Epidemiological mapping of mycetoma in Sudan

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Abstract

Mycetoma is a debilitating neglected tropical disease that ultimately destroys the bodies and lives of those it affects. It is caused by certain fungi or bacteria and classified into eumycetoma and actinomycetoma respectively. Although mycetoma is reported in many countries around the globe, information and experience on disease identification, diagnosis, and treatment are meagre. The primary disease epidemiological characteristics remain unknown, as do the true incidence, prevalence, and geographical distribution. Mycetoma is curable if discovered in its early stages. Therefore, early disease detection is the critical point in disease management that cannot be established without acknowledging the actual burden of the disease and its distribution. This thesis aimed to understand these aspects of mycetoma in Sudan using two approaches. Firstly, a community-based survey in Eastern Sennar Locality in Sennar State, Sudan, was undertaken, then historical data were compiled from the Mycetoma Research Centre (MRC) database including patients registered from all over Sudan and analysis included modelling of the distribution of the mycetoma cases and burden of mycetoma in Sudan.

To address the knowledge gap in the individual and environmental risk factors, a cross-sectional community-based study was conducted in sixty randomly selected villages within the five administrative units of Eastern Sennar Locality between June-July 2019. A total of 41,176 individuals were surveyed, and 359 mycetoma patients were identified. The overall prevalence was 0.87% (95%CI = 0.78-0.97%), the prevalence among males was 0.83% (95%CI = 0.71-0.96%), and females 0.92% (95% CI = 0.79-1.06%). The prevalence within individuals in the age group 31-45 years (1.52%, 95% CI = 1.23-1.86%) was the highest of all age groups. The prevalence map showed patients clustered within the central and north-eastern areas, while villages in the south-western part had few or no cases.

Then, a case-control study was conducted that included the 359 confirmed mycetoma cases and three healthy individuals for each case were selected from the same villages (n=1077) with no evidence of mycetoma as a control group to address disease risk factors. An increase of one year in age is associated with a 3% decrease in the odds of contracting mycetoma (adjusted odds ratio = 0.969; 95% CI= 0.961 - 0.977). A history of local trauma (adjusted odds ratio =1.957; 95% CI= 1.478 - 2.592), unmarried status (adjusted odds ratio =3.267; 95% CI= 2.459 - 4.341) and male sex (adjusted odds ratio =1.534; 95% CI= 1.177 -1.998) all increased the risk of contracting mycetoma.

The historical data were obtained from the MRC database in Khartoum, Sudan. Demographic and clinical data from confirmed mycetoma patients seen there between 1991 and 2018. Regression and machine learning techniques were used to model the relationship between mycetoma occurrence in Sudan and environmental predictors. The strongest predictors of mycetoma occurrence were aridity, proximity to water, low soil calcium and sodium concentrations and the distribution of various species of thorny trees. The models predicted the occurrence of eumycetoma and actinomycetoma in the central and south-eastern states of Sudan and along the Nile River valley and its tributaries. Based on the estimated suitable environment and area for mycetoma occurrence, the disease burden was estimated for eumycetoma and actinomycetoma separately.

Both mycetoma types were found to have a regionally varied and different distribution in Sudan. While most eumycetoma cases were predicted to occur in the Khartoum and Al Jazirah areas, the majority of actinomycetoma cases were predicted to occur in the rural North and West Kurdufan states. In Sudan and across all states, the burden of eumycetoma is four times that of actinomycetoma. In Sudan, the risk of mycetoma is limited to certain areas, though cases are found throughout the country. This work serves as an outline for future mycetoma control and preventive endeavours, with highly endemic areas identified and resources directed to high-demand areas.

This thesis provides an evaluation of the occurrence and distribution of mycetoma in Sudan and an estimated population at risk, which provides valuable insight into the mycetoma situation in Sudan and can aid in the development of surveillance and control programs. Furthermore, risk variables at the individual level have been identified, allowing for the creation of early case diagnosis and preventive strategies.

Table of contents

List of Figures

List of tables

List of acronyms

AFLP	**Amplification Fragment Length Polymorphism**
AOR	**Adjusted Odds Ratio**
AUC	**Area Under the Curve**
BIOMOD	**BIOdiversity MODelling**
BSMS	**Brighton and Sussex Medical School**
CCL5	**CC Chemokine ligand 5**
CGIARCSI	**Global-Aridity datasets**
CI	**Confidence Interval**
CR1	**Complement Receptor 1**
CT	**Computerised Tomography**
CXCL8	**Chemokine (C-X-C motif) Ligand 8**
CXCR2	**Chemokine (C-X-C motif) Receptor 2**
DNA	**Deoxyribonucleic Acid**
ENM	**Ecological Niche Model**
EVI	**Enhanced Vegetation Index**
FAO	**Food and Agriculture Organization**

FBA	**Fructose Bisphosphate Aldolase**
GAM	**Generalized Additive Models**
GBM	**Generalized Boosted Regression Model**
GLM	**Generalized Linear Models**
GLW	**Gridded Livestock of the World**
GPS	**Global Positioning System**
IL 8	**Interleukin 8**
IL-10	**Interleukin 10**
ILRI	**International Livestock Research Institute**
IQR	**Interquartile Range**
IRB	**Institutional Review Board**
ISRIC	**International Soil Reference and Information Centre**
MCP-1	**Macrophage Chemoattractant Protein-1**
MODIS	**Moderate Resolution Imaging Spectroradiometer**
MRC	**Mycetoma Research Centre**
MRI	**Magnetic Resonance Imaging**
NASA	**National Aeronautics and Space Administration**

NOS2	**Nitric Oxide Synthase 2**
NTD	**Neglected Tropical Disease**
ODK	**Open Data Kit**
OR	**Odds Ratio**
OSM	**OpenStreetMap project**
PCA	**Principal Components Analysis**
PCC	**Proportion Correctly Classified**
PCR	**Polymerase Chain Reaction**
PET	**Potential Evapo-Transpiration**
PK	**Pyruvate Kinase**
RAPD	**Random Amplified Polymorphic DNA**
REA	**Restriction Endonuclease Analyses**
RF	**Random Forest**
RNA	**Ribonucleic Acid**
ROC	**Receiver Operating Characteristic**
SDG	**Sudanese pounds**
SIR	**standardized incidence ratio**

SNP Single Nucleotide Polymorphism

SPSS Statistical Package for Social Sciences

SRTM Shuttle Radar Topography Mission

STROBE Strengthening the Reporting of Observational Studies in Epidemiology

SUH Soba University Hospital

TCTP Translationally Controlled Tumour Protein

TMP-SMX Trimethoprim-sulfamethoxazole

TNFα Tumour Necrosis Factor α

TSP-4 Thrombospondin-4

TSS True Skill Statistic

ULB-LUBIES Université Libre de Bruxelles

US Ultrasound

USD United State Dollars

WHO World Health Organization

Acknowledgement

At the beginning, I would like to express my appreciation the NIHR Global Health Research Unit for Neglected Tropical Disease at Brighton & Sussex Medical School for the PhD opportunity, for administering the grant and for all facilities. Definitely, without the kindness and the support of everyone around me, I could not have completed my doctoral thesis. Above all else, I would like to thank my God for giving me the strength and power to accomplish this task. I would also extend my appreciation to my husband, parents, and sisters as the support and belief I received from them were invaluable throughout the PhD process. I would also like to thank my friends and colleagues for their support.

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Author’s declaration

I declare that the research contained in this thesis, unless otherwise formally indicated within the text, is the author's original work. The thesis has not been previously submitted to these or any other university for a degree and does not incorporate any material already submitted for a degree.

The information displayed here is a result of an analysis of data collected from the field (Sennar State) by the author including part of chapter two and chapter three. Results chapters four and five were derived from analysis of data retrieved from the Mycetoma Research Centre (MRC) database, the analysis was guided by Dr. Jorge Cano and Miss Hope Simpson from London School of Hygiene and Tropical Medicine. The results of health economic analysis were generated by Dr. Natalia Ivashikina.

Signature: RFOH Date: 10 January 2023

Thesis layout

The thesis is displayed in chapters and each chapter includes the type of analysis, the results of each chapter are displayed within the chapter and the supporting information, and the published articles are included in the appendices. Chapter one explores the literature review and background knowledge of mycetoma pathogenesis, geographical distribution, environmental contributing factors, diagnosis, and management. Chapter two displays the results from the descriptive analysis of the data collected in the survey in the Eastern Sennar state locality along with the health economic analysis results. The results from these two chapters are included in two manuscripts and both are attached to the appendices (Appendix 3 and 4). While the third chapter included the individual risk factors for mycetoma occurrence in which a cases control study is displayed to determine risk factors for the occurrence of mycetoma and a manuscript was developed from the results and attached to the thesis (Appendix 5). In chapters four and five, the data for the analysis were retrieved from the Mycetoma Research Centre database, including the patients' data since 1991. In these two chapters, a modelling analysis was displayed to determine environmental factors implicated in mycetoma occurrence to predict the environmental distribution across Sudan and then estimate the population at risk and the results are used in the development of two manuscripts (Appendix 6 and 8). The final chapter in the thesis is the discussion which summarizes all the findings and discusses the impact and implications of the results.

Chapter one: Introduction

1.1. Background

Mycetoma was formally recognised by the World Health Organization (WHO) as a Neglected Tropical Disease (NTD) in 2016 after a long journey of neglect [1]. It is a chronic granulomatous inflammatory disease caused by more than 70 microorganisms. Mycetoma is a unique NTD, endemic in many tropical and subtropical regions[2, 3]. It is a localised disease; the inflammatory process is usually triggered by inoculation of infection, which spreads to involve the local subcutaneous tissue leading to a painless subcutaneous mass, sinus formation and purulent or seropurulent discharge that contains grains [4](Figure 1.1). It can spread to invade the skin, deeper tissues and structures such as tendons, and eventually bones [5]. If not treated appropriately, it can lead to enormous tissue damage, disfigurement and disability that hinder patients' normal daily activities [6, 7]. The foot and hand are the most frequently affected sites. Other body parts may be involved such as the knee, arm, leg, head and neck, and perineum [8, 9]. No age is exempt from mycetoma; however, it occurs more frequently in young adult men aged 20–40 years [10-12]. Mycetoma patients are unique. They are of low socioeconomic status with a low level of health education reside in remote, poor rural areas with meagre medical and health resources [13, 14].

Figure 1.1 Foot of a mycetoma patient with multiple sinus openings

The true incidence, prevalence, and geographical distribution of mycetoma worldwide are not precisely known [15-18]. Most of the reported mycetoma data are derived from hospitalised patients with advanced disease, which could be attributed to the nature of mycetoma, which is usually painless [19]. In addition to the patients' low socioeconomic status and poor health education level, lack of health facilities in endemic areas and the geographic distance from medical centres also contribute to the late presentation of mycetoma patients [20].

