cutting the overall cost associated with the management of suspected meningitis.

Ethical consideration

This will be a retrospective study, which will not affect patient's management.

Patients' consent will not be necessary, but consent will be sought from superintendent to access folders for review.

To maintain patient confidentiality, we will use alphanumeric codes to identify patients in the database and only the principal investigator will know their identity.

Limitations of the study

This is a retrospective study, therefore sample size has been estimated based on data collected from NHLS results, some folders may not be available, some necessary information may not be documented in the folders and the sample size may be insufficient to answer the question under review.

Negative results may not exclude the presence of the infection and some cases may be missed.

Institutional base

This will be collaborative study between the Department of Internal Medicine and NHLS, based at Groote Schuur Hospital.

Microbiology will also be consulted for more information on the disease.

The Department of Biostatistics of the University will be consulted to assist with complex statistical analysis.

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Case report form

Part 1: Demographics and Comorbidities

Folder Number	☐☐☐ ☐☐☐☐☐☐☐☐☐ ☐☐☐☐
Sex	☐ *Male* ☐ *Female*
Date of birth	☐☐ /☐☐/☐☐☐☐ *(dd/mm/yyyy)*
Address of patient	☐☐☐ ☐☐☐☐☐☐☐☐☐ ☐☐☐☐
Date of hospital visit	☐☐ / ☐☐/☐☐☐☐ *(dd/mm/yyyy)*
Date of discharge	☐☐/☐☐ /☐☐☐☐ *(dd/mm/yyyy)*
Hospital stay length	☐☐☐ *(days)*
Admission to ICU	☐*Yes* ☐ *No* ☐ *Not recorded*
Date of admission to ICU	☐☐/☐☐ /☐☐☐☐ *(dd/mm/yyyy)*
Date of discharge from ICU	☐☐/☐☐ /☐☐☐☐ *(dd/mm/yyyy)*
Race	☐ *Asian* ☐*Indian* ☐ *Black* ☐ *White* ☐ *Coloured*
Height	☐ ☐☐ *cm*
Weight	☐☐ ☐*kg*
Occupation	____ _____
Contact with animals	*Y* ☐ *N* ☐ *U* ☐ *If yes specify* _____
HIV status	☐*Negative* ☐ *Positive* ☐ *Unknown*
If positive, latest CD4 count	☐☐☐ *copies*
Latest viral load	*Copies* ☐☐☐☐ */Log* ☐☐☐☐
ARVS	☐*Yes* ☐ *No* ☐ *Not recorded*
If yes, current ARV regimen	_____*/unknown*
TB	☐*Yes* ☐ *No* ☐ *Not recorded* *If yes specify when*---------- *Mode of diagnosis*---------- *Duration of treatment*----------
Liver disease	☐*Yes* ☐ *No* ☐ *Not recorded* *If yes specify* ----------
Hypertension	☐*Yes* ☐ *No* ☐ *Not recorded*
Diabetes	☐*Yes* ☐ *No* ☐ *Not recorded*
Chronic renal diseases	☐*Yes* ☐ *No* ☐ *Not recorded*
Other comorbidities	----------

Part 2: Signs and Symptoms

Headache	☐ *Yes*	☐ *No*	☐ *Not recorded*
Confusion	☐ *Yes*	☐ *No*	☐ *Not recorded*
fever	☐ *Yes*	☐ *No*	☐ *Not recorded*
Siezures	☐ *Yes*	☐ *No*	☐ *Not recorded*
Vomiting	☐ *Yes*	☐ *No*	☐ *Not recorded*
Lethargy	☐ *Yes*	☐ *No*	☐ *Not recorded*
Myalgia	☐ *Yes*	☐ *No*	☐ *Not recorded*
Arthralgia	☐ *Yes*	☐ *No*	☐ *Not recorded*
Rash	☐ *Yes*	☐ *No*	☐ *Not recorded*
conjunctival suffusion	☐ *Yes*	☐ *No*	☐ *Not recorded*
Loss of weight	☐ *Yes*	☐ *No*	☐ *Not recorded*
Loss of appetite	☐ *Yes*	☐ *No*	☐ *Not recorded*
Coughing	☐ *Yes*	☐ *No*	☐ *Not recorded*
Night sweats	☐ *Yes*	☐ *No*	☐ *Not recorded*
Diarrhea	☐ *Yes*	☐ *No*	☐ *Not recorded*
Abdominal pain	☐ *Yes*	☐ *No*	☐ *Not recorded*
Jaundice	☐ *Yes*	☐ *No*	☐ *Not recorded*
hemorrhage	☐ *Yes*	☐ *No*	☐ *Not recorded*

