UNIVERSITY OF CAPE TOWN

INCIDENCE AND DISTRIBUTION OF HUMAN LEPTOSPIROSIS IN THE WESTERN CAPE PROVINCE, SOUTH AFRICA, (2010-2019): A RETROSPECTIVE STUDY.

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PREAMBLE

Declaration

I, Jacob Mugoya Gizamba (GZMJAC001), hereby declare that the work on which this dissertation 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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Date: 25 May 2022

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Abstract

Background

Leptospirosis is an emerging zoonotic infection of global importance. Among humans, the infection is associated with varying clinical manifestations ranging from mild self-limiting febrile illness to severe illness mainly characterized by pulmonary hemorrhagic syndrome and acute kidney injury due to Weil's disease. In addition, leptospirosis presents with symptoms that mimic commonly known infections that cause febrile illnesses such as malaria, influenza, hepatitis, yellow fever, and viral hemorrhagic diseases. This has consequently, led to under-estimation of the burden of leptospirosis hence contributing to it neglected status. The burden of leptospirosis is reported to be substantially high in tropical regions and resource limited settings. In Africa, few countries have data and reports on human leptospirosis and research studies are scarce. In South Africa, the infection is an important underreported public health concern however information on the incidence trend and distribution of the infection is lacking. Yet such epidemiological description is essential for effective prevention of the infection. This study aimed at determining the incidence of Human Leptospirosis from 2010 to 2019, and to compare the incidence based on seasonal and demographic factors in Western Cape Province (WCP), South Africa.

Methods

The study was a retrospective secondary analysis of all data on ELISA IgM tests that were positive for leptospirosis between January 2010 and December 2019 in WCP, South Africa. Data was obtained from the National Health Laboratory Services

(NHLS), where all serological tests on serum samples of patients who are clinically suspected to be having a leptospirosis infection are conducted. All leptospirosis positive results were grouped, and the incidence proportion of leptospirosis estimated according to sex, age, season, and year of occurrence. The provincial population sizes were used as the denominator when estimating the incidence and it was expressed as leptospirosis cases per 100,000 population. Negative binomial regression was used to estimate the effect of sex, year of occurrence and season on the incidence of human leptospirosis over the study period. The results were presented as incidence rate ratios (IRR) with 95% confidence intervals (CI).

Results

A total of 254 cases of human leptospirosis were recorded by the NHLS in the WCP, South Africa between 2010 and 2019. The highest number of cases was recorded in 2015 (42 cases, 16.5%) and lowest in 2012 (9 cases, 3.5%). The incidence of leptospirosis fluctuated widely across all the 10 years with the annual incidence ranging between 0.15 and 0.66 per 100,000 population and an average annual incidence of 0.40 per 100,000 population. The incidence was significantly higher among males compared to females (0.55 and 0.25 per 100,000 population respectively; incidence rate ratio (IRR) 2.2, 95% CI: 1.66,3.03) and the overall male to female ratio was 2.14:1. The average incidence of leptospirosis was highest among the 18-44-year-old age cohort (0.56 cases per 100,000 population), and lowest among the ≤17-year-old age cohort (0.07 cases per 100,000 population). The 18-44 (IRR 8.0, 95% CI: 4.65,15.15) and ≥ 45 (IRR 7.4, 95% CI: 4.17,14.17) age cohorts were more at risk of infection compared to ≤17age cohort. The incidence proportion in fall,

summer and spring seasons were slightly higher compared to what was observed in winter season. However, and there was no significant association between season and incidence of leptospirosis.

Conclusions

The incidence of leptospirosis widely fluctuated between 2010 and 2019, with males and those above 18 years of age substantially at risk of infection. The results show that leptospirosis is an important zoonotic disease within the province and potentially disproportionately affecting males and the productive age demographic groups. These findings emphasize the need to enhance targeted prevention strategies and provoke further investigation on the importance of environmental and socioeconomic factors on the occurrence of leptospirosis within Western Cape Province and South Africa at large.

List of abbreviations

CI	Confidence interval
IQR	Interquartile range
ELISA	Enzyme-linked Immunosorbent assay
ID	Identification number
IgM	Immunoglobulin M
IP	Incidence Proportion
IRR	Incidence Rate Ratio
NHLS	National Health Laboratory Services
SSA	Sub-Saharan Africa
UCT-HREC	University of Cape Town Human Research Ethics Committee
WCP	Western Cape Province
WC-DoH	Western Cape Department of Health

Organization of the dissertation

The dissertation is divided into 3 parts (part A, part B and part C).

Part A is the research protocol outlining the background literature, rationale of the study, objectives of the study, the research methodology, statistical analysis plan, the ethical consideration, and relevant references.

Part B is the journal manuscript formatted using submission guidelines of PLOS Neglected Tropical Diseases peer-review journal. This part outlines the study background, the methods used in the study, the study results, discussion of the findings and the conclusions from the findings.

Part C is the appendices section, and it includes documents of ethical approvals, supplementary tables, and submission guidelines for the PLOS Neglected Tropical Diseases peer-review journal. The Vancouver referencing style was used in the entire dissertation as delineated in the journal submission guidelines.

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PART A: RESEARCH PROTOCOL

Protocol Synopsis

Leptospirosis is an emerging zoonotic infection of global importance and is a cause of febrile illness in sub-Saharan Africa [1-3]. It is estimated that globally 1.03 million cases and 58,900 deaths due to leptospirosis occur annually [4, 5]. The infection among humans is associated with varying clinical manifestations and can mimic commonly known infections that cause febrile illnesses such as malaria, influenza, hepatitis, yellow fever, viral haemorrhagic diseases among others [1, 6]. Consequently, the infection has often been underdiagnosed and underreported [1]. Tropical regions and resource limited settings are reported to have the highest burden of leptospirosis [7]. However, in Africa, few countries have data and reports on human leptospirosis [2, 8] and research studies are scarce [3, 7]. Leptospirosis in South Africa has been suggested to be moderately high within the population [9-11]. Despite this, studies focusing on incidence trends and distribution are scarce, and the infection is largely underdiagnosed [9-11], yet such epidemiological description is essential for effective prevention of the infection. In addition, the effect of season, geographical location, gender, and age on the occurrence of leptospirosis has rarely been described in such setting.

The aim of the proposed study is to estimate the annual incidence of human leptospirosis cases and assess trends over a 10-year period in Western Cape province (WCP), South Africa. Furthermore, the incidence will be compared across gender, age, and seasons of the year.

This study will be a retrospective secondary analysis of data on human positive leptospirosis tests obtained by the National Health Laboratory Services (NHLS) in WCP, South Africa between 1st January 2010 and 31st December 2019. The study will not involve recruitment of study participants and there will be no contact with patients. All leptospirosis positive cases record by NHLS in WCP over the study period will be included in the analysis.

The identified positive leptospirosis cases will be described based on their sex, age, season, and year of occurrence. The incidence proportion of human leptospirosis will be estimated for each year, each season of the year, sex, and age over the 10-year period (2010-2019). This will be expressed as leptospirosis cases per 100,000 population. The population sizes to be used for calculating the incidence proportion will be extracted from data obtained from the Western Cape Province Department of Health, Population Circular H102 of 2020 [12]. Poisson regression models will be used to compare the incidence proportion of human leptospirosis between year of occurrence, age, sex, as well as between seasons of the year.

There will be no recruited study participants, as deidentified secondary data will be used; thus, minimal risk related to loss of privacy and anonymity. Data received from NHLS will be anonymized by NHLS whereby all patients' names, identification (ID) numbers and all other patient identifying information will not be part of the dataset received from NHLS. Participants in the study will be identified by unique random generated identifiers which cannot be linked to the participant's original identifiers. Furthermore, the data will only be accessed by a password-protected Microsoft excel

database kept on a password-protected personal computer. Since this is routinely collected data and anonymized as well, informed consent will not be obtained.

The findings of this study will contribute to the scientific knowledge about the occurrence and trends of leptospirosis within WCP population in the past 10 years (2010-2019) and how the occurrence varies based on seasons, location and demographically. This will inform policy on prevention and control of the Leptospirosis infection among the WCP as well as South Africa at large.

1. Introduction

1.1 Background

Leptospirosis is an emerging zoonosis of global importance [13, 14]. It is estimated that globally 1.03 million cases and 58,900 deaths occur annually [4, 7]. Occurrence of the disease is highest in tropical regions where the incidence ranges between 10 and 100 cases per 100,000 population per year compared to temperate regions where the incidence ranges between 0.1 and 1 cases per 100,000 population per year [14]. This is because the leptospires' survival is longer in warmer and moist environments compared to colder and drier environments [15]. However, leptospirosis has a seasonal occurrence in both tropical and temperate regions, with notable peaks occurring during the wet season and during summer respectively [16, 17].

With a ubiquitous distribution, leptospirosis transmission occurs either through direct exposure to urine or aborted tissues of reservoir animals; or indirect exposure such as contact with contaminated water, soil, and food [14]. Due to these modes of transmission, the infection has been reported to occur mostly among farmers, slaughterhouse workers, veterinarians, hunters, butchers, fish industry workers, construction workers and miners, hence its largely regarded as an occupational hazard [1, 8, 16, 18]. Additionally, the general population is at risk of the infection through recreational exposures such as camping, kayaking, adventure travelling, hiking, cave exploration or other activities done in infected water [1, 18]. Occupational and recreational exposures contribute between 30-50% of the leptospirosis cases [1]. Furthermore, environmental exposures such as improper waste management or living

in informal settlements in urban areas infested with rodents increase the risk of human leptospirosis infection [19, 20]. Despite the vast avenues through which the human population can be exposed to the leptospires, there is still limited information on occurrence and distribution of human leptospirosis within the general population [2].

Few countries in Africa have data and reports on human leptospirosis [2, 8] and research studies are significantly scarce within the continent [3, 7]. A previously published systematic review of peer reviewed studies conducted in Africa, reported a prevalence of acute human leptospirosis ranging from 2.3% to 19.8% among hospital patients presenting with febrile illness and an estimated total of 750,000 cases per annum [7, 8]. The review further reported that the incidence was highest in the East African region (25.7 cases per 100,000 population), followed by Central Africa with an estimated incidence of 13.5 cases/100,000 inhabitants; Southern Africa had the lowest estimated incidence, 3.4 cases/100,000 inhabitants [7]. However, its estimated that the morbidity is higher than what is recorded in all regions of Africa [21]. Lack of human disease data in most of the countries, little clinician or veterinarian awareness and challenges with clinical diagnosis are some of the factors contributing to the low reported morbidity levels hence more data is needed [8].