1.2. Pathogenesis

Mycetoma is a multifactorial disease resulting from environmental, biological, behavioural, and sociodemographic factors (Figure 1.2). The causative organisms of mycetoma are either bacteria or fungi, causing actinomycetoma and eumycetoma, respectively [21, 22]. Common actinomycetoma causative species include *Streptomyces somaliensis*, *Actinomadura madurae*, *Actinomadura pelletieri*, *Nocardia brasiliensis* and *Nocardia asteroides*. On the other hand, *Madurella mycetomatis*, *Madurella grisea*, *Pseudoallescheria boydii* and *Leptosphaeria senegalensis* are common eumycetoma causative agents [23-25]. Eumycetoma accounts for 60-70% of all mycetoma cases in Sudan, and *Madurella mycetomatis* is the most common organism [12]. The mode of mycetoma transmission is still unclear, but it is believed that organisms reside in the soil [26], and for mycetoma disease to occur a portal for the causative organisms is needed [27]. The incubation period for mycetoma is unknown due to the difficulty of establishing the time of initial infection. In animal model experiments with athymic mice, mycetoma grains appeared as early as nine days and were well developed by 21 days from the inoculation of *Madurella mycetomatis* [28]and mycetoma patients are usually immunologically competent. The infection spreads locally and through the lymphatics, leading to the development of satellite lesions that occur more commonly in actinomycetoma, especially in cases with repeated surgery [29]. Haematological spread has also been described. *Madurella mycetomatis* and *Actinomadura* *pelletierii* grains have been detected in an intact blood vessel, and in cases of spinal mycetoma, lesions occurred without the obvious involvement of nearby skin or surrounding tissues suggesting haematogenous spread [5, 30, 31].

1.2.1. Immunology

The immune system in mycetoma patients has been studied to some extent, but the role of the immune response in pathogenesis is not clearly defined. Serological studies with *Madurella mycetomatis*-specific antigenic proteins, including Translationally Controlled Tumour Protein (TCTP), fructose bisphosphate aldolase (FBA) and pyruvate kinase (PK), showed that the Immunoglobulin G (IgG) against all three antigenic proteins was elevated in mycetoma cases compared to the control groups [32]. The abundance of these antigenic proteins in mycetoma patients might lay the opportunity of using them as a candidate for vaccines that could work in the prevention of mycetoma.

Mahgoub and his colleagues in 1973 studied the cell-mediated immunity of a group of mycetoma patients using the tuberculin test, 2,4-dinitrochlorobenzene sensitisation and lymphocyte proliferation induced by phytohemagglutinin, and found defective T cell-mediated responses, especially in severely infected patients and in those patients who did not respond well to treatment [33]. These findings were supported by animal studies since mycetoma was more successfully induced in athymic mice than in immunocompetent mice [28].

1.2.2. Genetics

Host genetic factors may play a role in mycetoma pathogenesis [34], and it has been observed that mycetoma cases occur more frequently in certain families in endemic areas whilst neighbouring families living in the same environment have no cases.

Studies initially focused on neutrophil function, given its importance in early defence against mycetoma [13, 14]. Studies were undertaken on 11 single nucleotide polymorphisms (SNPs) in 8 genes: complement receptor 1 (CR1), MBL, tumour necrosis factor α (TNFα), macrophage chemoattractant protein-1 (MCP-1), interleukin (IL)-8, C-X-C Motif Chemokine Ligand 8 (CXCL8), C-X-C motif chemokine receptor 2 (CXCR2), nitric oxide synthase 2 (NOS2), and thrombospondin-4 (TSP-4). Five SNPs in CR1, CXCL8, its receptor CXCR2, TSP-4, and NOS2 had significant differences in allele distribution between 125 Sudanese mycetoma patients and 140 matched controls. Furthermore, a SNP in NOS2 was associated with lesion size. The SI1 and McCa alleles of CR1 SNPs rs17047661 and rs17047660, respectively, had higher distribution in mycetoma patients than in matched endemic controls, and the authors speculated that these polymorphisms could result in conformational changes in the receptor that eventually might lead to a defect in the efficacy of *Madurella mycetomatis* phagocytosis. CXCL8 and TSP-4 are chemoattractant for neutrophils. The −251A allele in CXCL8 and the 29929C allele in TSP4, in addition to the +785C allele in CXCR2 that encodes the CXCL8 receptor, were associated with mycetoma in this study. The final polymorphism for which there were differences in allele frequency between mycetoma patients and healthy controls was found in NOS2, encoding nitric oxide synthase, which produces nitric oxide (NO), which mediates tumoricidal and bactericidal actions. This finding was confirmed by measuring high levels of CXCL8 in serum of mycetoma patients. In contrast, the NOS2 genotype found more frequently in patients was associated with lower nitric oxide production. This finding was also confirmed by lower nitrite and nitrate levels in mycetoma patients' sera [35].

Mahmoud and colleagues determined the role of interleukin-10 (IL-10) and CC Chemokine ligand 5 (CCL5) in granuloma formation in mycetoma. Two SNPs in the promoter region of IL-10 and three SNPs in the promoter region of CCL5 were genotyped. Significant differences in allele distribution were demonstrated for one of the SNPs in the IL-10 promoter and two of the SNPs in the CCL5 promoter between mycetoma patients and healthy controls. Both IL-10 and CCL5 were elevated in mycetoma patients' serum compared to healthy controls. Since both CCL5 and IL-10 play essential roles in granuloma formation in general, it appears that granuloma formation might have a role in the pathology of mycetoma [36]. Based on the results of these genetic association studies performed in mycetoma patients, it is not possible to conclude strong explanations as these associations are underpowered and studies are not replicated. Hence, the exact role of these genetic differences in the development and progression of mycetoma requires further study.

1.2.3. Socio-economic factor

In mycetoma endemic areas, individuals usually work as farmers, shepherds and labourers, exposing them to mycetoma causative organisms. Local villages are characterised by an abundance of cattle, goats, sheep, dogs, chickens, and donkeys, and some human settlements are dried cow dung, thorns and trash [37]. Inhabitants of the villages mostly walk barefoot among the thorny bushes and are more prone to trauma. And due to poor sanitation practices without access to clean water sources, exposure to hazardous conditions is prolonged. Furthermore, access to health facilities may be compromised by the low economic status of individuals living in endemic regions who often have to travel long distances to reach the facilities. Moreover, due to their spiritual and religious beliefs, many individuals do not seek medical treatment and instead apply traditional herbal remedies that might increase the chances of secondary bacterial infections.

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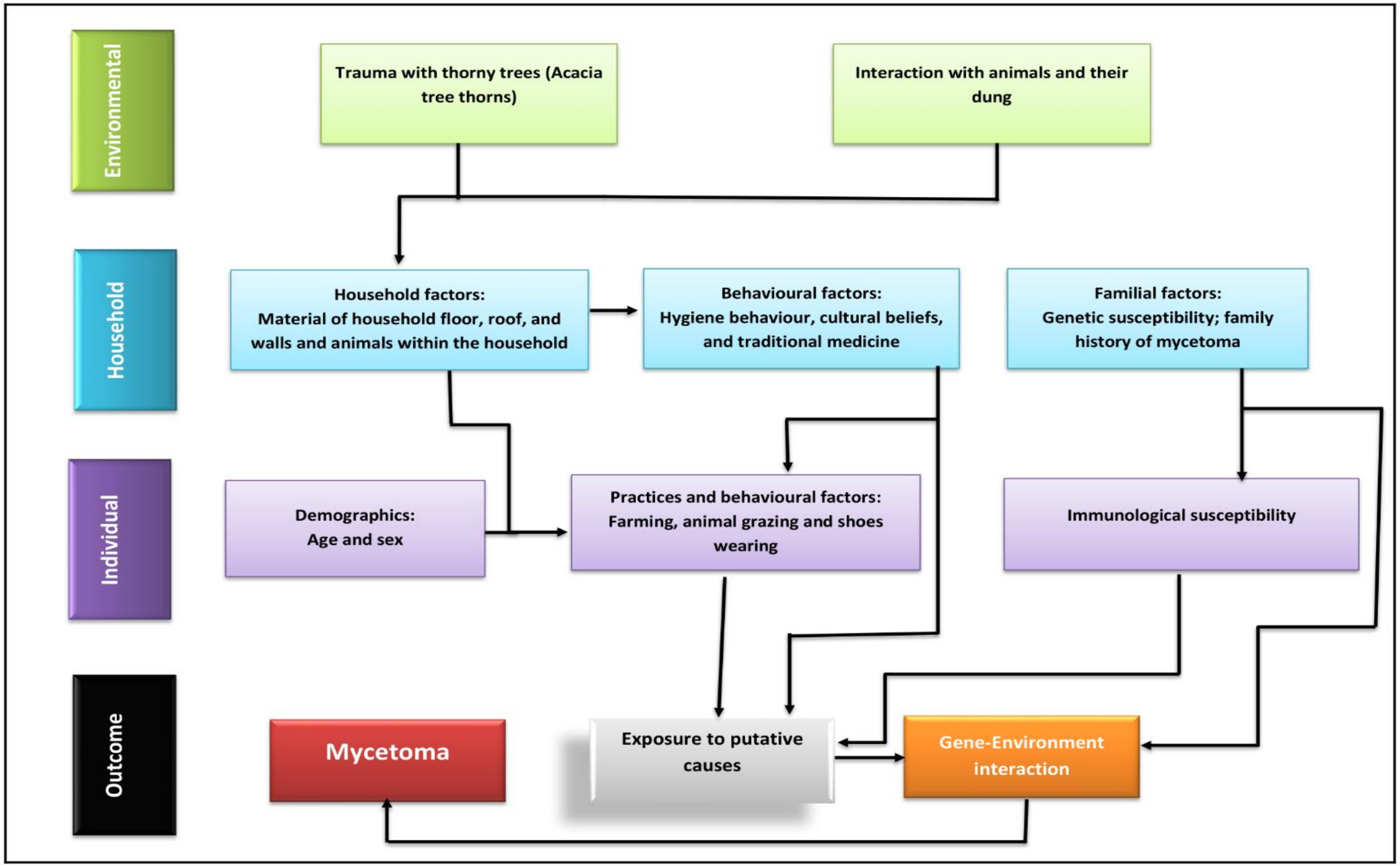

Figure 1.2 Conceptual framework representing the relationship between environmental, household, and individual-level variables. Environmental variables such as interaction with thorny trees and animal dung are essential factors facilitating the introduction of the causative organism through skin wounds. Socioeconomic status affects household factors such as materials used for floors or roofs and walls, sanitation, hygiene and ownership of animals. Individual factors such as shoe-wearing practices, occupational practices such as farming, and animal grazing can be risk factors for mycetoma. Genetic and immunological susceptibility are essential factors in determining the outcome of the exposure.

1.3. Geographical distribution and prevalence

1.3.1. Background on Sudan

Sudan is a vast but sparsely populated country compared to the rest of Africa. It is the largest nation in Africa and covers 967,495 square miles (2,505,813 square kilometres) with 43.85 million people [38]. Seven countries border it, and Egypt bounds it to the north. It borders Libya and Chad to the northwest and west, respectively. South Sudan and Ethiopia are south and southeast, respectively, while Eritrea borders Sudan to the east. The north-eastern boundary is formed by the Red Sea [39].

Sudan has a tropical climate characterised by generally high temperatures with significant seasonal and regional differences. Temperatures in the dry northern region can exceed 45˚C and there is negligible rainfall. High temperatures also occur throughout the semi-arid central plains region, which is generally of low humidity. The semi-wet region covers the southern parts of the country, along the border with South Sudan. The Red Sea coast has a different climate from the rest of the country, associated with the formation of Lake Nubia behind the Aswan High Dam due to the humidifying effect of the lake waters [40].

Sudan is divided into eighteen states and 189 localities, each divided into smaller administrative units [41]. The largest state is North Darfur, yet Khartoum has the biggest population (7,687,547) [41].