Part 3: Vital signs on admission

Temperature	☐☐,☐ *°C*
Blood pressure	☐☐☐/☐☐☐ *mm/hg*
Heart rate	☐☐☐ *beats / min*
Respiratory rate	☐☐☐ *breaths / min*
glucose	☐☐ *mmols*
Oxygen saturation	☐☐☐ *%*

Part 4: Blood results on admission

Test	*Results with 24 hours of admission*
Na	☐☐☐
K	☐☐☐
Urea	☐☐☐
Creat	☐☐☐
Wcc	☐☐☐
Hb	☐☐☐
plt	☐☐☐
Tbil	☐☐☐
albumin	☐☐☐
alt	☐☐☐
Ast	☐☐☐
Alp	☐☐☐
Ggt	☐☐☐
Inr	☐☐☐
Crp	☐☐☐
Ldh	☐☐☐
Amylase	☐☐☐
Ck	☐☐☐
Trop	☐☐☐

Part 5: Leptospirosis serology results and outcomes

Leptospirosis IgM	☐ Positive ☐ negative
Dialysis	☐ yes ☐ no
date of death	☐☐/☐☐/☐☐☐☐ *(dd/mm/yyyy)*

Ethics approval document

UNIVERSITY OF CAPE TOWN
Faculty of Health Sciences
Human Research Ethics Committee

Room E53-46 Old Main Building
Groote Schuur Hospital
Observatory 7925
Telephone [021] 406 6492
Email: sumayah.ariefdien@uct.ac.za
Website: www.health.uct.ac.za/fhs/research/humanethics/forms

16 January 2017

HREC REF: 006/2017

Dr S Diamini
Division of Infectious Diseases & HIV Medicine
G17.63
NGSH

Dear Dr Diamini

PROJECT TITLE: EPIDEMIOLOGY OF LEPTOSPIROSIS IN TERTIARY HOSPITAL (MMed candidate- Dr Z Mteshana)

Thank you for submitting your study to the Faculty of Health Sciences Human Research Ethics Committee (HREC) for review.

It is a pleasure to inform you that the HREC has **formally approved** the above-mentioned study.

Approval is granted for one year until the 30 JANUARY 2018.

Please submit a progress form, using the standardised Annual Report Form if the study continues beyond the approval period. Please submit a Standard Closure form if the study is completed within the approval period.
(Forms can be found on our website: www.health.uct.ac.za/fhs/research/humanethics/forms)

We acknowledge that the student, Dr Z Mteshana will also be involved in this study.

Please quote the HREC REF in all your correspondence.

Please note that the ongoing ethical conduct of the study remains the responsibility of the principal investigator.

Please note that for all studies approved by the HREC, the principal investigator **must** obtain appropriate institutional approval before the research may occur.

Yours sincerely

PROFESSOR M BLOCKMAN
CHAIRPERSON, FHS HUMAN RESEARCH ETHICS COMMITTEE

Federal Wide Assurance Number: FWA00001637.
Institutional Review Board (IRB) number: IRB00001938

This serves to confirm that the University of Cape Town Human Research Ethics Committee complies to the Ethics Standards for Clinical Research with a new drug in patients, based on the Medical Research Council (MRC-SA), Food and Drug Administration (FDA-USA), International Convention on Harmonisation Good Clinical Practice (ICH GCP), South African Good Clinical Practice Guidelines (DoH 2006), based on the Association of the British Pharmaceutical Industry Guidelines (ABPI),and Declaration of Helsinki (2013) guidelines.
The Human Research Ethics Committee granting this approval is in compliance with the ICH Harmonised Tripartite Guidelines E6: Note for Guidance on Good Clinical Practice (CPMP/ICH/135/95) and FDA Code Federal Regulation Part 50, 56 and 312.

HREC:006/2017

Instructions for authors (South African Medical Journal)

South African Medical Journal: Author Guidelines

For full instructions, click the following link:
http://www.samj.org.za/index.php/samj/about/submissions

General article format/layout

Accepted manuscripts that are not in the correct format specified in these guidelines will be returned to the author(s) for correction, which will delay publication.