In South Africa, the incidence of leptospirosis is moderately high within the population [9-11]. A rodent-related zoonosis study conducted between 2003 and 2006 in three Provinces (Eastern Cape, Kwazulu-Natal and Limpopo) showed a seroprevalence of 19.8% among people sampled in Durban [10]. Furthermore, a study conducted between 2009 and 2011 reported a seroprevalence that ranged from 9% to12.5% among all clinical samples sent to the Special Bacterial Pathogens laboratory at the

National Institute for Communicable Diseases (NICD) for IgM ELISA testing from all over the country [10]. The most recent retrospective study conducted between 2005-2015, focused on patients presenting at a tertiary referral hospital in Western Cape Province (WCP), and a seroprevalence of 20% was reported [22]. However, these studies did not describe the incidence distribution within the country and how the incidence has changed over a period.

Studies outside Africa have highlighted the influence of geographical location, season, gender and age on the distribution and occurrence of human leptospirosis [23-25]. Annual seasonal changes have been reported to have a significant effect on the annual occurrence of this infection [15, 17, 23, 26-28]. Such association have scarcely been studied in Africa despite changes in climatic factors, demographic shifts, urbanization, and globalization [1-3, 5, 19]. Environmental conditions in Western Cape South Africa, (the coastal, and temperate conditions with wet winters and warmer summers) have been suggested to favour transmission of pathogenic *Leptospira* species; *Leptospira borgpetersenii* and *Leptospira interrogans* traditionally associated with rats [4, 9, 11]. The risk conferred by rodent infestation and overcrowding have been demonstrated in a recent leptospirosis outbreak that occurred in a prison in WCP, South Africa in 2015 [9]. Irrespective of this information, studies describing leptospirosis occurrence in relation to demographic, geographic location and seasonal factors are lacking in WCP.

To address these knowledge gaps, a retrospective study of human leptospirosis cases will be conducted using data generated from diagnostic tests done in public healthcare setting in WCP, South Africa. The study will aim to determine the incidence and trends

of human leptospirosis over a 10-year period (2010-2019), to describe the demographic characteristics of those diagnosed with leptospirosis, and to compare the incidence across sex, age, districts, and seasons.

1.2 Rationale of the study

Leptospirosis is an emerging zoonotic infection of global importance and is an important cause of febrile illness in Sub Saharan Africa (SSA) [1-3]. The infection among humans is associated with varying clinical manifestations and can mimic commonly known infections that cause febrile illnesses such as malaria, influenza, hepatitis, yellow fever, viral hemorrhagic diseases among others [1, 6]. Consequently, infections with leptospires have been misdiagnosed and under-reported. The incidence of human leptospirosis has been found to be increasing in South America and South-East Asia due to changes in climatic factors, demographic drifts, massive urbanization, and globalization in a form of international travel [1-3, 5, 6, 19]. However, little has been documented about the trend of leptospirosis incidence in Africa although reports indicate that the occurrence is moderately high and largely underestimated within the population in SSA [2, 8] as well as in South Africa [9, 10]. In addition, an outbreak of leptospirosis was reported in a prison in WCP in 2015, and this was linked to rodent infestation, increased prevalence of infected rodents and overcrowded settings [9]. Subsequent circulation of pathogenic *Leptospira spp* among rodents and presence of overcrowded settings [9] necessitates investigation of incidence of leptospirosis within WCP population. Furthermore, information on incidence and its relationship with demographics, geographical location and seasons of the year is largely significant in guiding prevention and control strategies of leptospirosis in low

middle-income countries but this information is scarce [2, 8]. By assessing the incidence of leptospirosis and its relationship with demographics, geographical and seasonal factors over time, we aim to provide information that will guide in formulation of prevention strategies of the infection within South Africa. The results from the study will as well provide insights prompting further epidemiological research on human leptospirosis within the country.

2. Aims and objectives

2.1 Aim

The aim of the proposed study is to estimate the annual incidence of human leptospirosis cases and assess trends over time and to compare the incidence based on demographics, season of occurrence and geographical location in WCP, South Africa between 2010 and 2019.

2.2 Objectives

1) To determine the annual incidence of human leptospirosis in Western Cape Province between 2010 and 2019
2) To describe the demographic characteristics of individuals with human leptospirosis in the Western Cape Province between 2010 and 2019
3) To determine and compare the incidence of human leptospirosis across sex and age demographics in Western Cape Province between 2010 and 2019
4) To determine and compare the incidence of human leptospirosis across districts of the Western Cape Province between 2010 and 2019

5) To determine and compare the incidence of human leptospirosis cases across seasons in the Western Cape Province between 2010 and 2019

2.3 Research questions

1) What is the annual incidence of human leptospirosis in Western Cape Province between 2010 and 2019 and how does this change over time?
2) What are the demographic characteristics of human leptospirosis cases in Western Cape Province between 2010 and 2019?
3) What is the incidence of human leptospirosis across sex and age demographics within Western Cape Province between 2010 and 2019?
4) What is the incidence of human leptospirosis across districts of Western Cape Province between 2010 and 2019 and does the incidence differ by district?
5) What is the incidence of human leptospirosis cases in Western Cape Province between 2010 and 2019 and does the incidence differ by season?

3. Methodology

3.1 Study design

A retrospective study will be conducted to estimate the incidence and describe the distribution of human leptospirosis cases diagnosed by the NHLS in WCP, South Africa between 1st January 2010 and 31st December 2019. The NHLS does serological testing on all serum samples of patients who are clinically suspected to be having a leptospirosis infection. Specimens from suspected patients are collected from different public healthcare facilities within the province and sent to a central NHLS laboratory in Cape Town.

3.2 Study area

The WCP is located on the south-Western coast of South Africa and is boarded by the Northern Cape and Eastern Cape provinces. The province has an estimated population of approximately seven million inhabitants three quarters of whom use public-sector healthcare services [29]. WCP is approximately 129,462 km^2 and has a population density of 45 inhabitants per km^2 [30]. Administratively, the province is divided into one metropolitan municipality (City of Cape Town) and five district municipalities (Central Karoo, Garden Route, Overberg, West Coast and Cape Winelands) [30]. The province has a diverse climate but is mostly dominated by a Mediterranean climate with a cool, wet winter and a warm, dry summer. The inland daily maximum temperatures range from 20 ◦C in winter to 32 ◦C in summer and the mean annual rainfall is less than 350 mm [31].

3.3 Sampling and study population

This is a retrospective study and will use routinely collected data. A sample size calculation is unnecessary because all leptospirosis cases recorded by NHLS between 1st January 2010 to 31st December 2019 will be included in the analysis. Participants in this study will include all patients who tested positive for leptospirosis in WCP during the 10-year period of the study.

3.4 Inclusion criteria

All patients meeting the following criteria will be included in the study: Cases with an ELISA IgM test positive for leptospirosis between 1st January 2010 and 31st December 2019, whose test was conducted at a central NHLS laboratory in WCP.

3.5 Data collection

3.5.1 Human Leptospirosis cases

Data on all ELISA IgM serological tests that are positive for leptospirosis during the study period (2010 to 2019) within WCP will be obtained from NHLS database. ELISA IgM test is the serological test conducted on all samples submitted to NHLS for leptospirosis screening and it is sensitive in detecting of new onset of illness [1]. Patient data extracted from the NHLS database will include the patient age, sex, the name of the health facility at which the test was taken, the date when the test was done and the test result.

Table A 1: Variables to be included in the analysis

Variables	Scale	Categories
Age (years)	Numerical-continuous	
	Categorical ordinal	≤ 17-year-old
		18-44-year-old
		≥45-year-old
Sex	Categorical binary	Male, Female
Name of health facility	Categorical nominal	
District of the health facility	Categorical nominal	City of Cape Town, Central Karoo, Garden Route, Overberg, West Coast, Cape Winelands
Month test conducted	Categorical ordinal	January to December
Season test was conducted	Categorical nominal	summer, winter, spring, fall
Year test was conducted	Categorical-discrete	2010 to 2019
Test results	Categorical binary	ELISA IgM positive

3.5.2 Demographical data

The population sizes based on year, sex, age and district in WCP for the 10-year period (2010-2019) will be obtained from the Western Cape Department of Health

(WC-DoH) population circular H102 of 2020 [12]. This data will be used as the denominator when calculating the incidence of leptospirosis infection.

3.6 Exposures of interest

3.6.1 Objective one:

To determine the annual incidence of human leptospirosis in WCP between 2010 and 2019.

The exposure of interest will be the year in which the case was diagnosed. This variable will be conceptualised as a consecutive 12-month period from 1st January to 31st December, thus there will be ten categories for year (2010 to 2019).

3.6.2 Objective two and three:

To describe the demographic characteristics of individuals with human leptospirosis and to determine and compare the incidence of the infection across sex and age demographics in WCP between 2010 and 2019.

The exposure of interest will be the age and sex of the cases. Sex will include two categorises, Male and Female while age will be categorized into three age groups: ≤17-year-old age, 18–44-year-old age and ≥ 45-year-old.

3.6.3 Objective three:

To determine and compare the incidence of human leptospirosis across districts of the WCP between 2010 and 2019**.**

The exposures of interest will be the district municipalities of the health facility that submitted the sample to the laboratory for testing. This will be categorized as: City of Cape Town, Central Karoo, Garden Route, Overberg, West coast, and Cape Winelands.

3.6.4 Objective four:

To determine and compare the incidence of human leptospirosis cases across seasons in the Western Cape Province between 2010 and 2019

The exposure of interest will be the season of the year in which the case was diagnosed. This will be categorized as:

- Summer (1st December-28/29th February)
- Autumn/Fall (1st March-31st May)
- Winter (1st June-31st August)
- Spring (1st September-30th November).

3.7 Outcomes of interest

The outcomes of interest in this analysis will be the incidence of human leptospirosis. A case in this study will be defined as a suspected case with laboratory detected IgM antibodies against *Leptospira* spp. by Enzyme-linked Immunosorbent Assay (ELISA) [32].