The Sudanese society is a young society, where 42.01% of the population is under the age of fifteen according to 2020 estimates [42].

The country's soils can be divided into three categories. The soil of the desert zones is loose sand or a mixture of sand and gravel or rocks. A deep clay soil (black cotton) of river origin extends from the east westwards across most of the central region, particularly the Gezira area, and similar clay soil can be found around the Nuba Mountains. In the west, sandy soil is dominant except in the Marrah Mountain area, covered in rich volcanic soil [43].

Sudan's vegetation is closely related to the climatic zones. From the desert in the north, the vegetation gradually improves through semi-arid shrubs to low woodland savannah characterised by acacia trees and short grasses. On the uplands of the southern border, rainfall is sufficient to support tropical rainforests [43].

The secession of South Sudan significantly affected the country's economy because the south contained over 80% of the nation's oilfields. Because of this, the Gross Domestic Product (GDP) has been decreasing, and inflation has been increasing since 2010 [38]. Agriculture is the most important economic sector in the country, creating 39% of GDP, employing more than one-third of the workforce. Animal husbandry is another essential part of the national economy. Its production increased during recent years because of better veterinary treatment, more liberal credit policies and higher market prices. The livestock sector of Sudan provides a livelihood for about 17% of the population. Sudanese livestock products meet the domestic demand for meat in addition to a substantial excess for export, both amounting to about 25% of total country exports in 2015 [44].

The latest official estimates of poverty in Sudan are based on the 2014-2015 National Budget and Household Survey. This survey showed that an estimated 36.1 percent of the population had per capita expenditure below the national poverty line. The World Bank expects Sudan's GDP growth rate estimated to be 3.5% in 2020 [44].

1.3.2. Mycetoma global distribution

The worldwide distribution of mycetoma varies extensively. It is endemic in many tropical and subtropical regions [10]. The term "mycetoma belt" has been introduced in the literature to define the global distribution of mycetoma. It has been described using different parameters, including countries within this belt, geographical latitude, or even more comprehensive tropical and subtropical labels [45]. The global distribution of mycetoma is not precisely known. The burden of mycetoma worldwide was first explored by Magana in 1984, who produced a map with the distribution of mycetoma cases [46]. The map showed that mycetoma was frequently reported from Africa, central and south America, and India had the highest burden [46]. In 2013 Van de Sande conducted a meta-analysis to determine the global distribution of mycetoma in which she studied 8763 published mycetoma cases. Mexico had the highest number of reported cases, followed by Sudan and then India, while Uganda, Rumania and Nigeria had the least reported cases [25].

In 2014, Lopez Martinez analysed mycetoma cases from 14 dermatological centres throughout Mexico over 30 years, and the estimated prevalence was 0.6 cases per 100,000 inhabitants [47]. Sudan is considered highly endemic for mycetoma, yet the incidence and prevalence rates are not well established. The history of mycetoma in Sudan is long and interesting. Treatment by cautery and amputation was practised during the Mahdeya (1885-1899). However, the first documented case of mycetoma in Sudan was published by Balfour in 1904 [48]. Since that report, only sporadic cases were reported until further studies were conducted by Abbott and Fahal (details in chapter 2).

1.3.3. Environment

Mycetoma usually prevails in relatively arid areas with a short rainy season of 4–6 months, rainfall of 50–1000 mm per year, relative humidity of 60–80% and constant temperatures of 30–37°C, followed by a dry season of 6–8 months with a relative humidity of 12–18%, day temperatures of 45–60°C and night temperatures of 15–18°C [7, 49]. Mycetoma is transmitted to humans through penetrating skin injuries that allow entry of the causative organisms. Thorns, specifically Acacia tree thorns, are implicated in the transmission, especially in endemic areas [50]. The presence of

Madurella species' DNA on Acacia tree thorns has suggested that infection results from inoculation by infected thorn pricks [51, 52]. Samy *et al.* constructed an Ecological Niche Model (ENM) to estimate the potential distribution of mycetoma in important endemic areas such as Sudan, assess risk factors associated with mycetoma infections, and test Acacia-mycetoma associations based on the overlap of the ecological niche of mycetoma infections with Acacia trees. The geographic distributions of Acacia trees and mycetoma cases appeared to overlap only in central Sudan. Mycetoma ENM predictions indicated a band of highest environmental suitability in central Sudan between 11°S and 17°N latitude [53].

Furthermore, it has been suggested that cattle dung may have a role in the ecology of mycetoma based on the observation that *Madurella mycetomatis* is phylogenetically closely related to dung-inhabiting fungi such as Chaetomiaceae [51]. Recently, a study was conducted in one of the endemic areas for mycetoma in Sudan to compare infestation rates of ticks in domestic animals in high and low mycetoma-prevalence villages. In the village with a high mycetoma prevalence rate, there were high infestation rates of ticks in domestic animals. *Hyalomma* and *Rhipicephalus* species were the most prevalent species in houses with mycetoma patients, and together they constituted 83% of the total collection. *Madurella mycetomatis* recombinant RNA genes were detected in *Rhipicephalus evertsi* and *Hyalomma rufipes.* This suggests for the first time that ticks might have a role in transmitting mycetoma to humans, especially those ticks implicated in transmission in other pathogens such as protozoa, rickettsia and others [54]. This study provides an indication to the possibility of disease transmission through ticks but yet further studies are required to explore this interesting observation.

1.4. Clinical presentation

The clinical presentation of mycetoma is almost identical irrespective of the causal organism, with actinomycetoma progressing more rapidly than eumycetoma [3]. All mycetoma cases present with a swelling that may have sinuses discharging grains. The swellings are usually firm and rounded, but they may be soft, lobulated and, rarely, cystic and are often mobile in the early stages [55, 56]. In eumycetoma, the lesion grows slowly with clearly defined margins and remains encapsulated for an extended period, whereas, in actinomycetoma, the lesion is not encapsulated, is more inflammatory, more destructive and invades the bone earlier [57]. The discharge is usually serous, serosanguinous or purulent. During the active phase of the disease the sinuses discharge grains, the colour of which depends on the causative organism. The grains can be black, yellow, white, or red and of variable size and consistency. Black grains are usually due to *Madurella mycetomatis*; red grains are due to *Actinomadura. pelletierii*, while yellow grains are due to *Streptomyces somaliensis*, and white grains can be due to *Actinomadura. madurae* [58].

Mycetoma is usually painless, and it has been suggested that mycetoma causative agents produce substances that have an anaesthetic action [56]. In the later stages of the disease, the nerve damage resulting from entrapment in the intense fibrous tissue reaction or decreased vascular supply leads to an absence of any pain sensation [3, 19]. The pain may become negligible at a late stage of the disease due to nerve damage by the dense fibrous tissue reaction. Pain may be produced due to a secondary bacterial infection [59]. The infection remains localised but can disseminate due to open sinus tracts, fistula formation in some patients, or generalised immuno-suppression [58].

Males are more commonly affected by mycetoma than females as recorded in the literature, while in some community-based studies, the male to female ratio is almost the same [60]. In endemic areas, males are responsible for farming and animal husbandry activities, which are considered risk factors for mycetoma, though females can also be involved in these activities [10]. The foot is the most common site affected by mycetoma, especially the dorsal aspect of the forefoot. The hand ranks as the second commonest site; the right hand is more commonly affected than the left [61]. Some diseases can have a similar presentation to mycetoma, which can delay the diagnosis; hence a high index of suspicion in endemic areas is essential. The differential diagnosis of mycetoma includes many soft tissue tumours such as Kaposi's sarcoma, fibroma, malignant melanoma, fibrolipoma, and thorn granuloma. The radiological features of advanced mycetoma may be comparable to osteogenic sarcoma and bone tuberculosis. Primary osseous mycetoma needs to be differentiated from chronic osteomyelitis, osteoclastoma, bone cysts, and syphilitic osteitis [2, 62].

1.5. Diagnosis

The distinctive clinical presentation usually makes the diagnosis of mycetoma straightforward, followed by different imaging techniques to confirm the disease presence and extent. A series of radiological changes are seen in mycetoma. In early disease, the plain X-ray is essentially normal, but the obliteration of fascial planes may be seen [63]. In 2003 a study was conducted to define the patterns of spread and stages of bone involvement, organising them from soft tissue swelling with no bone involvement (stage 0) to total disorganisation and mutilation of the foot (stage VI) [64]. Ultrasound imaging is used to differentiate between eumycetoma and actinomycetoma and between mycetoma and other non-mycetoma lesions [65]. In eumycetoma lesions, the grains produce numerous sharp, bright, hyperreflective echoes, while for actinomycetoma lesions, the findings are similar, but the grains are less distinct due to their smaller size and consistency [66]. Magnetic Resonance Imaging (MRI) is useful for determining the extent of the lesions and the invasion of structures with greater sensitivity than radiographs, ultrasound, and computerised tomography (CT). In MRI, the pathognomonic dot-in-circle sign (a sign of a small round- to oval-shaped T2-weighted hyperintense lesion with surrounding low-signal intensity and a central low-signal dot) or macro- and micro-abscesses on a hypointense matrix background are diagnostic of mycetoma [67, 68]. CT scanning is used to diagnose mycetoma, but the technique is not specific, and the differential diagnosis includes many other conditions. However, bone involvement can be determined more accurately with this modality [66].

Fluid aspirated from mycetoma lesions has a distinct appearance in a cytology smear characterised by grains and polymorphous inflammatory cells consisting of an admixture of neutrophils, lymphocytes, plasma cells, histiocytes, macrophages, and foreign body giant cells [56]. Cytological smears taken by fine-needle aspiration (FNA) from the lesion can help identify the causative organism and tissue reaction. The technique is simple, quick, economical, and can be used for rapid diagnosis of mycetoma, particularly in remote, endemic regions [23].

Surgical biopsies taken under general or spinal anaesthesia are essential to obtain grains for culture, histopathological identification and molecular identification of the causative organism and the tissue reaction [69]. In culture, grains are required for a successful procedure, and they should be viable and free of contaminants to ensure adequate growth. The culture technique is cumbersome and time-consuming, and chance contamination may result in false positives. It also requires experience to identify the causative organisms [4, 23]. To overcome these difficulties, histological examination is often used to complement culture, which requires a deep biopsy specimen containing grains to establish a diagnosis [70, 71]. Polymerase chain reaction (PCR) can be used for molecular typing and speciation of the causative agents. Different techniques are in use, which includes restriction endonuclease analyses (REA), random amplified polymorphic DNA (RAPD), and amplification fragment length polymorphism. Still, all these techniques are expensive, and are neither field-friendly or available in endemic areas [24, 72].

1.6. Management

The treatment of mycetoma depends mainly on the aetiological agent, the site of infection, and the extent of the disease. Until recently, the only available treatment for mycetoma was amputation or multiple mutilating, disfiguring surgical excisions [73, 74]. Combined medical treatment in the form of antifungals for the eumycetoma, and antimicrobial agents for actinomycetoma, and various surgical excisions is the gold standard in mycetoma [75]. The drug of choice for eumycetoma are itraconazole and posaconazole, which showed some success in treating eumycetoma caused by *Madurella mycetomatis,* while terbinafine showed a good effect [76-78]. For actinomycetoma, combined drug therapy is always preferred to a single drug to avoid the development of drug resistance and for disease eradication. Combined medical and surgical treatment accelerates healing and reduces the chance of relapse; however, many patients respond to medical treatment alone [25]. Trimethoprim-sulfamethoxazole (TMP-SMX) became the gold standard for treatment, replacing sulphonamides. Then streptomycin was added as a second drug and replaced by amikacin sulphate later [78].