General:

- Manuscripts must be written in UK English.
- The manuscript must be in Microsoft Word format. Text must be single-spaced, in 12-point Times New Roman font, and contain no unnecessary formatting (such as text in boxes).
- Please make your article concise, even if it is below the word limit.
- Qualifications, ***full*** affiliation (department, school/faculty, institution, city, country) and contact details of ALL authors must be provided in the manuscript and in the online submission process.
- Abbreviations should be spelt out when first used and thereafter used consistently, e.g. 'intravenous (IV)' or 'Department of Health (DoH)'.
- Include sections on Acknowledgements, Conflict of Interest, Author Contributions and Funding sources. If none is applicable, please state 'none'.
- Scientific measurements must be expressed in SI units except: blood pressure (mmHg) and haemoglobin (g/dL).
- Litres is denoted with an uppercase L e.g. 'mL' for millilitres).
- Units should be preceded by a space (except for % and ºC), e.g. '40 kg' and '20 cm' but '50%' and '19ºC'.
- Please be sure to insert proper symbols e.g. µ not u for micro, a not a for alpha, b not B for beta, etc.
- Numbers should be written as grouped per thousand-units, i.e. 4 000, 22 160.
- Quotes should be placed in single quotation marks: i.e. The respondent stated: '...'
- Round brackets (parentheses) should be used, as opposed to square brackets, which are reserved for denoting concentrations or insertions in direct quotes.
- If you wish material to be in a box, simply indicate this in the text. You may use the table format –this is the *only* exception. Please DO NOT use fill, format lines and so on.

Research

Guideline word limit: 4 000 words

Research articles describe the background, methods, results and conclusions of an original research study. The article should contain the following sections: introduction, methods, results, discussion and conclusion, and should include a structured abstract (see below). The introduction should be concise – no more than three paragraphs – on the background to the research question, and must include references to other relevant published studies that clearly lay out the rationale for conducting the study. Some common reasons for conducting a study are: to fill a gap in the literature, a logical extension of previous work, or to answer

an important clinical question. If other papers related to the same study have been published previously, please make sure to refer to them specifically. Describe the study methods in as much detail as possible so that others would be able to replicate the study should they need to. Results should describe the study sample as well as the findings from the study itself, but all interpretation of findings must be kept in the discussion section, which should consider primary outcomes first before any secondary or tertiary findings or post-hoc analyses. The conclusion should briefly summarise the main message of the paper and provide recommendations for further study.

Select figures and tables for your paper carefully and sparingly. Use only those figures that provided added value to the paper, over and above what is written in the text.

Do not replicate data in tables and in text.

Structured abstract

- This should be 250-400 words, with the following recommended headings:
  - **Background:** why the study is being done and how it relates to other published work.
  - **Objectives:** what the study intends to find out
  - **Methods:** must include study design, number of participants, description of the intervention, primary and secondary outcomes, any specific analyses that were done on the data.
  - **Results:** first sentence must be brief population and sample description; outline the results according to the methods described. Primary outcomes must be described first, even if they are not the most significant findings of the study.
  - **Conclusion:** must be supported by the data, include recommendations for further study/actions.
- Please ensure that the structured abstract is complete, accurate and clear and has been approved by all authors.
- Do not include any references in the abstracts.

Main article

All articles are to include the following main sections: Introduction/Background, Methods, Results, Discussion, Conclusions.

The following are additional heading or section options that may appear within these:

- Objectives (within Introduction/Background): a clear statement of the main aim of the study and the major hypothesis tested or research question posed
- Design (within Methods): including factors such as prospective, randomisation, blinding, placebo control, case control, crossover, criterion standards for diagnostic tests, etc.
- Setting (within Methods): level of care, e.g. primary, secondary, number of participating centres.
- Participants (instead of patients or subjects; within Methods): numbers entering and completing the study, sex, age and any other biological, behavioural, social or cultural factors (e.g. smoking status, socioeconomic group, educational attainment, co-existing disease indicators, etc)that may have an impact on the study results. Clearly define how participants were enrolled, and describe selection and exclusion criteria.

- Interventions (within Methods): what, how, when and for how long. Typically for randomised controlled trials, crossover trials, and before and after studies.
- Main outcome measures (within Methods): those as planned in the protocol, and those ultimately measured. Explain differences, if any.