3.8 Data analysis and management plan

3.8.1 Statistical analysis

All data related to human leptospirosis tests from the NHLS database will be extracted by NHLS personnel and will be obtained in a password protected Microsoft excel. All statistical analysis will be conducted using R software version 1.2.5033.

Summary statistics of the data will be described whereby all continuous variables will be summarized using either mean and standard deviation or median and interquartile range depending on the distribution of the variable. Categorical variables will be summarized using frequency and proportions. Demographic data of all the leptospirosis cases will be summarized and case for each year will be stratified by sex and age group.

Using the name of the health facility where the test was done, the district of the health facility will be identified and therefore all health facilities will be grouped based on the district municipality where they are located. Depending on how the NHLS dataset will look like, we intend using the district of the healthcare facility that submitted the specimen to the central NHLS laboratory, to infer district location of the case. The total number of leptospirosis cases in each district municipality will then be summarized. Using the date when the test was done, the case will be assigned to a particular month of the year. Furthermore, the months will be categorized into seasons; Summer (1st December-28/29th February), Autumn/Fall (1st March-31st May), Winter (1st June-31st August), and Spring (1st September-30th November) and the cases in each

season will be calculated. Subsequently, cases in each year (2010-2019) will be summarized by sex, age group, district municipality and season of occurrence.

The incidence proportion of human leptospirosis will be estimated for each year (2010 to 2019). The incidence will be estimated for each season of the year, sex and age group and district municipality over the 10-year period. The incidence proportion will be expressed as leptospirosis cases per 100,000 population. The population sizes to be used for calculating the incidence proportion will be extracted from data obtained from the WC-DoH, Population Circular H102 of 2020 [12].

Poisson regression models will be used to compare the incidence proportion of human leptospirosis between sex, between age groups, between years, between district municipalities as well as between seasons of the year. The cut-off value for statistical significance will be 0.05 and the 95% confidence interval will be calculated for all point estimates.

3.8.2 Data management

All data obtained from NHLS will be already anonymized, such that all patient identifiable information will not be part of the dataset that will be used to conduct the analysis. Only unique identifiers that cannot be linked to direct identifiers will be available on the dataset to assist with analysis. The data will only be accessed by a password-protected Microsoft excel database kept on a password-protected personal computer.

4. Ethical considerations

4.1 Informed consent

Individual informed consent will not be obtained because this will be a study analysing routinely collected secondary data from NHLS.

The study protocol will be submitted to University of Cape Town Human Research Ethics Committee (UCT-HREC) for ethical approval, and to the Western Cape Department of Health for Provincial approval.

4.2 Benefits

There will be no direct benefits to the study participants because the study involves analysis of secondary data hence no participants will be enrolled or recruited. However, indirect benefits to the participants and the general population will be provided by the results from this study. The study will contribute to the scientific knowledge about the occurrence and trend of leptospirosis within WCP population and how the occurrence varies based on seasons of the year, sex, age, and district municipality. This will inform policy on effective prevention and control of the occurrence and transmission of leptospirosis by indicating risk groups, locations, and seasons with high incidence within the province.

4.3 Risks

Risks to the study participants will be minimal because the study will involve analysis of secondary data hence no participants will be enrolled. There will be some minimal indirect risk related to loss of privacy and anonymity. This will be mitigated by anonymization whereby all patients' names, ID numbers and all other patient identifying information will not be part of the dataset received from NHLS. Participants in the study will be identified by unique random generated identifiers which cannot be linked to the participant's original identifiers.

4.4 Dissemination of findings

The findings of this study will be submitted as a mini dissertation in partial fulfilment of a Master's in Public Health degree at the University of Cape Town (UCT). The findings will also be submitted for publication in a peer reviewed journal and to the UCT's Institutional Repository (OpenUCT). The findings will be presented at local and international conferences as well.

4.5 Strengths and Limitations

This study is analysing data over a 10-year period; therefore, it will allow understanding of the incidence trends and epidemiological characteristics of human leptospirosis where prevention strategies can be implemented to curb the spread of leptospirosis in South Africa. However, there are shortcomings to this study; firstly, the study is utilizing routinely collected data therefore data quality may be variable and there may be missing data. This also means that there will be limited measurement of

possible confounders such as occupation, co-morbidities, socio-economic status, and physical location among others. Secondly, the study is utilizing data collected from passive surveillance, hence there is a great potential of underestimation of the exact occurrence of leptospirosis in WCP. The passive surveillance system only records cases who seek medical assistance, therefore missing out on cases that might have had mild clinical symptoms and those who were unable to seek treatment immediately more especially in rural areas. Thirdly, the study is intending to use district location of the health facility that submitted the specimen to infer district location of the cases. This assumption has implications on the study objective seeking to describe leptospirosis incidence by district, since not all patients access healthcare services from a health facility within their district of residence.

4.6 Timeframe

Table A 2: Proposed timetable from start to end of the study

	2021												2022		
Research phases	**Jan-Mar**			**Apr-Jun**			**Jul-Sept**			**Oct-Dec**			**Jan-Mar**		
Proposal development															
Department protocol approval															
Ethical committee submission															
NHLS Data acquisition process															
Data management															
Data analysis															
Manuscript write-up															
Submission for examination															
Addressing Examiners comments															

4.7 Budget

The research activities in the study will be self-funded and the primary author will personally be conducting the analysis of the study in partial fulfilment of requirements for a Master of Public Health degree. The study will be using secondary data from NHLS, hence there are no direct costs expected to arise from the data acquisition process.

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PART B: MANUSCRIPT

Incidence and distribution of human leptospirosis in the Western Cape Province, South Africa, (2010-2019): A retrospective study.

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Key words: Human leptospirosis, Incidence, South Africa

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[1] The target peer reviewed journal that was chosen to guide the formatting of this manuscript is the **PLOS Neglected Tropical Diseases**. The journal submission guidelines for authors are included in **Part C (Appendix 4)** of the dissertation. As per the MPH guidelines, the co-authors are not listed in the main manuscript, but their contributions are recognized in the acknowledgement section of this dissertation.

Abstract

Background

Leptospirosis is an emerging zoonosis of global importance. In South Africa, the infection is an underreported public health concern, with limited information on its incidence and distribution. This study aimed to determine the incidence of human leptospirosis from 2010 to 2019 in Western Cape Province (WPC), and to compare the incidence based on seasonal and demographic factors.

Methods

A retrospective study was conducted with data on leptospirosis diagnoses by sex, age, season, and year in WCP obtained from the National Health Laboratory Services. With the provincial population sizes as the denominator, the incidence of leptospirosis was estimated and expressed as cases per 100,000 population. Negative binomial regression was used to estimate the effect of sex, season, and year on the incidence of leptospirosis.

Results

A total of 254 cases of leptospirosis were reported between 2010 and 2019, with the highest number of cases being in 2015. The annual incidence ranged between 0.15 and 0.66/100,000 population with a 10-year average incidence of 0.40/100,000 population. The incidence was higher among males than in females (0.55 vs. 0.25/

100,000 population; incidence rate ratio (IRR) 2.2, 95% CI: 1.66,3.03). The 18-44 years age cohort had the highest average incidence (0.56/100,000 population), while the ≤17 years age cohort had the lowest incidence (0.07/100,000 population). The 18-44 (IRR 8.0, 95% CI: 4.65,15.15) and ≥45 (IRR 7.4, 95% CI: 4.17,14.17) years age cohorts were more at risk of infection compared to ≤17 years age cohort. The average incidence of the infection was similar among seasons and there was no significant association between season and incidence of leptospirosis.

Conclusions

The results highlight that leptospirosis is an important zoonosis within the province disproportionately affecting males and the productive age demographic groups. These findings should enhance targeted prevention and provoke further investigation on the importance of environmental and socioeconomic factors on leptospirosis burden.

Author Summary

Leptospirosis is an emerging zoonotic bacterial disease of global importance. Despite its wide distribution, the disease is largely underestimated because its clinical manifestations mimic certain commonly known febrile illnesses such as malaria, influenza, hepatitis, and yellow fever. Leptospirosis burden in South Africa has been suggested to be moderately high however studies on the burden of the infection are lacking. This study sought to determine the incidence and trends of leptospirosis in the Western Cape Province, South Africa between 2010 and 2019. Overall, leptospirosis average incidence was 0.4 cases per 100,000 population (ranging from 0.15 to 0.66 cases per 100,000 population). Leptospirosis incidence was highest among male individuals and among those who were aged 18-years and above, however, the incidence did not differ by seasons. These estimates highlight that leptospirosis is an important zoonotic disease within the province and potentially disproportionately affecting males and productive age demographic groups. Therefore, this indicates the need for an all-encompassing One Health Approach to obtain all relevant information concerning leptospirosis distribution and risk factors in South Africa and in Africa at large to effectively enhance leptospirosis prevention strategies.

1 Introduction

Leptospirosis is an emerging, global zoonosis with an estimated 1.03 million cases and 58,900 deaths occurring annually [1-5]. The disease is caused by pathogenic spirochetes *Leptospira* species currently reported to comprise 66 different species with more than 300 serovars [6]. The infection in humans is associated with varying clinical manifestations ranging from a mild self-limiting febrile illness to severe illness characterized by dysfunction of multiple organs such as liver, kidneys, lungs, and the brain [7] potentially leading to pulmonary hemorrhagic syndrome and acute kidney injury due to Weil's disease [3, 8-10]. In addition, leptospirosis presents with symptoms that can mimic commonly known infections that cause febrile illnesses such as malaria, influenza, hepatitis, yellow fever, and viral hemorrhagic diseases among others [11, 12]. Consequently, the infection has often been underdiagnosed and underreported [11]. Despite the life-threatening nature of leptospirosis, there is little published data on morbidity associated with the infection [2, 3, 13] contributing to its neglected status.

The risk of leptospirosis infection among humans occurs either through direct exposure to urine or aborted tissues of wild or domestic reservoir animals such as rodents and livestock/pets [2, 14], or through indirect exposure such as contact with contaminated water, soil, and food [5, 14]. In South Africa, a recent prison outbreak of leptospirosis was associated with exposure of inmates to rat urine in an overcrowded prison [15]. Due to the predominant modes of transmission, leptospirosis is regarded as an occupational hazard affecting mainly farmers, sewer workers, veterinarians, and military personnel [11, 16, 17]. However, the transmission patterns are changing

because there are increasing reports of leptospirosis in the general population. These cases are due to recreational exposures such as camping, kayaking, adventure travelling, hiking, cave exploration and other activities done in infected water [11, 17, 18]. Due to the diverse avenues through which humans can be exposed to the leptospires, occurrence of leptospirosis within the general population requires consideration [7, 19].