The duration of treatment varies with the severity of the infection and response of each patient. In general, the duration is 18–24 months and sometimes more, and the liver function of the patients needs regular monitoring, especially with long-term itraconazole treatment in cases of advanced mycetoma [79]. Medical treatment for both types of mycetoma must continue until the patient is clinically, ultrasonically and cytologically cured [80]. Recurrence is common after an incomplete or irregular course of medical treatment. There is a good chance the organism may develop drug resistance [81].

1.7. Control strategies

There are no control or preventive programmes for mycetoma worldwide except for Sudan, but there is an urgent need to develop such programmes. However, populations in endemic areas should be advised to wear protective shoes during outdoor activities, avoid thorn pricks, maintain general hygiene, including cleaning sites of superficial injury, and keep animals in separate areas away from peoples' dwellings. In addition, health education and raising awareness, early case detection and management may provide the best means of reducing the high morbidity and complications of mycetoma.

1.8. Rationale

- Affected patients are usually young adults of low socioeconomic status in whom mycetoma leads to severe economic and social consequences.

- Most patients present late with advanced disease and serious complications due to the painless nature of the disease, low socioeconomic status, low health education and lack of health facilities in endemic areas.

- Mycetoma is curable if discovered in its early stages, early detection of mycetoma improves management outcomes and reduces morbidity.

- Education of the population at risk about the risk factors that include occupational hazards such as contact with thorns and animals and about proper sanitation and hygiene practices will help prevent the disease.

- The primary disease epidemiological characteristics remain unknown, and the true incidence and prevalence globally are not defined. Early disease detection is critical to the management but cannot be established without knowing the actual burden of the disease and its distribution.

- Mapping of mycetoma and understanding the geographical distribution is a cornerstone in establishing prevention and disease control.

1.9. Thesis objectives

1.9.1. Aim

The study aimed to determine the epidemiology and spatial distribution of mycetoma in Sennar State, Sudan.

1.9.2. Objectives

1. To determine the clinical epidemiology and health economic risk factors of mycetoma in Sennar State, Sudan.
2. To determine the individual risk factors of mycetoma in Sennar State, Sudan.
3. To model the distribution of mycetoma in Sudan.
4. To determine the burden of mycetoma in Sudan.

1.10. Thesis outline

To achieve the objectives of this study, a retrospective study was conducted using data from the MRC clinic database, followed by an epidemiological survey which was carried out in one of the endemic areas in Sudan, and 60 villages were randomly selected to collect data on community, household and individual levels. The data collected in the survey were used to determine the prevalence and risk factors for mycetoma in the endemic area, and modelling was undertaken on the data obtained from the MRC to determine the burden of disease.

Chapter 2: Clinical epidemiology and health economic risk factors of mycetoma in Sennar State

2.1. Overview

This chapter describes the clinical features and prevalence of mycetoma in addition to the health economic factors contributing to mycetoma in Eastern Sennar Locality, Sennar State. Mycetoma prevalence and geographical distribution data worldwide are still lacking. Few studies have been conducted in a community-based setting to explore the epidemiological features and risk factors for mycetoma in Sudan. This study was conducted to report the clinical epidemiological characteristics of mycetoma patients and the disease burden in Sennar state. It provides information on the typical clinical features of mycetoma at a community level and will help in the early detection of cases. Moreover, knowledge of the mycetoma burden and the health economic factors contributing to the development of mycetoma in the state can be used to guide the planning of prevention and control programs.

2.2. Introduction

Mycetoma prevalence and incidence are still unknown worldwide [82]. In Sudan, there are few studies reporting prevalence and incidence. Although mycetoma was first reported in Sudan in1904 [83], the first attempt to assess its prevalence was not made by Abbott until 1952 [84]. He studied 1321 mycetoma cases from different parts of Sudan and he reported a disease prevalence of 0.51% among hospital patients seen in Khartoum during a study period of 36 months. He reported higher prevalences in Atbara, Ed Dueim, and Wad Madani cities (within central Sudan states) with estimates of 0.92%, 0.93% and 1.18%, respectively. In 2014, Fahal and colleagues conducted a community-based study in one of the villages of White Nile State, Sudan, to determine the burden of mycetoma, and reported a prevalence of 1.45% [12]. However, the prevalence did not capture the actual burden of the disease in the state or Sudan because this village had a high incidence. In the early 1970s, the Sudanese medical community responded to the mycetoma threat by establishing The Khartoum North Mycetoma Clinic for patient medical care and research. Following that, The MRC was established in 1991 under the umbrella of the University of Khartoum.

Studies that estimated the prevalence of mycetoma in Sudan were limited and underestimated the actual magnitude of the problem. Cases used to estimate the prevalence were identified through hospital records, hence only patients who were able to seek medical care were included, leaving out several unidentified cases. People living in rural areas in Sudan usually have low socioeconomic status and low access to health care facilities. Most individuals work in either arable farming or animal grazing with a constant risk of contracting mycetoma through injuries during work in the field. Hence, early detection of mycetoma rarely occurs due to financial barriers and inability to access medical care. This study aimed to conduct an extensive survey in 60 villages in Eastern Sennar Locality, Sennar State, to detect mycetoma cases in and determine prevalence. The second aim was to determine the clinical and epidemiological features of mycetoma at the community level. The output of the study would give accurate measures of the disease burden and help formulate accurate prevention and control measures.

2.3. Material and Methods

2.3.1. Study setting

Eastern Sennar Locality is one of seven localities in Sennar State. This state is situated in the southeast part of Sudan. Jazeera State in the north borders it, the Blue Nile State in the south, Al-Gadaref State in the east and the White Nile State & the Upper Nile State of South Sudan in the west [85]. It has a total area of 37,844 square kilometres and a population of approximately

1,918,692 inhabitants. Eastern Sennar Locality covers an area of 2,670 square kilometres and represents 7% of the total area of Sennar State [43]. The locality comprises 292 villages distributed into five administrative units with a total population of 353,196 individuals. Eastern Sennar Locality is subdivided into five administrative units: Wad Alabbas, Wad Onsa, Wad Taktok, Elreif Elshargi and Doba (Figure 2.1, A and B).

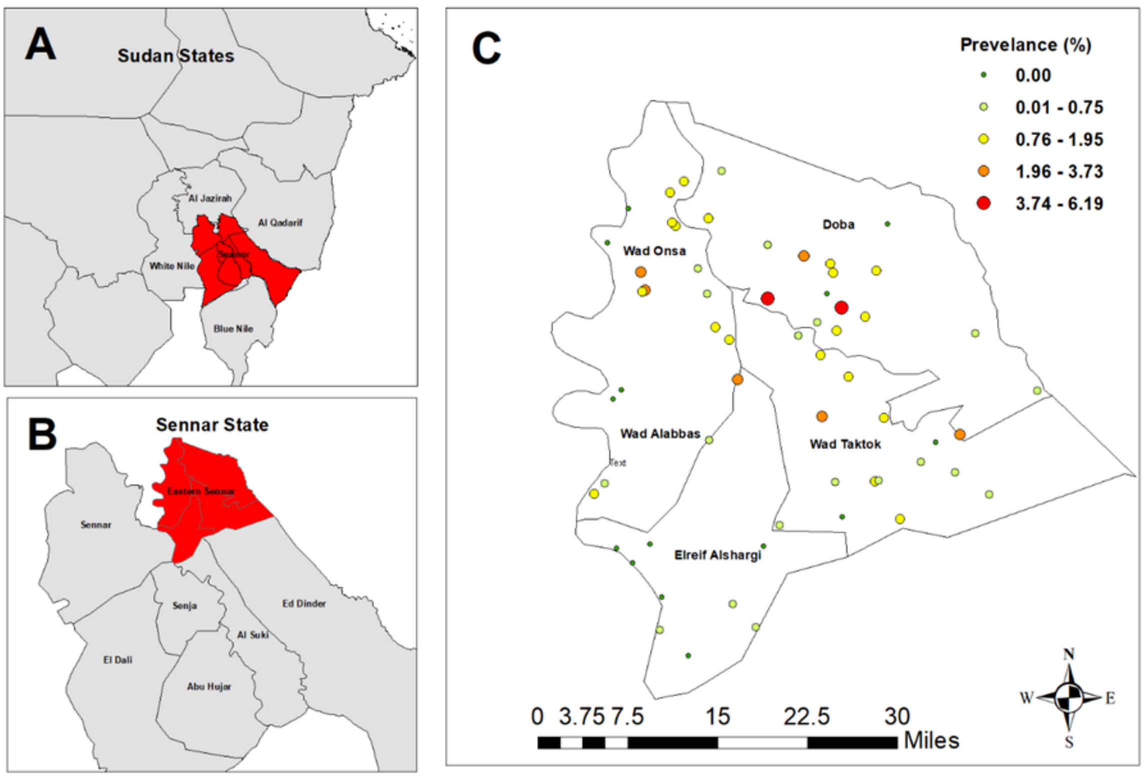

Figure 2.1 Geographical distribution of mycetoma in the sixty study villages in Eastern Sennar Locality, Sennar State, with a total population of 41,176 individuals. Base map of Sudan downloaded from Diva.GIS[86]

A- Map of states around Sennar state with Sennar State in red

B- Sennar State Localities with Eastern Sennar Locality in red

C- Geographical distribution of mycetoma in Eastern Sennar locality

2.3.2. Sampling strategy and participant selection

A cross-sectional community-based study was conducted. Cluster sampling was used to select sixty villages randomly within the five administrative units of Eastern Sennar Locality. The sample size was calculated based on the estimated prevalence of 5 cases of mycetoma per 10,000 population, an average population size of each village of 935 people, a population of Eastern Sennar Locality of 353,196 people, a design effect of 2.5, a precision of 0.03 and a community participation rate of 80%. The calculations suggested that sampling 60 villages would give 80% power to detecting mycetoma at a 5% significance level.

A household-to-household survey was conducted across all households in the 60 selected villages. Data were collected using pre-designed validated questionnaires at three levels: community, household, and individual. All individuals living in the selected villages who provided written informed consent were included in the study. The survey team consisted of a coordinator responsible for organising the survey activities and communication with village leaders and a medical doctor responsible for obtaining the written consent, interviewing individuals within the household and performing the clinical examination. Also, the team had a village guide who was responsible for the facilitation of movement within the village and communication with villagers. The teams visited the villages before the actual day of the survey, where the local leaders and community representatives were informed of the survey objectives. Teams identified a suitable day and time for the survey process and recorded geo-coordinates of the village using a Global Positioning System (GPS) device. All households were then visited. To conduct the survey, informed written consent was obtained from each household's head. After verbal consent, all individuals residing in the study area were screened for mycetoma by careful clinical examination. (Appendix 1)

2.3.3. Diagnosis of mycetoma patients

All individuals with swelling involving any part of the body or sinus formation with or without grains were classified as suspected mycetoma cases. All suspected cases were referred to Wad Onsa Mycetoma Satellite Centre, where they were clinically examined, and an expert radiologist performed ultrasound (US) examinations of any lesions to ascertain a mycetoma diagnosis. Confirmed mycetoma cases were defined as individuals with swelling involving any part of the body with or without sinus formation or multiple sinuses with or without grain discharge that was evident by ultrasound examination in the form of a pocket of fluid containing echogenic grains [12]. Data collection was done using electronic questionnaires (appendix 2), and it included suspected cases' demographic data (age, gender, marital status, educational level, and occupation) to describe the characteristics of the targeted population. The clinical data collected concerning mycetoma were duration of the disease (the duration from when the symptoms started), lesion site, history of trauma by a sharp object (such as thorn, glass or metal sharp object) and family history. For the behavioural practices, data on footwear use, arable farming activities, animal grazing, and direct contact with animals and thorny trees were collected. Animal grazing involved tending to domestic livestock that roamed freely outdoors and consumed wild vegetation. The data were collected by medical doctors who received training on the data collection process and consent form administration. (Appendix 1)

2.3.4. Data Analysis

2.3.4.1. Clinical epidemiology and prevalence

Data were sent directly to a server at the MRC Data Centre, Khartoum and visualised using Microsoft Excel (Microsoft Corp., Redmond, WA). Data verification, cleaning and analysis were done using Statistical Package for Social Sciences, SPSS 25 (SPSS, Chicago, IL, USA).