Results

- Start with description of the population and sample. Include key characteristics of comparison groups.
- Main results with (for quantitative studies) 95% confidence intervals and, where appropriate, the exact level of statistical significance and the number need to treat/harm. Whenever possible, state absolute rather than relative risks.
- Do not replicate data in tables and in text.
- If presenting mean and standard deviations, specify this clearly. Our house style is to present this as follows:
- E.g.: The mean (SD) birth weight was 2 500 (1 210) g. Do not use the ± symbol for mean (SD).
- Leave interpretation to the Discussion section. The Results section should just report the findings as per the Methods section.

Discussion

Please ensure that the discussion is concise and follows this overall structure – sub-headings are not needed:

- Statement of principal findings
- Strengths and weaknesses of the study
- Contribution to the body of knowledge
- Strengths and weaknesses in relation to other studies
- The meaning of the study – e.g. what this study means to clinicians and policymakers
- Unanswered questions and recommendations for future research

Conclusions

This may be the only section readers look at, therefore write it carefully. Include primary conclusions and their implications, suggesting areas for further research if appropriate. Do not go beyond the data in the article.

Illustrations/photos/scans

- If illustrations submitted have been published elsewhere, the author(s) should provide consent to republication obtained from the copyright holder.
- Figures must be numbered in Arabic numerals and referred to in the text e.g. '(Fig. 1)'.
- Each figure must have a caption/legend: Fig. 1. Description (any abbreviations in full).
- All images must be of high enough resolution/quality for print.
- All illustrations (graphs, diagrams, charts, etc.) must be in PDF or jpeg form.

- Ensure all graph axes are labelled appropriately, with a heading/description and units (as necessary) indicated. Do not include decimal places if not necessary e.g. 0; 1.0; 2.0; 3.0; 4.0 etc.
- Scans/photos showing a specific feature e.g. *Intermediate magnification micrograph of a low malignant potential (LMP) mucinous ovarian tumour. (H&E stain).* –include an arrow to show the tumour.
- Each image must be attached individually as a 'supplementary file' upon submission (not solely embedded in the accompanying manuscript) and named Fig. 1, Fig. 2, etc.

Tables

- Tables should be constructed carefully and simply for intelligible data representation. Unnecessarily complicated tables are strongly discouraged.
- Large tables will generally not be accepted for publication in their entirety. Please consider shortening and using the text to highlight specific important sections, or offer a large table as an addendum to the publication, but available in full on request from the author
- Embed/include each table in the manuscript Word file - do not provide separately as supplementary files.
- Number each table in Arabic numerals (Table 1, Table 2, etc.) and refer to consecutively in the text.
- Tables must be cell-based (i.e. not constructed with text boxes or tabs) and editable.
- Ensure each table has a concise title and column headings and include units where necessary.
- Footnotes must be indicated with consecutive use of the following symbols: * † ‡ § ¶ || then ** †† ‡‡ etc.

Do not: Use [Enter] within a row to make 'new rows':

Rather:

Each row of data must have its own proper row:

Do not: use separate columns for *n* and %:

Rather:

Combine into one column, *n* (%):

Do not: have overlapping categories, e.g.:

Rather:

Use <> symbols or numbers that don't overlap:

References

NB: *Only complete, correctly formatted reference lists in Vancouver style will be accepted. Reference lists must be generated manually and not with the use of reference manager software. Endnotes must **not** be used.*

- Authors must verify references from original sources.
- Citations should be inserted in the text as superscript numbers between square brackets, e.g. These regulations are endorsed by the World Health Organization,[2] and others.[3,4-6]
- All references should be listed at the end of the article in numerical order of appearance in the Vancouver style (not alphabetical order).
- Approved abbreviations of journal titles must be used; see the List of Journals in Index Medicus.
- Names and initials of all authors should be given; if there are more than six authors, the first three names should be given followed by et al.
- Volume and issue numbers should be given.
- First and last page, in full, should be given e.g.: 1215-1217 not 1215-17.
- Wherever possible, references must be accompanied by a digital object identifier (DOI) link). Authors are encouraged to use the DOI lookup service offered by CrossRef:
  - On the Crossref homepage, paste the article title into the 'Metadata search' box.
  - Look for the correct, matching article in the list of results.
  - Click Actions > Cite
  - Alongside 'url =' copy the URL between { }.
  - Provide as follows, e.g.: https://doi.org/10.7196/07294.937.98x