Globally, tropical regions as well as resource limited settings are reported to have a higher burden of leptospirosis compared to temperate regions [3]. However, in Africa, few countries have data and reports on human leptospirosis [19, 20] and research studies are scarce [3, 4]. A previously published systematic review, of peer reviewed studies conducted in Africa, reported a prevalence of acute human leptospirosis ranging from 2.3% to 19.8% among hospital patients presenting with febrile illness and an estimated total of 750,000 cases per annum [20]. It was further concluded in this study that the morbidity of human leptospirosis in Africa is likely to be high relative to other global regions [20]. The need of more studies on the occurrence of leptospirosis in Africa was emphasized in another systematic review by De Vries et al.[19] to reliably understand the extent of the problem.

The incidence of human leptospirosis in South Africa has been suggested to be moderately high within the population [15, 21, 22]. Findings about the continued circulation of highly pathogenic *Leptospira* spp. among rodents suggests that the infection maybe an important underreported public health concern in the country [15, 21]. A study conducted between 2009 and 2011 reported a seroprevalence that ranged from 9% to12.5% among all clinical samples sent to the Special Bacterial

Pathogens laboratory at the NICD for IgM ELISA testing from all over the country [22]. Environmental conditions in the Western Cape Province (WCP), South Africa, (the coastal, and temperate conditions with wet winters and warmer summers) have been suggested to favor transmission of pathogenic *Leptospira* species *Leptospira borgpetersenii* and *Leptospira interrogans* traditionally associated with rats [2, 15, 21]. The most recent retrospective study conducted between 2005-2015, focused on patients presenting at a tertiary referral hospital in WCP, and a seroprevalence of 20% was reported [23].

Studies outside Africa have highlighted the influence of season, gender and age on the distribution and occurrence of human leptospirosis [24-26]. Such associations have scarcely been studied in Africa despite changes in climatic factors, demographic shifts, urbanization, and globalization [4, 11, 19, 27-29]. The risk conferred by rodent infestation and overcrowding have been demonstrated in a recent leptospirosis outbreak that occurred in a prison in WCP, South Africa [15]. Despite this information, studies describing leptospirosis occurrence in relation to demographic and seasonal factors are lacking in WCP.

To address these knowledge gaps, a retrospective study on human leptospirosis cases in the WCP, South Africa was conducted using data generated from diagnostic tests done in a public health care setting in Cape Town, South Africa. The study aimed at determining the incidence and trends of human leptospirosis over a 10-year period (2010-2019), with respect to year and seasonality and to describe the demographic characteristics of leptospirosis cases.

2 Methods

2.1 Study setting

WCP is located on the south-western coast of South Africa and is bordered by the Northern Cape and Eastern Cape provinces. WCP has a population of approximately seven million inhabitants, three quarters of whom use public-sector healthcare services [30]. The province is approximately 129,462 km^2 and has a population density of 45 inhabitants per km^2 [31]. Administratively, the province is divided into one metropolitan municipality (City of Cape Town) and five district municipalities (Central Karoo, Garden Route, Overberg, West Coast and Cape Winelands) [31]. The province has a diverse climate but is dominated by a Mediterranean climate with a cool, wet winter and a warm, dry summer. The inland daily maximum temperatures range from 20 ◦C in winter to 32 ◦C in summer and the mean annual rainfall is less than 350 mm [32].

2.2 Study design and study population

A retrospective study was conducted by using data provided by the data warehouse of the National Heath Laboratory Services (NHLS), on all human leptospirosis tests conducted at public healthcare facilities in the WCP, South Africa. The NHLS does serological testing on all serum samples of patients who are clinically suspected to be having a leptospirosis infection. Specimens from suspected patients are collected from different public healthcare facilities within the province and sent to a central NHLS laboratory in Cape Town. In this analysis, data included are from patients who were

positive for *Leptospira* IgM antibodies (presumptive leptospirosis cases) between 1st January 2010 and 31st December 2019.

2.3 Data collection

Human leptospirosis cases were considered for all ELISA IgM serological tests that were positive for Leptospirosis during the study period (2010 to 2019). Pan Bio ® *Leptospira* IgM ELISA (Abbott, Illinois, USA) testing is the serological test conducted on all samples submitted to NHLS for leptospirosis screening and is sensitive in detecting new onset of illness [11]. Patient data extracted included the patient's age, sex, year, date of test (year, month, and day) and the name of the health facility that submitted the specimen for testing.

The population sizes based on year, sex, and age in WCP for the 10-year period were extracted from the Western Cape Department of Health population circular H102 of 2020 [33]. This data was used as the denominator when calculating the incidence of leptospirosis infection.

2.4 Statistical analysis

All human leptospirosis cases were tabulated according to sex, age, season, and year of occurrence (2010-2019) and their frequency and proportions were calculated. The cases were not grouped based on their geographical location (district municipality) because the patient address information was not part of received data. Furthermore, the location of the health facility that submitted the specimen could not be assumed to be the same as the patient's location because of the possibility of patients seeking

healthcare or being hospitalized in a facility outside of their actual district of residence. The date when the test was done was used to assign the case to a particular year and season. Season was categorized as Summer (1st December-28/29th February), Autumn/Fall (1st March-31st May), Winter (1st June-31st August), and Spring (1st September-30th November). The variable year was conceptualized as a consecutive 12-month period from 1st January to 31st December, thus there were ten categories for year (2010 to 2019).

The incidence proportion of human leptospirosis for each year (2010-2019) was estimated and expressed as leptospirosis cases per 100,000 population. The incidence proportion by sex and age group as well as for each season of the year was also estimated. The Kruskal-Wallis Rank Sum Test was used to compare the average annual incidence between seasons of the year, age groups and between years. The Wilcoxon Rank Sum Exact Test was used to compare the average annual incidence between sex demographics. The negative binomial regression was used to estimate the effect of sex, gender, year of occurrence and season of the year on the incidence of human leptospirosis over the study period. This is a suitable regression model to use instead of the traditional Poisson regression in situations where modelling involves a count variable that is over-dispersed (the mean is less than the variance). The results were presented as incidence rate ratios (IRR) with 95% confidence intervals (CI). The cut-off value for statistical significance was 0.05. All statistical analysis was conducted using R software version 1.2.5033.

2.5 Ethical considerations

Ethical approval was obtained from the University of Cape Town Human Research Ethics Committee (UCT-HREC) (reference number: HREC REF: 303/2021). The Western Cape Provincial approval was granted by Groote Schuur Hospital Research Ethics Committee. The approval to use data from NHLS database was given by the NHLS Academic Affairs and Research office (reference number: PR2118953).

3. Results

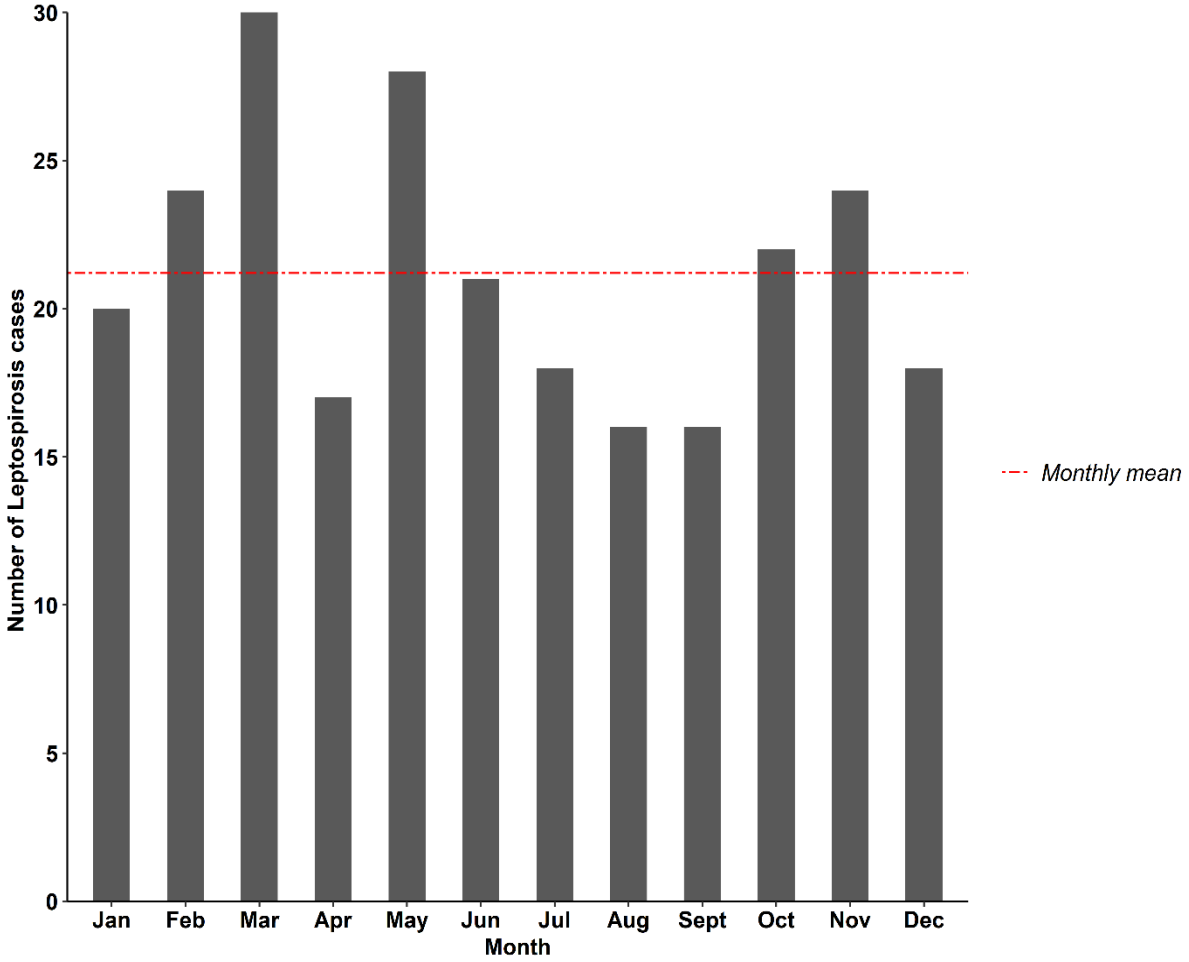
3.1 Distribution of leptospirosis cases in WCP, 2010-2019