Descriptive statistical analysis was performed to calculate the overall prevalence and determine the individual data variables. Maps for mycetoma prevalence were developed using ArcGIS 10.5 ([ESRI] Inc., Redlands CA, USA).

2.3.4.2. Health economic risk factors

The health economic analysis was performed on my data by my colleague Dr Natalia Hounsome. She is a specialist in economic evaluation, statistical analysis and economic modelling, her expertise was utilized in the analysis of health economic-related data. She conducted the analysis using Microsoft Excel 2010 and SPSS 25 (SPSS, Chicago, IL, USA). Then questionnaire data were anonymised, checked for misspelling and coded (when required). The data from the individual questionnaire was merged with the data from the household questionnaire using the household identification number to enable comparisons of households with suspected mycetoma cases and the general survey households. At the data analysis point, the mycetoma diagnosis was not confirmed for 23 survey participants (21 were travelling outside Sudan, and two had died). Therefore, the following analytical approaches were used to compare the data:

1. The primary comparisons were conducted between the households with confirmed mycetoma cases and the general survey households and between participants with confirmed mycetoma cases and suspected cases in whom the diagnosis of mycetoma was in fact excluded.

2. Multiple imputations were conducted for the 23 participants in whom the diagnosis of mycetoma could not be confirmed. Subgroup analyses were conducted to compare participants with and without a diagnosis of mycetoma.

Single-level multiple imputations were conducted using SPSS 25. Missing diagnoses were assumed to be missing at random. For each missing diagnosis, five datasets were imputed. Covariates included in the imputation were: the presence of swelling, sinuses either with or without discharge, history of trauma, family history and practices such as arable farming and animal grazing. Animal grazing is considered a farming activity where domestic livestock is allowed to roam around and consume wild vegetation outdoors.

Statistical significance of differences between the samples was tested using the Mann-Whitney-Wilcoxon test (continuous variables), chi-squared test (binary variables) and logistic regression (multiple categorical variables). Opportunity costs were calculated using the minimum monthly wage of 425 Sudanese pounds (SDG) [87]. Costs were converted to US dollars (USD) using the exchange rate of 1USD/47.65 SDG (December 31, 2018) [88].

2.3.5. Ethical considerations

Ethics approval for this study was obtained from the MRC Sudan IRB (Approval no. SUH 11/12/2018) and from the BSMS Research Governance and Ethics Committee (ER/BSMS435/1). Written informed consent was obtained from each adult patient and parents or guardians of participants under 18 years old. Confirmed mycetoma cases were referred for management at Wad Onsa Satellite Centre or the MRC in Khartoum.

2.4. Results

A total of 41,176 of the expected population of 56,100 individuals were surveyed, from which 515 suspected mycetoma cases were detected. Of these, 359 patients (69.7%) proved to have mycetoma, the diagnosis was not confirmed in 133 (25.8%), and 23 cases did not attend for confirmation of the diagnosis (Figure 2.2).

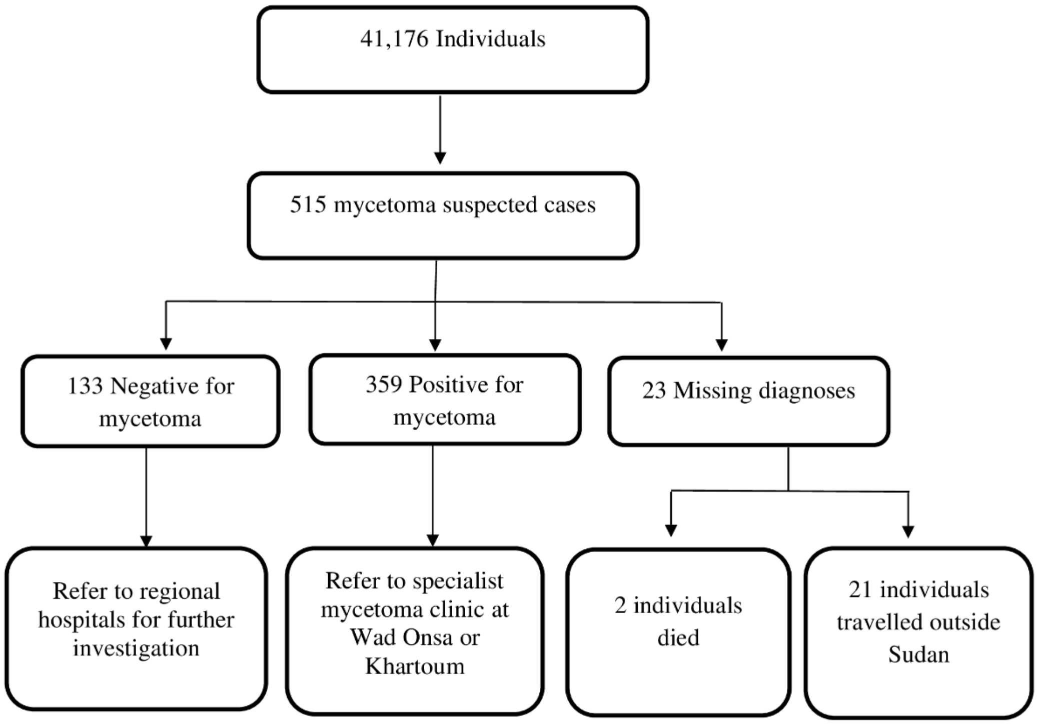

Figure 2.2 Flow diagram showing overall numbers and outcomes for the total population covered in the survey. Negative cases were referred to the medical facility for further investigation and management. Positive cases were referred to the specialist mycetoma clinic at Wad Onsa or Khartoum.

The median age of the participants was 17 years (interquartile range [IQR], 8 to 34). The male to female ratio of the respondents in the study population was 1:1. The sex ratio of the cases was 0.9:1.0, male to female. Only 36.2% experienced pain, and 35.7% had a family history of mycetoma. Student aged 10-20 years was the commonest occupation and constituted 19.2% of mycetoma cases, followed by farmers and shepherds (17.5%) (Table 2.1).

Most of the lesions were on the lower extremities (68.2%), followed by the upper extremities (26.2%), and 1.7% of patients had multiple lesions at different sites. Females had more lesions in the upper extremities than males with a percentage of (58.5%) (Figure2.3). Most of the patients (80.8%) had swelling, 38.4% had sinuses, 33.9% had to discharge sinuses only following previous surgical excisions, 32% had grains discharge of which 97.4% were black.

Table 2.1 The demographic features of mycetoma cases seen in Eastern Sennar Locality, Sennar State, Sudan. (N=359 mycetoma cases and 41,176 surveyed individuals)

Variable	**No. (%)**
Visible swelling	
Yes	290 (80.8%)
No	69 (19.2%)
Visible sinuses	
Yes	138 (38.4%)
No	221(61.6%)
Discharge	
Grains	115 (32%)
Fluid, pus, blood	7 (1.9%)
No discharge	237 (66%)
Grains	
Black	112 (97.4%)
White	3 (2.6%)
Site of the lesion	
Upper extremities	94 (26.2%)
Lower extremities	245 (68.2%)
Head, neck, trunk, back and perineal area	14 (3.9%)
Multiple sites	5 (1.7%)
Pain	
Yes	130 (36.2)
No	229 (63.8%)
Family History	
Yes	128 (35.7%)
No	231 (64.3%)

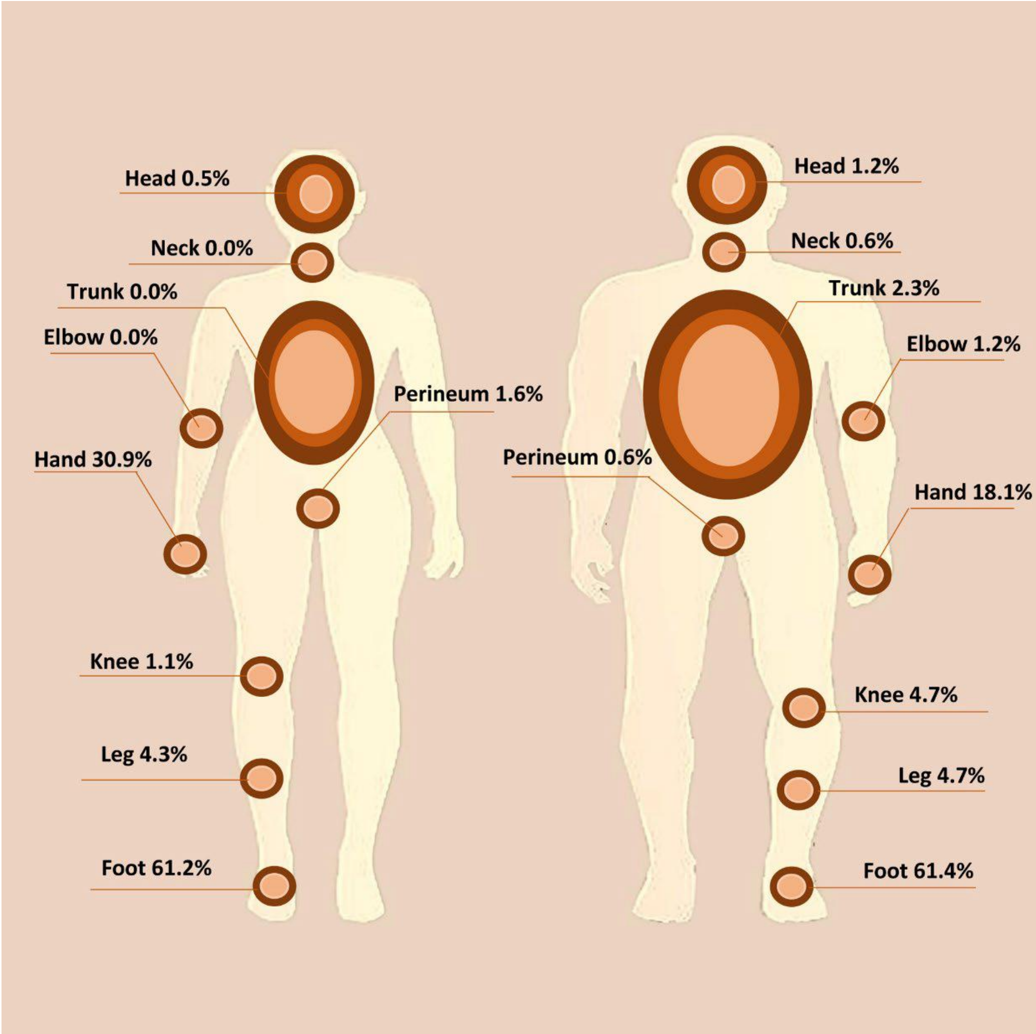

Figure 2.3 Physical distribution of mycetoma lesions for female (left side) and male (right side) cases. The feet constituted more than 60% of the lesion sites in both males and females. The fewest lesions were recorded on the neck and perineum for males and no lesion noticed in the neck, trunk, and elbows for females.