During the 10-year study period (2010-2019), a total of 254 presumptive cases of human leptospirosis were recorded by the National Health Laboratory Services (NHLS) (**Table B 1**) for persons in the Western Cape Province, South Africa. Most of the cases reported had their specimen submitted from healthcare facilities located within the City of Cape Town Metropolitan (**S2 Table 2**). The highest number of cases was recorded in 2015 (42 cases, 16.5%) and lowest in 2012 (9 cases, 3.5%). When grouped by month of occurrence, the month of March (30 cases) had the highest number of cases over the 10-year period while September (16 cases) had the lowest number of the cases (**Fig B 1**). When grouped into seasons, more cases were observed in fall season (75 cases), followed by summer, spring, and winter (64, 62, 53 cases respectively) (**Table B 1**). More males were infected than females, with the males accounting for 173 (68.1%) cases compared to 81 (31.9%) cases who were females, and the overall male to female ratio was 2.136:1 (**Table B 1**). The median age of the observed cases was 37.0 (Interquartile range (IQR) 28.0-48.0) years. Overall, 163 cases (64.2 %) were in the 18–44-year-old age group, 78 cases (30.7%) were in ≥ 45-year-old age group while 13 cases (5.1%) were in the ≤17-year-old age group over the 10-year study period (**Table B 1**).

Table B 1*:* Distribution of leptospirosis cases by age, sex, season, and year of occurrence in Western Cape Province South Africa (2010-2019)

Variables	All Cases (n=254) n (%)
Age (years) median (IQR)	37.0 (28.0,48.0)
Age group	
≤17	13 (5.1)
18-44	163 (64.2)
≥ 45	78 (30.7)
Sex	
Male	173 (68.1)
Female	81 (31.9)
Season of the Year	
Summer	64 (25.2)
Fall	75 (29.5)
Winter	53 (20.9)
Spring	62 (24.4)
Year of occurrence	
2010	34 (13.4)
2011	19 (7.5)
2012	9 (3.5)
2013	12 (4.7)
2014	28 (11.0)
2015	42 (16.5)
2016	37 (14.6)
2017	22 (8.7)
2018	31 (12.2)
2019	20 (7.9)

IQR: Interquantile Range; n: frequency.

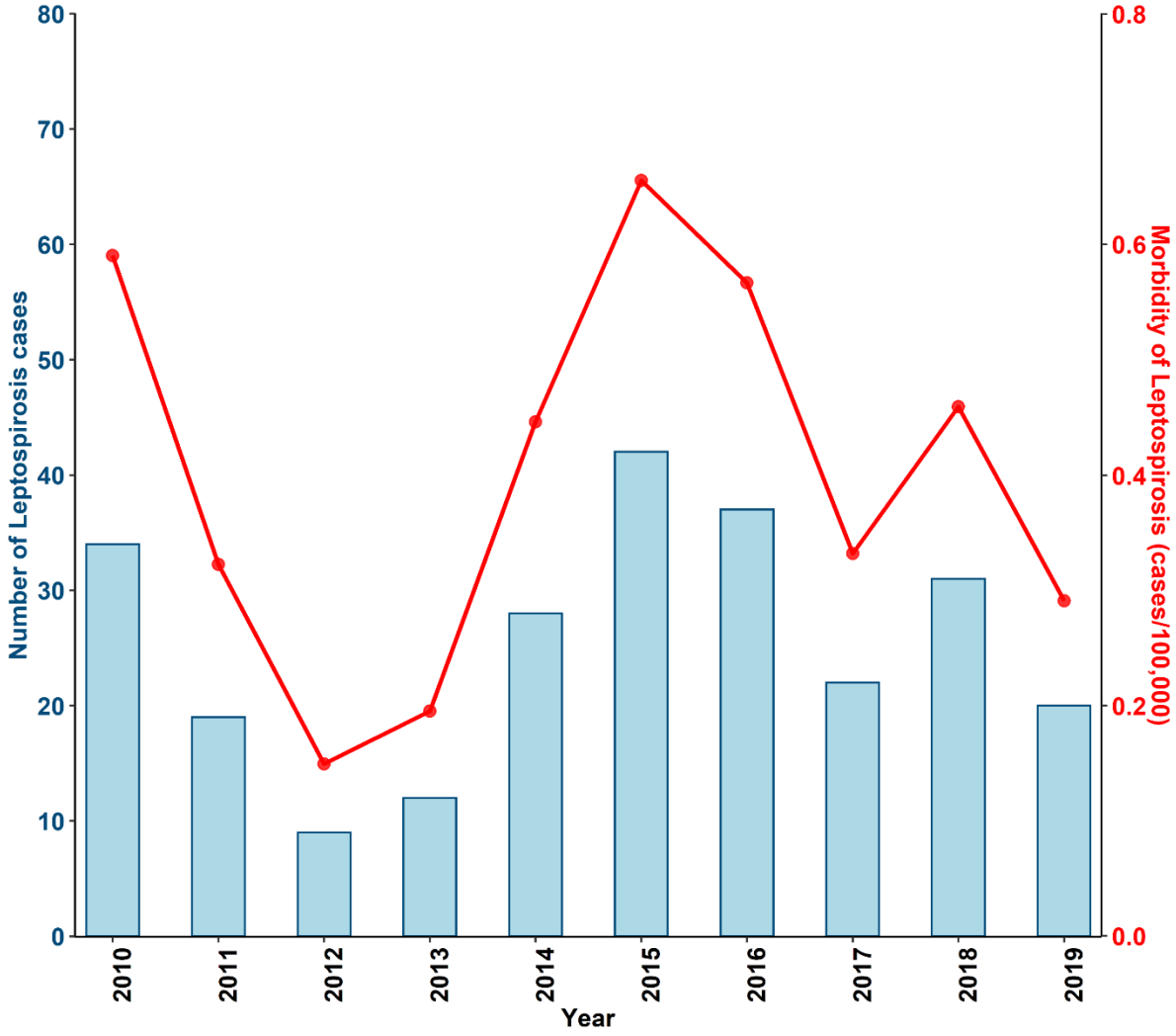


224 **Fig B 1*:* Monthly distribution of leptospirosis cases in Western Cape Province**

225 **over a 10-year period (2010-2019)**

3.2 Incidence of leptospirosis in the WCP, 2010-2019

The incidence of leptospirosis fluctuated widely across the 10 years. The annual incidence ranged from 0.15 to 0.66 cases per 100,000 population, with an average annual incidence of 0.40 cases per 100,000 population. The annual number of cases and the incidence are plotted against the year of occurrence to show the trends across the years (**Fig B 2**). The incidence followed a downward trend between 2010 (0.59 cases per 100,000 population) and 2012 (0.15 cases per 100,000 population). However, there was an increase in the incidence from 2013 (0.20 cases per 100,000 population) which peaked in 2015 (0.66 cases per 100,000 population). This was followed by a gradual decrease in the incidence between 2015 and 2019 (0.66-0.27 cases per 100,000 population) except for year 2018 where the annual incidence was higher than the one observed in 2017.

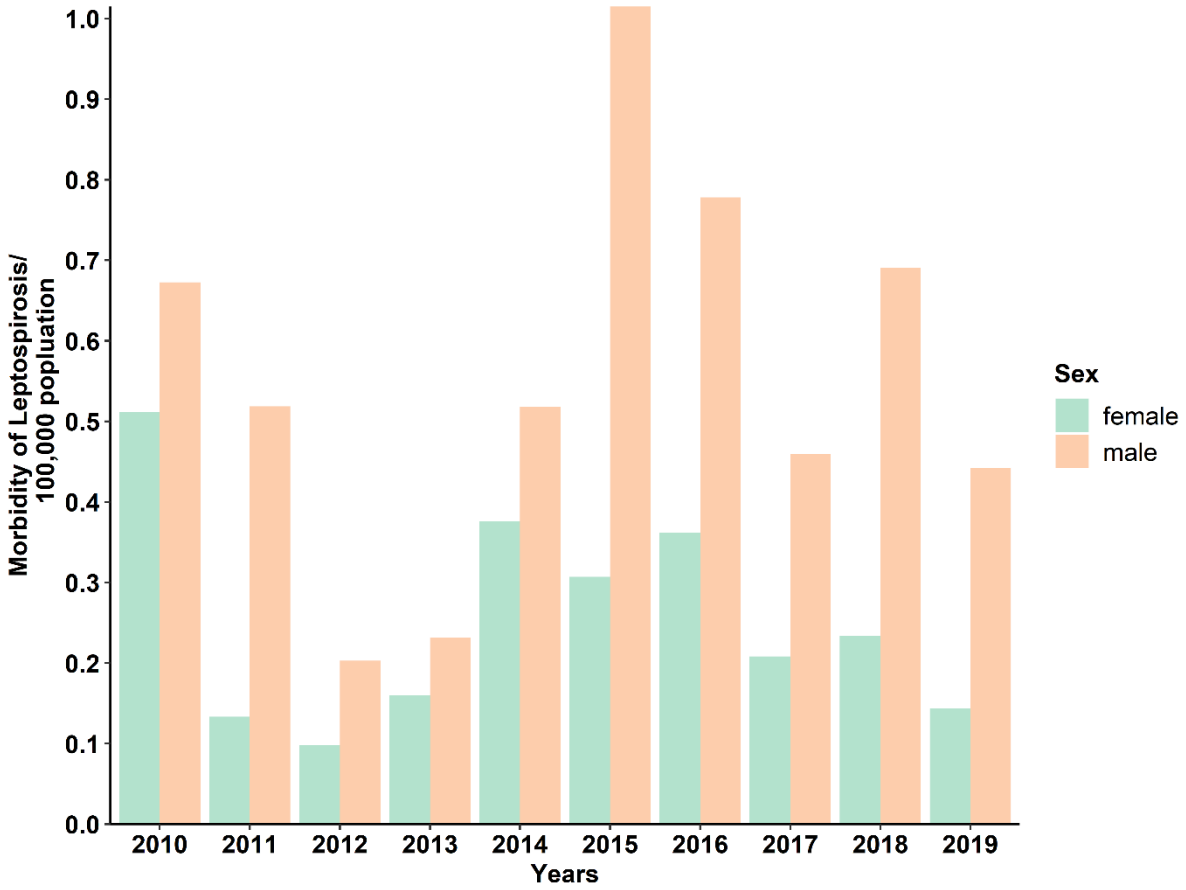


238 **Fig B 2: The number of cases and incidence of leptospirosis per year (2010 to**

239 **2019) in Western Cape Province, South Africa.**

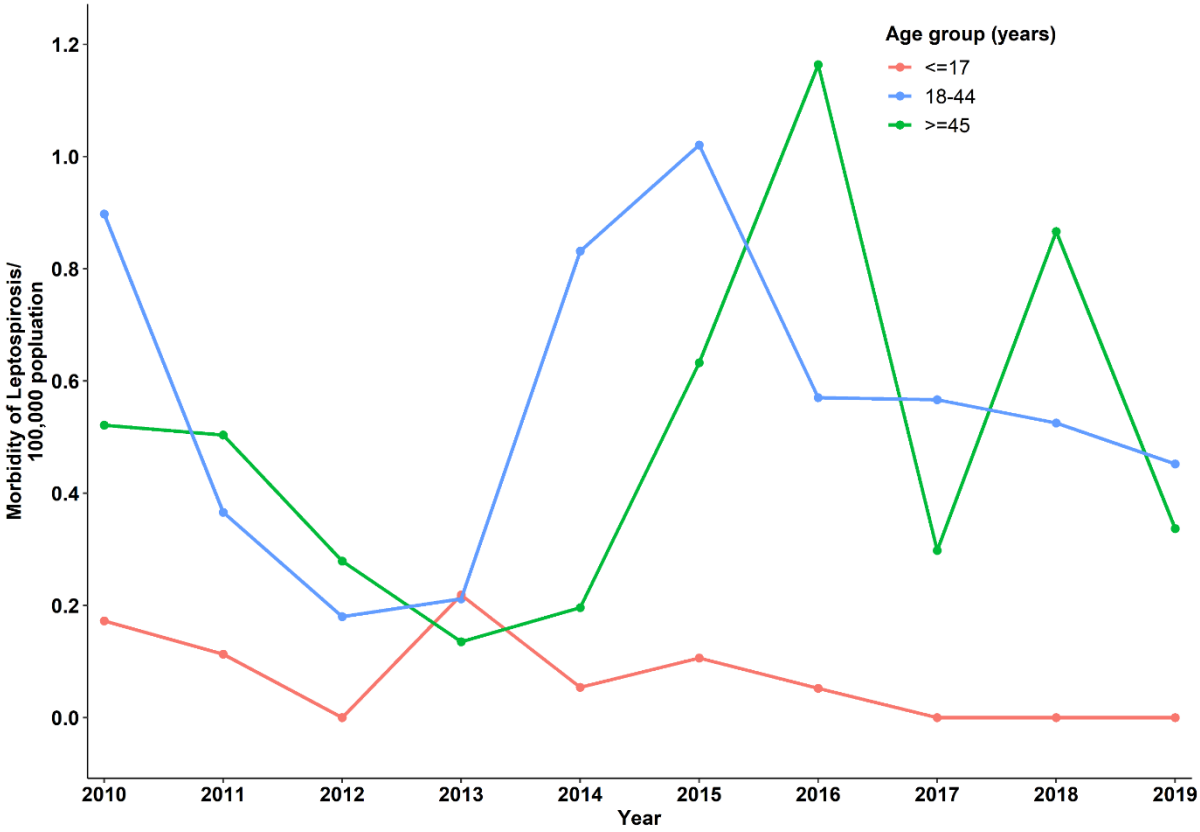
Throughout the study period, the incidence was highest among males compared to females (**Fig B 3**), with the average incidence among males being 0.55 cases per 100,000 population and 0.25 cases per 100,000 population among females (p-values=0.004). During the entire study period, the incidence was highest in the 18-44-year-old and ≥ 45 years-old age groups compared to the ≤17-year-old age group (**Fig B 4** and **S1 Table 2**). The average incidence differed substantially between the age groups with the highest average incidence being among the 18-44-year-old age group (0.56 cases per 100,000 population) over the 10-year period, followed by ≥ 45 years-old age group (0.49 cases per 100,000 population) and the ≤17-year-old age group had the lowest incidence (0.07 cases per 100,000 population) (**S1 Table 2**).

The results indicate that on average, the annual incidence was higher during the fall season (0.12 cases per 100,000 population) and lowest in the winter season (0.08 cases per 100,000 population) over the 10-year period (**Table B 2**). However, the observed difference in the average incidence of leptospirosis between the seasons of the year was not statistically significant.



255 **Fig B 3: Incidence of leptospirosis in males and females from 2010 to 2019 in**

256 **Western Cape Province.**



257 **Fig B 4*:* Incidence of leptospirosis by age group from 2010 to 2019 in Western**

258 **Cape Province, South Africa.**

Table B 2*:* Seasonal incidence of leptospirosis for the years 2010-2019, in Western Cape Province, South Africa.

Year	Summer	Fall	Winter	Spring
	IP/100,000 (95% CI)	IP/100,000 (95% CI)	IP/100,000 (95% CI)	IP/100,000 (95% CI)
2010	0.10 (0.02,0.19)	0.28 (0.14,0.41)	0.12 (0.03,0.21)	0.09(0.01,0.16)
2011	0.12 (0.03,0.21)	0.03 (-0.01,0.08)	0.07 (0.001,0.13)	0.10 (0.02,0.18)
2012	0.07 (0.001,0.13)	0.03 (-0.01,0.08)	0.02 (-0.02,0.05)	0.03 (-0.01,0.08)
2013	0.05 (-0.01,0.10)	0.05 (-0.01,0.10)	0.07 (0.001,0.13)	0.03 (-0.01,0.08)
2014	0.02 (-0.01,0.05)	0.10 (0.02,0.17)	0.14 (0.05,0.24)	0.19 (0.08,0.30)
2015	0.20 (0.09,0.31)	0.17 (0.07,0.27)	0.16 (0.06,0.25)	0.13 (0.04,0.21)
2016	0.18 (0.08,0.29)	0.21 (0.10,0.33)	0.03 (-0.01,0.07)	0.14 (0.05,0.23)
2017	0.12 (0.03,0.18)	0.09 (0.02,0.16)	0.08 (0.01,0.14)	0.06 (0.001,0.12)
2018	0.12 (0.04,0.20)	0.15 (0.06,0.24)	0.09 (0.02,0.16)	0.10 (0.03,0.18)
2019	0.04(-0.01,0.09)	0.07 (0.01,0.14)	0.07 (0.01,0.14)	0.10 (0.03,0.18)
Average incidence	**0.10 (0.03,0.18)**	**0.12 (0.04,0.20)**	**0.08 (0.02,0.15)**	**0.10 (0.02,0.17)**

IP: incidence proportion; CI: confidence interval

3.3 Effect of sex, age, year, and season on leptospirosis incidence in WCP, 2010-2019

Table B 3 shows the results from the Multivariable Negative Binomial Regression (MNBR) model that was run to identify factors associated with the incidence of leptospirosis during the 10-year period. The incidence rate of leptospirosis among the male demographic was 2.2 (95% CI: 1.66,3.03) times higher than among females during the entire study period. The model results indicated that on average the incidence rate of leptospirosis among 18-44 and ≥ 45 age groups was 8.0 (95% CI: 4.65,15.15) and 7.4 (95% CI: 4.17,14.17) times higher than the incidence rate among ≤17 years age group. The incidence was substantially lower in 2012 (IRR 0.25; 95% CI: 0.11,0.54), 2013 (IRR 0.34; 95% CI: 0.16,0.68), 2017 (IRR 0.53; 95% CI: 0.28,0.98) and 2019 (IRR 0.47; 95% CI: 0.24,0.87) compared to the incidence in 2010. From the model results, the incidence rate in fall, summer and spring seasons was 1.4 (95% CI: 0.95,2.15), 1.2 (95% CI: 0.78,1.81) and 1.2 (95% CI: 0.77,1.78) times higher than what was observed in winter season, however the observed difference was not statistically significant.

Table B 3*:* MNBR analysis results between year, season, sex, and age group with incidence of leptospirosis

Variables	IRR	95% CI	*P.value*
Year of occurrence			
2010	Ref	_	_
2011	0.543	(0.281,1.026)	0.061
2012	0.25	(0.106,0.536)	**0.001**
2013	0.337	(0.157,0.684)	**0.003**
2014	0.73	(0.402,1.314)	0.294
2015	1.033	(0.597,1.796)	0.907
2016	0.956	(0.546,1.677)	0.874
2017	0.53	(0.282,0.98)	**0.046**
2018	0.748	(0.418,1.334)	0.323
2019	0.466	(0.244,0.872)	**0.019**
Season of the year			
Winter	Ref	_	_
Spring	1.166	(0.765,1.782)	0.475
Summer	1.184	(0.778,1.808)	0.431
Fall/autumn	1.422	(0.947,2.147)	0.091
Sex			
Female	Ref	_	_
Male	2.239	(1.664,3.034)	**<0.001**
Age group (years)			
≤17	Ref	_	_
18-44	8.045	(4.654,15.147)	**<0.001**
≥45	7.391	(4.167,14.169)	**<0.001**

IRR: incidence rate ratio; CI: confidence interval; ref: reference category; MNBR: Multivariable Negative Binomial Regression.

4. Discussion

This study provides a first description of the incidence of human leptospirosis in the Western Cape Province (WCP), South Africa across a 10-year period. Here we report an average incidence of 0.40 leptospirosis cases per 100,000 population, ranging from 0.15 in 2012 to 0.66 in 2015 cases per 100,000. There was no overall increase in the annual incidence of leptospirosis, however the incidence fluctuated widely across the 10-year period. The incidence was significantly higher in males compared to female. The 18-44-year-old and ≥ 45 years-old age groups had a higher incidence of the infection compared to the ≤17-year-old age group. The incidence did not vary based on season over the 10-year period.