The study showed that most houses, 334 (93%), had soil or sand floors, and most of them (83%) had roofs made of traditional material such as tree branches and palm leaves. Surrounding walls, when present, were made of mud and animal dung (29.8%), red bricks and concrete (27.7%) or tree branches (8.6%) and 33.7% had no surrounding walls.

More than half (51%) of the mycetoma patients owned animals, and 34.8% raised animals within the household. The study showed that most cases (64.9%) practised arable farming while only 29.5% practised animal grazing.

Approximately 36% of households used water from multiple sources, and only 7.7% of households purified water for drinking. The most common purification methods were adding bentonite clay to absorb impurities and settling [89]. Straining, boiling, and adding chemical agents (chlorine and alum) were rarely used (<1%). Approximately half of the surveyed households (53.1%) had no toilet facilities, 42.7% had pit latrines, 3.4% ventilated pit latrines and 0.7% flush toilets.

The primary approach to waste disposal was open dumping (88.5%), followed by burning (28.8%) and burying (0.3%). Approximately 13% of households combined open dumping with burning or burying.

Electricity was available in 69.2% of households, a television in 43.8%, radio in 21.9%, refrigerator in 27.7% and mobile phone in 76.1% of households. Vehicles were owned by 33.1% of households. These were animal-drawn carts (25.2%), cars/trucks (10.8%) and "rakshas" (three-wheeled motorised rickshaw-type vehicles, 1.4%).

Approximately 59% of households owned agricultural land, varying from 46.5% in El-Reif El-Shargi to 75.6% in Doba. Farm animals were raised by 48.3% of households, from 39.5% in Wad Taktok to 57.9% in Doba. The commonest farm animals owned were goats, cows, donkeys, chickens, pigeons, camels, horses, rabbits and ducks. Approximately 41% of households had animal sheds. Of these, 33.1% were inside houses and 10.6% outside. Outside sheds were located near the houses (6.1%) or further away from the houses within the village's boundaries (4.1%).

The overall mycetoma prevalence was 0.87% (95% CI = 0.78-0.97%), the prevalence among males was 0.83% (95%CI = 0.71-0.96 %), and among females was 0.92% (95% CI = 0.79-1.06%). The mycetoma prevalence was highest in the age group 31-45 years (1.52%, 95% CI = 1.23-1.86%)

followed by the age group 16-30 years (1.11%, 95% CI = 0.93-1.33%). Most of the males with mycetoma were in the younger age groups compared to females (Figure2.4).

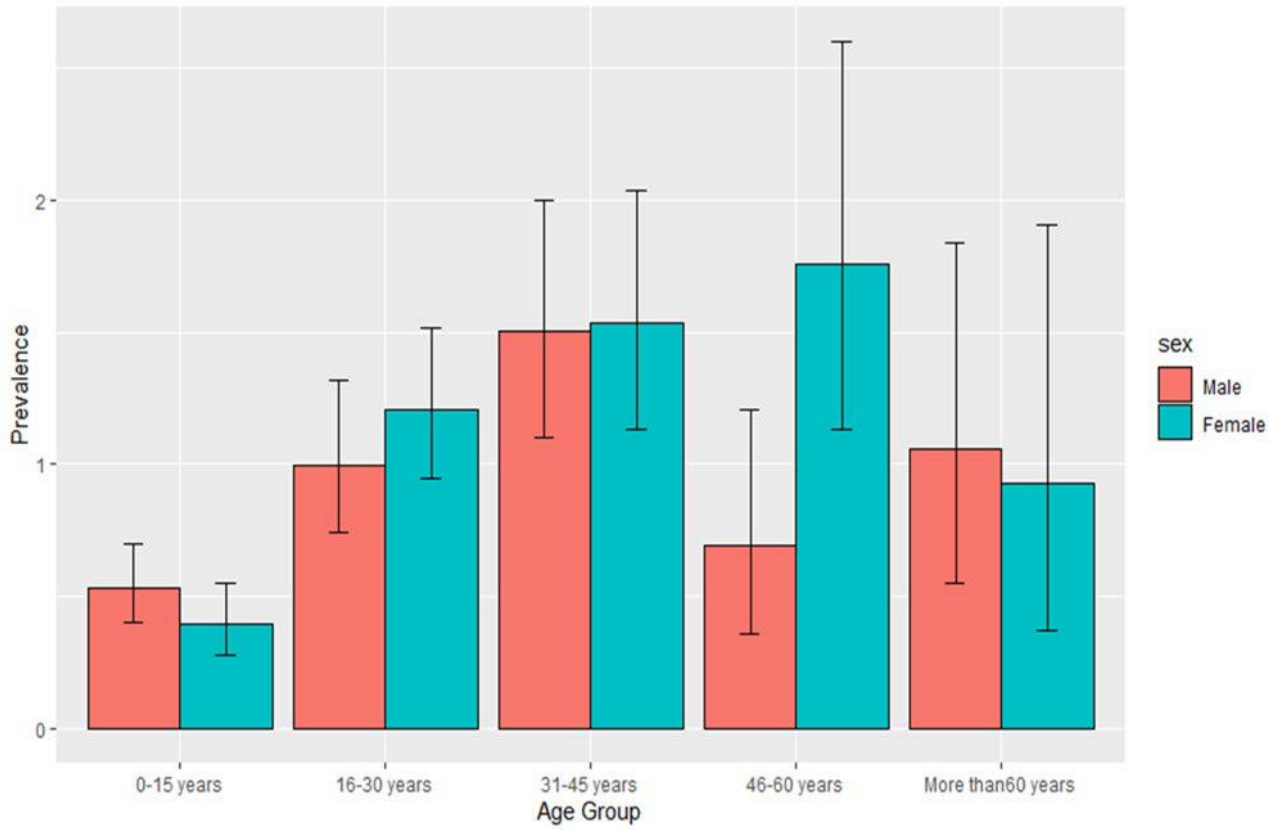

Figure 2.4 Bar plot of the prevalence of mycetoma among different age groups according to gender. Males had a higher prevalence among the age groups 0-15 years and more than 60 years, and females recorded a higher prevalence among the other age groups.

Married and illiterate individuals had a higher mycetoma prevalence (1.34%, 95% CI = 1.16-1.53%) and (1.24%, 95% CI = 1.04-1.48 %) respectively. Prevalence was higher among individuals with a history of trauma with sharp objects (1.63%, 95% CI = 1.36-1.95%). Wearing shoes did not affect the mycetoma prevalence as individuals who wore shoes most of the time had a prevalence of 0.94% (95% CI = 0.84-1.06), while individuals who wore shoes either at home or work only had a prevalence of 0.74% (95% CI = 0.50-1.08) (Table 2.2).

Table 2.2 Number of cases and population of each category and prevalence of mycetoma patients (N=359) with 95%CI among the studied individuals (41,176) in Eastern Sennar locality, Sennar State, Sudan.

Variable	**Cases**	**Population**	**Prevalence %(95%CI*)**
Gender			
Male	172	20753	0.83 (0.71-0.96)
Female	187	20423	0.92 (0.79-1.06)
Age group			
0-15 years	90	19227	0.47 (0.38-0.57)
16-30 years	121	10859	1.11 (0.93-1.33)
31-45 years	93	6111	1.52 (1.23- 1.86)
46-60 years	36	3094	1.16 (0.82-1.61)
>60 years	19	1885	1.01 (0.61-1.57)
Marital Status			
Currently married	198	14830	1.34 (1.16-1.53)
Currently unmarried	161	26346	0.61 (0.52-0.71)
Education			
Literate	208	23805	0.87 (0.76-1.00)
Illiterate	124	9976	1.24 (1.04-1.48)
Underage of school	27	7395	0.37 (0.25-0.53)
History of trauma			
Yes	123	7527	1.63 (1.36-1.95)
No	236	33649	0.70 (0.61-0.80)
Wearing shoes/ slippers			
Both work and home	264	28027	0.94 (0.84-1.06)
At work or home only	27	3647	0.74 (0.50-1.08)
Not at all	68	9502	0.72 (0.56-0.91)

Geographical distribution of mycetoma

The study included sixty villages distributed among all the administrative units of the locality. Doba had the highest prevalence of mycetoma among all the administrative units (1.14%), and Elreif Elshargi had the lowest (0.15%). The highest village prevalence was recorded in Awlad El-Tai village (6.2%), followed by Wad Yagoub (4.9%), and the lowest prevalence was estimated in Kasab Garbi (0.11%). There were nine villages with no cases and an average of seven cases per village across the other 51 surveyed villages (range 1-39). The mycetoma prevalence map showed cases clustered within the central and north-eastern locality, while the south-western part had few or no cases (Figure2. 1C). In the figure, more than nine villages have a prevalence of 0.00, and that was because eight villages recorded only one case.

2.5. Discussion

Mycetoma is one of the neglected tropical diseases increasingly recognised by the international scientific and funding communities. Most of its epidemiological characteristics are an enigma [20]. Globally, its incidence and prevalence are not well known. Furthermore, the infection route, incubation period and factors contributing to susceptibility and resistance to mycetoma are not well documented [50]. This is due to a lack of international attention, research funding, and interested institutes to work on mycetoma, leading to a scarcity of data on the disease's basic epidemiological features and its seriousness and magnitude, promoting the negligence cycle.

Furthermore, due to the patients' low socio-economic and health education levels, the painless and slow-progressing nature of the disease, the lack of health facilities in endemic regions and the patients' inability to reach central hospitals for management, they tend to present late with advanced disease [90]. Hence, most of the mycetoma epidemiological characteristics were obtained from case reports and a series of hospital patients with advanced disease, representing the tip of the iceberg.

The present study is distinctive as it is community-based, and multi-level data were collected to determine the mycetoma clinical, epidemiological characteristics in the study area.

This study documented a mycetoma prevalence of 0.87% in the studied locality, higher than previous Sudan estimates [25]. Abbott studied individuals who managed to reach health facilities for diagnosis and reported a disease prevalence of 0.51% among hospital patients seen in Khartoum during a study period of 36 months. In addition to the higher prevalence in Atbara, Ed Dueim, and Wad Madani cities (within central Sudan states) with estimates of 0.92%, 0.93% and 1.18%, respectively [84]. Still, the reported prevalence could have underestimated the burden of disease because all of these cases are recorded in hospital records and show only the cases that reached the medical facility for diagnosis and management. The reported prevalence (1.45%) by Fahal and associates in 2014 is higher than that reported in the present study. That could be attributed to the high endemicity of mycetoma in the village where that survey was conducted [78].

Van de Sande in 2014 conducted a systemic review in an attempt to determine the global burden of mycetoma, reviewing 8,763 cases from different countries around the world, and estimated the prevalence for endemic countries such as Sudan and Mexico to lie between 0.0015 and 0.018 cases per 1000 inhabitants [25]. The difference between these studies and the current study is the study's design, which was performed at a community level, examining all the individuals in all the households of the selected communities.