The annual incidence observed during this 10-year period lies within the range of the incidence that has been reported globally to be occurring in temperate regions (0.1-1 cases per 100,000 population) [34]. The cold wet winters and warm dry summers might allow transmission and survival of pathogenic *Leptospira* species [21]. WCP is known for its harsh drought, and different types of floods such as river floods, flash floods and these occur in each year at varying intensities [35]. Annual fluctuation in such environmental conditions could partly explain the observed wide fluctuation in the annual incidence of leptospirosis because increase in incidence is normally reported after occurrence of extreme weather events such as heavy rains and high temperatures [28, 34, 36]. In addition, rodent infestation, increased prevalence of infected rodents and overcrowded settings were reported as risk factors for human leptospirosis outbreak in a one of the prisons in WCP in 2015 [15]. This outbreak could have contributed to the observed increase in the incidence in 2015. In a study

conducted after this outbreak, Naidoo et al. further highlighted the importance of continued circulation of pathogenic *Leptospira spp* among rodents in maintaining the status of leptospirosis transmission particularly in informal settlements [15].

Previous studies have indicated a significant relationship between leptospirosis incidence and seasonality [34, 36, 37]. In Western Cape, seasonality was not associated with the incidence of leptospirosis between 2010 and 2019, however the incidence was slightly higher in fall and summer as compared to winter and spring. This is consistent with some reports from other countries in temperate regions [34, 38]. Studies on correlation between leptospirosis incidence and seasonality require consideration of other variables such as rodent seasonal population, temperature, rainfall, and other climate related parameters to be included in the analysis [37]. This could help in understanding the importance of the interaction of different environmental and climatic factors in determining the incidence of leptospirosis in resource limited settings.

The results showed that the overall incidence was significantly higher in males as compared to females (0.55 cases vs 0.25 cases per 100,000 population) during the study period. Similar results have been reported in both seroprevalence studies in Africa and in incidence studies conducted using surveillance data globally [24, 25, 28, 34, 38]. The higher incidence among males has been largely attributed to occupational or environmental exposure, whereby males engage in activities that may put them at higher risk of contracting the infection [36, 39]. Epidemiological studies collecting data on occupation, underlying medical conditions, and place of settlement could aid in

categorically establishing risk groups hence guiding policies on targeted prevention of leptospirosis.

The incidence of leptospirosis was highest among those within the 18-44 and ≥ 45 -year-old age groups compared to those below ≤17 years of age. These findings are in line with a systematic review by Costa et al. that reported the highest incidence to be occurring among adult males aged 20-49 years [3]. The observed results also correspond with other studies, where incidence has been shown to be higher among the active adult population [34, 38]. People belonging to the 18-44 and ≥ 45 age groups would theoretically have an increased environmental exposure compared to those belonging to ≤17 age group, thus increasing their risk of being infected.

The data presented here are the first results to inform on the incidence trends and distribution of leptospirosis in WCP. The data covers a 10-year period, hence could help to guide designing of preventive strategies. However, there are some study limitations that need to be considered. Firstly, the study utilized data collected from passive surveillance and from a public health laboratory setting excluding data from private laboratories, hence there is a great potential for underestimation of the real occurrence of leptospirosis in WCP. Some bias may still be inherent in the analysis due to ambiguity in clinical presentation; the latter contributes to underestimation of actual incidence. Secondly, use of the IgM ELISA has limitation because of the risk of obtaining false positive results and also antibodies may not be detectable in acute phase of the disease and once present, they can persist for long period, this could potentially result into misdiagnosis. Thirdly, the analysis does not provide information on the geographical distribution of the cases within the province as such information

was not included in provided data. Consideration of the geographical distribution of the incidence would have given insights into which specific districts are at risk, hence informing targeted interventions.

Findings from this analysis highlight the continued circulation of leptospirosis infection within WCP with age and sex being significant risk factors for infection. However, there is need of a good knowledge about the epidemiology of leptospirosis (such as the geographical and seasonal patterns, the specific risk populations, circulating *Leptospira* strains and the importance of reservoir animals) [13] within the province to improve prevention strategies, prediction, and detection of leptospirosis burden and outbreaks. For instance, future studies focusing on the impact of heavy rainfall, floods, seasonal fluctuation in weather-related factors, occupation, geographical location, and rodent dynamic parameters on leptospirosis incidence could help address the knowledge gaps on the actual burden of this emerging zoonosis.

In conclusion, the incidence of leptospirosis in WCP fluctuated within the range that has been reported to be occurring in temperate regions. The incidence was strongly related to sex and age; however, it did not differ across the seasons of the year. These results show that leptospirosis is an important zoonosis within the province and potentially disproportionately affecting males and the productive age demographic groups. The findings, therefore, can guide targeted intervention strategies within the province to decrease the burden of human leptospirosis.

5. Acknowledgement

Special thanks to the National Health Laboratory Services (NHLS), South Africa for providing all the necessary data on leptospirosis tests conducted in Western Cape Province.

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7. Supporting information

S1 Table 2: Summary of leptospirosis incidence distribution by age group from 2010 to 2019 in Western Cape Province, South Africa

Age group	≤17-year-old	18-44-year-old	≥ 45-year-old
	IP/100,000 (95% CI)	IP/100,000 (95% CI)	IP/100,000 (95% CI)
Year of occurrence			
2010	0.172 (-0.02,0.37)	0.898 (0.54,1.26)	0.521 (0.14,0.91)
2011	0.113 (-0.04,0.27)	0.366 (0.14,0.59)	0.504 (0.13,0.88)
2012	0	0.180 (0.02,0.34)	0.279 (0.01,0.55)
2013	0.218 (0.004,0.43)	0.212 (0.04,0.38)	0.135 (-0.05,0.32)
2014	0.054 (-0.05,0.16)	0.831 (0.50,1.16)	0.196 (-0.03,0.42)
2015	0.106 (-0.04,0.25)	1.020 (0.66,1.39)	0.632 (0.24,1.02)
2016	0.052 (-0.05,0.15)	0.570 (0.3,0.84)	1.160 (0.64,1.69)
2017	0	0.567 (0.30,0.84)	0.298 (0.04,0.56)
2018	0	0.525 (0.27,0.78)	0.867 (0.43,1.31)
2019	0	0.452 (0.22,0.69)	0.337 (0.07,0.61)
Mean Incidence	**0.072 (-0.02,1.6)**	**0.562 (0.16,0.83)**	**0.493 (0.3,0.83)**

IP; incidence proportion per 100,000 population: CI; confidence interval

S2 Table 2: Leptospirosis cases per healthcare facility between 2010-2019 in Western Cape Province, South Africa.

District	Health Facility	n	%
Cape Town Metro	Groote Schuur Hospital	62	24.4
Cape Town Metro	Mitchells Plain Hospital	42	16.5
Cape Town Metro	New Somerset Hospital	34	13.4
Cape Town Metro	Tygerberg Hospital	27	10.6
Cape Town Metro	Victoria Hospital	26	10.2
Cape Town Metro	Dietrich Voigt Mia TPY	20	7.9
Cape Town Metro	GF Jooste Trauma Hospital	19	7.5
Cape Town Metro	Khayelitsha Hospital	10	3.9
Cape Town Metro	2 Military Hospital	4	1.6
Cape Town Metro	Helderberg Hospital	3	1.2
Cape Town Metro	Red Cross Children's Hospital	2	0.8
Cape Town Metro	Wynberg Clinic	2	0.8
Garden Route	George Hospital	1	0.4
Cape Town Metro	Karl Bremer Hospital	1	0.4
Cape Town Metro	Woodstock CDC	1	0.4
		254	**100.0**

n; frequency

PART C: APPENDICES

Appendix 1: UCT- HREC approval document

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

Room G50- Old Main Building
Groote Schuur Hospital
Observatory 7925
Telephone [021] 406 6492
Email: hrec-submissions@uct.ac.za
Website: www.health.uct.ac.za/fhs/research/humanethics/forms

19 May 2021

HREC REF: 303/2021

Dr J Odayar
Public Health & Family Medicine-FHS
5th Floor, Falmouth Building- FHS
Email: Jasantha.odayar@uct.ac.za
Student: gzmjac001@myuct.ac.za

Dear Dr Odayar

PROJECT TITLE: INCIDENCE AND DISTRIBUTION OF HUMAN LEPTOSPIROSIS IN THE WESTERN CAPE PROVINCE, SOUTH AFRICA, (2010-2019): A RETROSPECTIVE STUDY-MSC CANDIDATE-MR JACOB GIZAMBA

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.

This approval is subject to strict adherence to the HREC recommendations regarding research involving human participants during COVID -19, dated 17 March 2020 & 06 July 2020.

Approval is granted for one year until the 30 May 2022.

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)

The HREC acknowledge that the student: Mr Jacob M Gizamba will also be involved in this study.

Please quote the HREC REF 303/2021 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, where necessary, before the research may occur.

Yours sincerely

PROFESSOR M BLOCKMAN
CHAIRPERSON, FACULTY OF HEALTH SCIENCES HUMAN RESEARCH ETHICS COMMITTEE

Federal Wide Assurance Number: FWA00001637.
Institutional Review Board (IRB) number: IRB00001938
NHREC-registration number: REC-210208-007

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 Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use: 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/REF 303/2021sa

Appendix 2: Provincial Government of the Western Cape approval

Western Cape Government
Health

GROOTE SCHUUR HOSPITAL
Enquiries: Dr Bernadette Eick
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Dr Jasantha Odayar
PUBLIC HEALTH & FAMILY MEDICINE

E-mail: Jasantha.Odayar@uct.ac.za / gzmjac001@myuct.ac.za

Dear Dr Odayar,

RESEARCH PROJECT: Incidence And distribution Of Human Leptospirosis In The Western Cape Province, South Africa, (2010-2019): A Retrospective Study (MSc. Mr Jacob Gizamba)

Your recent letter to the hospital refers.