Previous studies suggested that the distribution of mycetoma is affected by environmental and climate factors and due to the multifactorial nature of the disease environmental and climatic factors are important contributors to the mycetoma distribution [91]. Although not addressed in this chapter, these factors are examined later in chapter 4 to determine implicated environmental and climatic factors. A previous modelling study predicted mycetoma occurrence in central and south-eastern states of Sudan and along the Nile River and indicated that arid areas proximal to water sources, soil with low concentrations of calcium and sodium and areas with a variety of thorny tree species provided the most suitable environment for the occurrence of mycetoma in Sudan [16].

The results showed that mycetoma is prevalent in the central and north-eastern locality, with Doba administrative unit having the highest prevalence recorded. The local environmental and sanitation conditions may explain this geographical distribution [8, 17]. People in those areas mainly work in farming and with animals, are exposed to organisms residing in soil and lack access to good sanitation. In the hygiene and sanitation analysis, most of the individuals had to get water from a central pipe in the village, and the rest had to get water from other sources that were not entirely clean such as nearby rivers and required walking for long distances to reach. A combination of poor access to clean water and exposure to mycetoma-causative organisms were likely to promote susceptibility to mycetoma.

Male predominance is a documented feature of mycetoma. In most studies, the reported male/female ratio is 3-4: 1 [4, 92]. In this study, the disease prevalence was slightly higher in females, contradicting all the previous reports. The prevalence reported here may be more accurate as it is a community-based study, and most of the previous studies were hospital-based. Females are less likely to seek medical treatment [90] and often present late, suggesting previous studies may have been biased by female health-seeking behaviour [4, 19].

In this study, individuals aged 16-45 years were most affected. This concurs with the literature, and the results are not surprising given that this is the most active group in society and is often involved in farming and animal grazing practices [93-95]. A fifth of the affected individuals were students who helped with farming and animal care during vacations. Also, students must walk long distances to and from schools in the rural communities and hence are more exposed to the environment.

The medical literature documents a high incidence of mycetoma among farmers and labourers. It was postulated that the direct and continuous contact with the environment and soil where the causative organisms reside, and minor trauma and thorn pricks are important disease predisposing factors [7]. In the present study, arable farmers had a higher prevalence of mycetoma than shepherds, and this is attributed to farmers being more prone to injuries with sharp objects when working the field. Furthermore, individuals with a history of local trauma and thorn pricks had a higher mycetoma prevalence, in line with the reported studies [53, 91, 96].

Several studies suggested a possible role for animal dung in the development of mycetoma as some organisms were isolated from the dung, which may act as a reservoir for them allowing the direct transmission to the human [51, 54, 91, 97]. Most individuals living in rural areas are in direct contact with animals such as cattle, donkeys, dogs, sheep, and chickens. I found no strong evidence supporting a role for animals or their dung in the development of mycetoma, which needs further in-depth study [50, 54].

In this study, the disease duration (defined here as time since first symptoms developed) ranged between one month and 40 years, with a mean duration of 4.5 years before presentation. A study conducted in West Bengal showed that the disease period could vary according to causative organisms such as Nocardia, Streptomyces, Actinomadura species, and *Madurella grisea*. The shortest duration was three months before presentation for all organisms and the longest was three years *for Nocardia* and *Actinomadura species,* while *Madurella grisea* organisms reached up to nine years [92]. However, due to the painless nature of the disease, duration is subject to memory bias.

Mycetoma was reported to affect different body parts, but the foot and hand are affected the most [24]. In this study, the obtained data align with this: the lower extremities (67%) and upper extremities (22.6%) were most affected. This result is expected since men primarily work as barefooted farmers, allowing exposure to injury and inoculation with mycetoma-causative organisms. In this study, only 2.3 % of male cases presented with trunk mycetoma, which could be attributed to the nature of rural residents' occupation that makes them prone to injuries of the upper or lower extremities. In contrast, trunk mycetoma was recorded in 19% of patients in a study conducted in Mexico analysing mycetoma patients seen in mycological centres from 1958 to 2012, and in 10% from a single centre study in patients seen in the period between 1980 to 2013. In Mexico wood for domestic activities is consistently carried on the backs, explaining these findings [47, 98].

An interesting observation was noted in this study, that females have more hand mycetoma. This may potentially be attributed to the fact that females are commonly responsible for cooking and getting wood from forests to be used as a fuel source and are prone to minor hand injuries. Also, they are involved in different indoor activities such as cleaning the floor and removing animal dung from within the houses where the organisms possibly reside.

The triad of subcutaneous swelling, multiple sinuses and discharge that contain grains is pathognomonic of mycetoma [10, 82, 99]. In our study, 80.8% of the patients presented with swelling, 38.4% had sinuses, and 32% of these sinuses produced purulent and sero-purulent discharge with mostly black grains. Black grains are usually produced in the fungal form of the disease eumycetoma, and in Sudan, eumycetoma accounts for 60-70 % of mycetoma cases and the same pattern is observed for patients in Eastern Sennar Locality [78][80]. Only 3% had white grain discharge, which could be attributed to actinomycetoma, the bacterial type of mycetoma. Actinomycetoma is described but not widespread in Sudan but constitutes most mycetoma cases in Central and South America [25, 47].

Currently, there is no evidence-based preventive or control programme or notification system for mycetoma as most of its epidemiological characteristics are not well known. Wearing shoes is considered a preventative measure for mycetoma since mycetoma is most commonly seen in the foot and is believed to be contracted via injuries by sharp objects [96].

In this study, I found that wearing shoes did not affect the prevalence of mycetoma in the region, as the prevalence in people who wore shoes was almost exactly the same as the prevalence in people that did not wear them. However, in mycetoma endemic regions, people frequently work in the fields and walk barefooted as the available shoes can be an obstacle to executing these activities and sometimes are regarded as a hindrance. Due to the hot weather in Sudan, people tend to wear light open shoes, which offer little foot protection.

Even though individuals living in endemic areas share the same environment and are exposed to similar risk factors, only a proportion develop mycetoma. This supports the hypothesis that genetic factors play a role in mycetoma. In our study, 35.7% of mycetoma patients had a family history of mycetoma. It is essential to highlight that consanguineous marriages are common in rural areas where mycetoma is endemic, which may explain this observation. In previous studies, family history was strongly associated with disease recurrence; individuals with a positive family history might be more genetically susceptible to initially contracting the disease and getting recurrent disease [81].

In conclusion, this clinical, epidemiological community-based study is the first of its kind to be reported. The disease prevalence reported here may be more accurate as it was generated from a large study population. It revealed an equal sex ratio which contradicts most of the previous reports. Other findings are in line with those reported previously. Further epidemiological studies are needed to determine mycetoma prevalence in Sudan nationally and to bridge the gaps in our understanding of the epidemiology of mycetoma, which is vital to design evidence-based control and prevention programmes. Furthermore, these surveys are also helpful in early case detection and treatment, health education, disease awareness, and advocacy, reducing the disease burden and improving the disease prognosis. However, implementing surveys in rural areas in Sudan could be difficult, particularly during the rainy seasons, and proper team training, good facilities, and collaboration between different stakeholders including village leaders, health care workers and ministry of health officials are all required.

Chapter Three: Individual Risk Factors of Mycetoma Occurrence in Eastern Sudan: A Case-Control Study

3.1. Overview

In this chapter, I employed a case-control study design to determine the risk factors for mycetoma based on the data obtained from the survey in Eastern Sennar Locality. Each mycetoma positive case was matched with three controls based on sex and village. Until now, predisposing factors for mycetoma have not been entirely understood. Recognition of risk factors is considered a cornerstone in the early detection of mycetoma and proper management.in this study the individual risk factors will be documented for the first time. Then prevention and control programmes could be formulated based on accurate data obtained from such a study. In addition, this work was conducted in one endemic state, while mycetoma cases occur in all states of Sudan. Replicating this study over a wider area would give a fuller picture of the situation, providing the control program with more comprehensive information on the risk factors for the disease.

3.2. Introduction

Mycetoma is reported worldwide, and Sudan is considered the most endemic country, though mycetoma is commonly reported from Mexico, Venezuela, Mauritania, Senegal, Chad, Ethiopia, Somalia, Yemen, and India [45]. The disease is associated with massive morbidity, stigma and reduced economic productivity [101]. The disease is most frequently reported in young adults aged 20-40 years, but people of all ages are at risk. Males are at higher risk, but a community-based study in White Nile, Sudan, showed an almost equal risk for both sexes [12]. Mycetoma is seen more frequently in the feet of patients of low socioeconomic status, with poor hygiene and in villages with animal enclosures made of thorny trees. These observations have led to the belief that thorn pricks and minor injuries are important routes of mycetoma infection. Beyond this, mycetoma is a multifactorial disease and it is likely that other factors also play a role in the development of mycetoma, determining which individuals develop disease while others stay healthy despite exposure to the same environment. However, other risk factors have not been established [14].

This lack of knowledge about factors favouring transmission, and determinants of susceptibility to the disease impede the design and implementation of effective prevention strategies [10, 50, 90, 100]. There are currently no dedicated control programmes for mycetoma, and the only available tools to reduce the disease burden are early case finding and appropriate management. However, knowledge gaps surrounding its incidence, prevalence, and determinants of susceptibility exist, hampering to ability to diagnose and treat the disease [12, 96, 101]. The promotion of early case detection is costly and relies on appropriate public health messaging and community education. A case-control study was conducted to address this issue to determine risk factors for mycetoma infection in area highly endemic areas for mycetoma in Sudan.

3.3. Material and Methods

This case-control study was conducted in Eastern Sennar Locality, Sennar State, central-eastern Sudan, and included the 359 mycetoma cases identified in the survey described in chapter 2, each matched to three healthy controls were selected from the same survey population by simple random sampling. The control group was selected from households where no suspected cases were detected and matched on community and sex.

Data were sent directly to a server at the MRC and imported to the Statistical Package for Social Sciences, SPSS 25 (SPSS, Chicago, IL, USA) for analysis. Descriptive analysis was performed, and bivariate analysis (chi-square test) was used to identify any statistically significant associations between explanatory variables and the outcome variable (confirmed mycetoma).

The independent variables of this study were: age, sex, marital status, education, occupation, wearing shoes, history of trauma, Source of fuel for cooking, primary material of the dwelling floor, main material of the dwelling roof, main material of dwelling exterior walls, Animal raised within the dwelling and Livestock ownership. A logistic regression model was produced with mycetoma status as an outcome variable (case and control). The univariate analysis included independent variables (individual and household-level variables) and the multivariate analysis conducted included all independent variables in the model to assess the strength of the association with the outcome (i.e. being a mycetoma case or control). I used stepwise regression which is a step-by-step iterative construction of a regression model that involves the selection of independent variables to be used in a final model. It involves adding or removing potential explanatory variables in succession and testing for statistical significance after each iteration. The goal of stepwise regression is to find a set of independent variables that significantly influence the dependent variable. Forward selection began with no variables in the model then variables were added one at a time and all variables with a P-value of 0.05 or less were kept in the model. The backward selection was conducted with all variables then removing variables one at a time when a p-value was 0.1 or above. The strength of association of each retained variable with mycetoma was expressed using the odds ratio with a confidence interval (CI) of 95% for the univariate analysis and in the multivariate analysis the adjusted odds ratios (adjusted for the other variables in the model) with a confidence interval (CI) of 95%. The STROBE case-control reporting guidelines were used in this study [102].