You are granted permission to proceed with your research, which is valid until **30 May 2022.**

Please note the following:

a) Your research may not interfere with normal patient care.
b) Hospital staff may not be asked to assist with the research.
c) Confidentiality must always be maintained.
d) No additional costs to the hospital should be incurred as indicated in your Annexure 2 i.e. Lab, consumables or stationery. **If access to TRACK Care/NHLS is required, kindly attach our letter of approval to the application form and approach Information Management to assist with data.**
e) **No patient folders may be removed from the premises or be inaccessible.**
f) Please provide the research assistant/field worker with a copy of this letter as verification of approval.
g) Should you at any time require photographs of your subjects, please obtain the necessary indemnity forms from our Public Relations Office (E45 OMB or ext. 2187/2188).
h) Should you require additional research time beyond the stipulated expiry date, please apply for an extension.
i) Please discuss the study with the HOD before commencing.
j) Please introduce yourself to the person in charge of an area before commencing.
k) On completion of your research, please forward any recommendations/findings that can be beneficial to use to take further action that may inform redevelopment of future policy / review guidelines.
l) Please contact Michelle Riley (Patient Fees) at ext. 2276 to ascertain if there will be charges for conducting the Research and to obtain a quote or to discuss charges
m) Kindly submit a copy of the publication or report to this office on completion of the research.
n) At no time should any posters encouraging patients to partake in research, be displayed within a clinical area.
o) Please adhere to ALL COVID-19 regulations and Groote Schuur Hospital policies.

I would like to wish you every success with the project.

Yours sincerely

DR BERNADETTE EICK
CHIEF OPERATIONAL OFFICER
Date: 10 June 2021

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Appendix 3: NHLS Access to data approval

NATIONAL HEALTH LABORATORY SERVICE

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30 August 2021

Applicant: Jacob Gizamba
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CC: Lynthia Paul, Jasantha Odayar, Sipho Dlamini

Re: Approval to access National Health Laboratory Service (NHLS) Data

Your application to undertake a research project **"Incidence and distribution of Human Leptospirosis in the Western Cape Province, South Africa, (2010-2019): A Retrospective Study, Ref No: PR2118953"** using data from the NHLS database has been reviewed. This letter serves to advise that the application has been approved and the required data will be made available to you ***without patient names*** to conduct the proposed study as outlined in the submitted application. Submissions should be made annually on the AARMS system – https://aarms.nhls.ac.za.

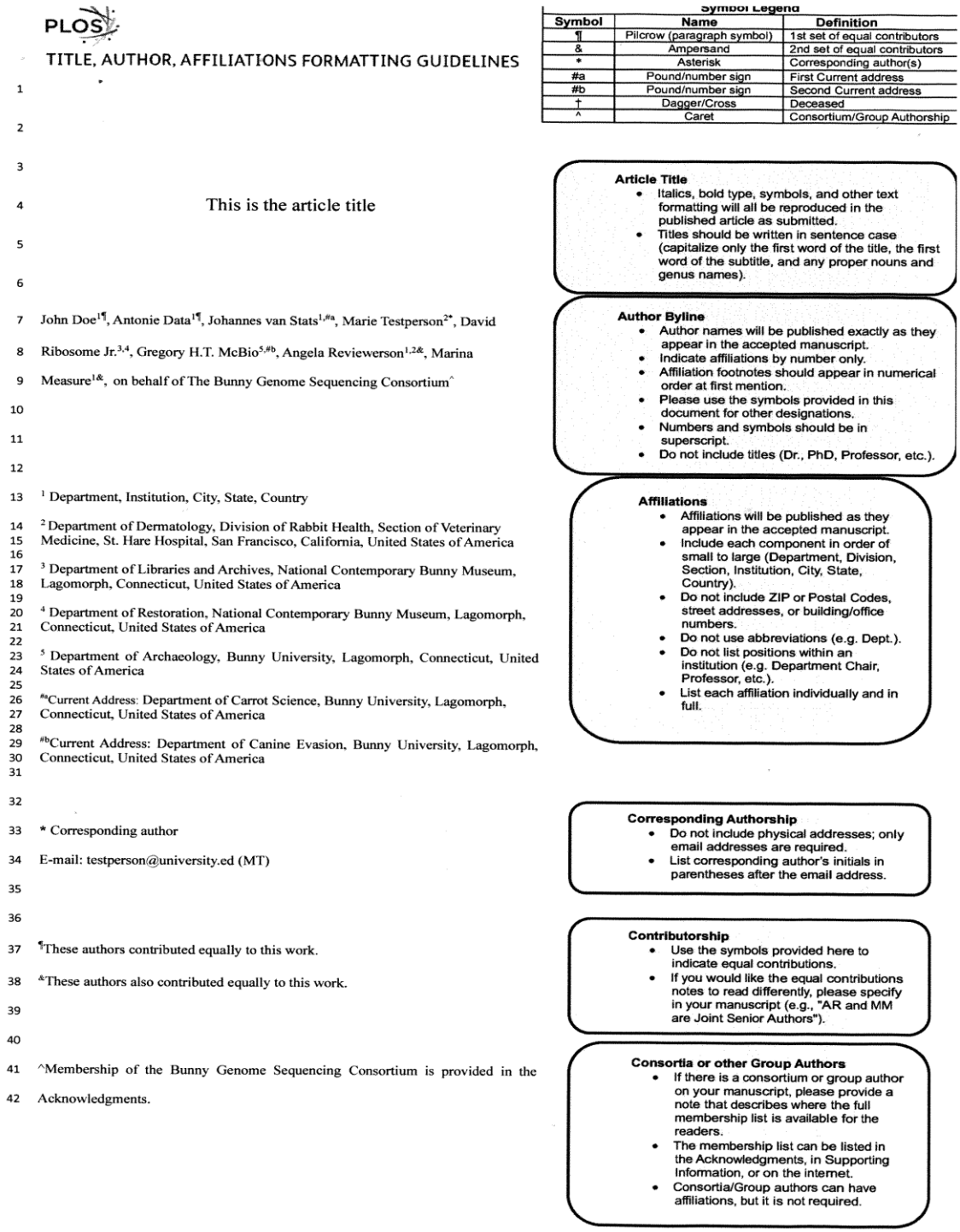
Please note that approval is granted on your compliance with the NHLS conditions of service and that the study can only be undertaken provided that the following conditions have been met.

- Processes are discussed with the relevant NHLS departments (i.e. Information Management Unit and Operations Office) and are agreed upon.
- Confidentiality is maintained at participant and institutional level and there is no disclosure of personal information or confidential information as described by the NHLS policy.
- NHLS Data cannot be used to track patients as no pre-approval/consent is obtained from Patients.
- All data requested should be in accordance with the research protocol submitted and approved by the relevant Ethics Committee.
- Request for the inclusion of the NHLS as a source of data in the original protocol to be approved by Ethics as NHLS does not have a Human Research Ethics Committee.
- A final report of the research study and any published paper resulting from this study are submitted and addressed to the NHLS Academic Affairs and Research office and the NHLS has been acknowledged appropriately.

Please note that this letter constitutes approval by the NHLS Academic Affairs and Research Office. Any data related queries may be directed to NHLS Corporate Data Warehouse, contact number: 011 386 6074 email: zarina.sabat@nhls.ac.za

Dr Babatyi Malope-Kgokong
National Manager: Academic Affairs and Research

Appendix 4: Instructions for Authors; PLOS Neglected Tropical Disease Journal.



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Symbol	Name	Definition
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John Doe[1¶], Antonie Data[1¶], Johannes van Stats[1,#a], Marie Testperson[2*], David Ribosome Jr.[3,4], Gregory H.T. McBio[5,#b], Angela Reviewerson[1,2&], Marina Measure[1&], on behalf of The Bunny Genome Sequencing Consortium[^]

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[1] Department, Institution, City, State, Country

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[3] Department of Libraries and Archives, National Contemporary Bunny Museum, Lagomorph, Connecticut, United States of America

[4] Department of Restoration, National Contemporary Bunny Museum, Lagomorph, Connecticut, United States of America

[5] Department of Archaeology, Bunny University, Lagomorph, Connecticut, United States of America

[#a]Current Address: Department of Carrot Science, Bunny University, Lagomorph, Connecticut, United States of America

[#b]Current Address: Department of Canine Evasion, Bunny University, Lagomorph, Connecticut, United States of America

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* Corresponding author

E-mail: testperson@university.ed (MT)

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[¶]These authors contributed equally to this work.

[&]These authors also contributed equally to this work.

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- Use the symbols provided here to indicate equal contributions.
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[^]Membership of the Bunny Genome Sequencing Consortium is provided in the Acknowledgments.

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- The membership list can be listed in the Acknowledgments, in Supporting Information, or on the internet.
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Abstract

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Level 1 Heading

- Use Level 1 heading for all major sections (Abstract, Introduction, Materials and methods, Results, Discussion, etc.).
- Bold type, 18pt font.
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NOTE: Do not cite figures, tables, supporting information, or references in the Abstract.

Introduction

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Figure Citations

- Cite figures as "Fig 1", "Fig 2", etc.
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Fig 1. This is the Fig 1 Title. This is the Fig 1 legend.

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Inline Equation

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Level 1 heading

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Table 1. This is the Table 1 Title.

	Chemical W	Chemical X	Chemical Y	Chemical Z
Chemical 1	Reaction 1W	Reaction 1X	Reaction 1Y	Reaction 1Z
Chemical 2	Reaction 2W	Reaction 2X	Reaction 2Y	Reaction 2Z
Chemical 3	Reaction 3W[a]	Reaction 3X	Reaction 3Y[b]	Reaction 3Z
Chemical 4	Reaction 4W	Reaction 4X	Reaction 4Y	Reaction 4Z
Chemical 5	Reaction 5W	Reaction 5X	Reaction 5Y	Reaction 5Z

This is the Table 1 legend.
[a]Table footnotes belong here.
[b]Footnotes should have corresponding symbols in the table.

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- Tables must be cell-based in Microsoft Word or embedded with Microsoft Excel.
- Do not use empty rows to create spacing.
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Conclusion

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Acknowledgments

- Do not include funding or competing interests information in Acknowledgments.

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References

93 1. Doe J, Data A, van Stats J, Testperson M, Ribosome D Jr,
94 McBio GHT, et al. This is the article title. PLoS ONE.
95 2017;12(12):e00000. doi: 10.1371/journal.pone.0000000
96 2. Doe J, Data A, van Stats J, Testperson M, Ribosome D Jr,
97 McBio GHT, et al. Bunny dynamics in cartoon landscapes.
98 PLoS ONE. Forthcoming 2017.

References

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