3.4. Results

3.4.1. Demographic characteristics of cases and controls

A total of 1,436 individuals (359 mycetoma patients and 1,077 controls) were included and the frequency distribution was normally distributed. The mean age of confirmed mycetoma patients was 27.0 (SD=16.3) years, ranging from 1- 85 years. The mean age of controls was 37.2 (SD=16.9) years, ranging from 1-105.

The patient group comprised 174 males (48.5%) and 185 females (51.5%), and the control group 537 (49.9%) males and 540 (51.1%) females. Sixty-seven (18.7%) cases and 236 (21.9 %) of controls worked as shepherds or farmers. One hundred and thirty-nine cases (38.7%) and 525 controls (48.7%) were illiterate.

3.4.2. Clinical characteristics of cases

In total, 290 cases (80.8%) presented with visible swelling, and 245 (68.2%) had lesions in the lower extremities. Sinuses were present in 138 (38.4%), and of those, 122 (33.9%) patients had discharge. One hundred and twenty-nine cases (35.9%) had a history of local trauma at the mycetoma site.

Age, history of trauma, marital status, education, raising animals within the household and livestock ownership were strongly associated with the odds of mycetoma (p-value <0.05). Compared to individuals aged 0- 15, the odds of mycetoma were more than six times higher in individuals in the age group 16-30 years (OR = 6.467, 95% CI= 3.421 - 12.224, $p < 0.001$), and around two times higher those aged 31-45 or 46-60 years. The likelihood of mycetoma in individuals with a history of trauma was 71% higher than in those without (OR = 1.710, 95% CI= 1.323 - 2.209, $p < 0.001$). The odds of mycetoma in unmarried individuals were over four times that in married individuals

(OR = 4.117, 95% CI= 3.151 - 5.381, p < 0.001), while illiterate individuals lower odds were observed (OR = 0.664, 95% CI= 0.521– 0.848, p =0.001). Compared to individuals in other occupations, housewives had increased odds of mycetoma (OR = 4.945, 95% CI= 2.747 – 8.902, p < 0.001), as did those who were desk employees (odds ratio, 1.251, 95% CI= 1.279 – 3.961, p = 0.005). Individuals living in households with animals were raised within the dwelling had lower odds (OR = 0.557, 95% CI= 0.435 – 0.713, p < 0.001). Ownership of animals increased the odds of mycetoma by more than two times (OR= 2.15, 95% CI= 1.687 – 2.742, p < 0.001). (Table 3.1)

Table 3.1 Demographic characteristics of confirmed cases and controls, showing unadjusted odds ratios (OR) for mycetoma risk factors (n=1436)

Characteristic	**Cases No. (%)**	**Controls No. (%)**	**Crude OR (95%CI)**	***p*-value**
Individual factors				
Age group				
0-15 Years	70 (19.5%)	70 (6.5%)	1.0	
16-30 Years	136 (37.9%)	391 (36.3%)	6.467(3.421 - 12.224)	**<0.001**
31-45 years	101 (28.1%)	344 (31.9%)	2.249 (1.262 - 4.008)	0.006
46-60 Years	37 (10.3%)	175 (16.2%)	1.899(1.055 - 3.416)	0.032
>60 Years	15 (4.2%)	97 (9.0%)	1.367(0.714 - 2.617)	0.345
Sex				
Female	185 (51.5%)	540 (50.1%)	1.0	
Male	174 (48.5%)	537 (49.9%)	1.069(1.414 - 2.290)	0.583
Trauma history				
Yes	129 (35.9%)	266 (24.7%)	1.710 (1.323 – 2.209)	**<0.001**
No	230 (64.1%)	811 (75.3%)	1.0	
Marital status				
Married	207 (57.7%)	905 (84.0%)	1.0	
Unmarried	152 (42.3%)	172 (16.0%)	3.864(2.963 – 5.038)	**<0.001**
Education level				
Literate	220 (61.3%)	552 (51.3%)	1.0	
Illiterate	139(38.7%)	525 (48.7)	0.664 (0.521– 0.848)	**0.001**
Wear shoes/ Slippers				
Both work and home	261 (72.7%)	828 (76.9%)	1.0	
At work or at home only	32 (9.8%)	84(7.8%)	0.788 (0.574 - 1.083)	0.142
Not at all	66 (18.4%)	165 (15.3%)	0.952 (0.579 - 1.566)	0.847

Occupation				
Farmers or shepherds	67 (18.7%)	236 (21. 9%)	1.0	
Students	67 (18.7%)	60 (5.6%)	1.257 (0.728 – 2.170)	0.411
Housewives	114 (31.5%)	447 (41.5%)	4.945 (2.747 – 8.902)	**<0.001**
Merchants	15 (4.2%)	76 (7.1%)	1.129 (0.674 – 1.893)	0.644
Unemployed and underage of work	61 (17.3%)	120 (11.1%)	0.874 (0.422 – 1.811)	0.717
Desk employee	5 (1.4%)	29 (2.7%)	1.251 (1.279 – 3.961)	**0.005**
Freelancer	21 (5.8%)	93 (8.6%)	2.491 (0.969 – 6.403)	0.058
Other jobs	9 (2.4%)	16 (1.5%)	0.764 (0.264 – 2.205)	0.618
Household factors				
Source of fuel for cooking				
Wood and animal dung	243 (67.7%)	433(40.2%)	0.600 (0.429 – 0.839)	**0.003**
Gas and coal	77 (21.4%)	130 (12.1%)	1.0	
Any source of fuel available	35 (9.7%)	500 (46.4%)	0.403 (0.286 – 0.569)	**<0.001**
No food cooked in the house	4 (1.1%)	14 (1.3%)	0.476 (0.151 – 1.498)	0.205
The primary material of the dwelling floor				
Brick/cement/ceramic	20(5.6%)	50 (4.6%)	2.160 (1.372 **-** 3.400)	**0.001**
Earth/soil and/or sand	326 (90.8%)	1007 (93.5%)	1.0	
Combination	13 (3.6%)	20(1.9%)	1.271 (0.554 – 2.913)	0.571
The main material of the dwelling roof				
Traditional/wood/zinc/plastic cover	256(71.3%)	760 (70.6%)	1.101 (0.764 – 1.588)	0.605
Concrete	42(11.7%)	145(13.5%)	1.0	
Combination	61 (17.0%)	172 (16.0%)	1.169 (0.748 – 1.826)	0.493

The main material of dwelling exterior walls				
Wood	40 (11.1%)	104 (9.7%)	0.824 (0.525 – 1.294)	0.401
Mud and/or animal dung	114(31.8%)	371 (34.4%)	0.847 (0.626 – 1.147)	0.284
Red bricks and/or concrete	97 (27.0%)	324 (30.1%)	0.786 (0.572 – 1.079)	0.137
No walls	108 (30.1%)	278 (25.8%)	1.0	
Animal raised within the dwelling				
Yes	127 (35.4%)	534 (49.6%)	0.557 (0.435 – 0.713)	**<0.001**
No	232 (64.6%)	543 (50.4%)	1.0	
Livestock ownership				
Yes	183 (51.0%)	351 (32.6%)	2.151 (1.687 – 2.742)	**<0.001**
No	176 (49.0%)	726 (60.4%)	1.0	

3.4.3. Multivariate analysis

After adjusting for other significant variables, being unmarried was the strongest risk factor for mycetoma (AOR = 3.179, 95% CI= 2.339 – 4.20, p <0.001). The odds of the disease were roughly double for patients with a history of local trauma compared to those without (AOR = 1.892, 95% CI= 1.425 – 2.513, p <0.001). Those aged 16-30 had higher odds (AOR = 2.804, 95% CI = 1.424 – 5.523, p =0.003) compared to those aged 0-15 years. Illiterate individuals had lower odds of mycetoma (AOR = 0.685, 95% CI= 0.521 – 0.900, p= 0.007). Individuals who owned animals had higher odds of mycetoma (AOR = 3.914 ,95% CI= 2.874 – 5.405, p< 0.001), but those keeping animals within their own dwelling had lower odds of disease (AOR = 0.310, 95% CI= (0.303, 95% CI= 0.220 – 0.416, p< 0.001) (Table 3.2).

Table 3.2 Individual risk factors for mycetoma at Eastern Sennar locality, Sennar State (n=1436)

Individual Characteristics	Cases No. (%)	Controls (%)	AOR (95%CI)	p-value
Age group				
0-15 years	70 (19.5%)	70 (6.5%)	1.0	
16-30 years	136 (37.9%)	391 (36.3%)	2.804 (1.424 – 5.523)	0.003
31-45 years	101 (28.1%)	344 (31.9%)	1.564 (0.852 – 2.871)	0.149
46-60 years	37 (10.3%)	175 (16.2%)	1.469 (0.791 – 2.726)	0.223
> 60 years	15 (4.2%)	97 (9.0%)	1.218 (0.617 – 2.405)	0.570
Trauma history				
Yes	129 (35.9%)	266 (24.7%)	1.892 (1.425 – 2.513)	<0.001
No	230 (64.1%)	811 (75.3%)	1.0	
Marital status				
Married	207 (57.7%)	914 (84.9%)	1.0	
Unmarried	152 (42.3%)	163 (15.1%)	3.179 (2.339 – 4.20)	<0.001
Education level				
Literate	220 (61.3%)	559 (51.9%)	1.0	
Illiterate	139 (38.7%)	518 (48.1)	0.685 (0.521 – 0.900)	0.007

Animal raised within the dwelling				
Yes	127 (35.4%)	534 (49.6%)	0.303 (0.220 – 0.416)	<0.001
No	232 (64.6%)	543 (50.4%)	1.0	
Livestock ownership				
Yes	183 (51.0%)	351 (32.6%)	3.941 (2.874 – 5.405)	<0.001
No	176 (49.0%)	726 (60.4%)	1.0	

3.5. Discussion

Although mycetoma is a severe disease, inflicting disability and stigma on patients across many parts of the world, there remain essential questions about its epidemiology, particularly the risk factors for the disease. In the current study, patients with confirmed mycetoma were compared to the community- and sex-matched controls to identify determinants of risk in the study population. To the best of our knowledge, this is the first population-based case-control study of sociodemographic risk factors for mycetoma, providing evidence likely to apply to other settings and support global control efforts.

One of the risk factors I identified was a history of trauma, which roughly doubled the odds of mycetoma. This finding represents the most substantial epidemiological evidence for the theory that skin trauma may facilitate the inoculation of mycetoma-causative organisms into the subcutaneous tissue- although this does not rule out other possible routes of transmission [2]. This hypothesis is supported by the fact that mycetoma-causative organisms typically reside in the soil and evidence that certain Acacia trees - whose thorns may facilitate inoculation - are associated with environmental suitability for the disease [16]. However, previous analyses of mycetoma cases have shown a history of trauma in only a few patients, hypothesised to reflect that the trauma may be minor and pass unnoticed by the patient [96]. The case-control design I employed, including many patients and community-matched controls, allowed us to demonstrate a significant difference in the history of trauma between these two groups.

Many reports in the literature show that young adults and children are the most affected populations [25, 45, 103, 104]. The data obtained in this study showed that the most common age group affected is the category 16-30 years. This higher odds in younger age groups is likely to reflect that these individuals are more likely to be actively engaged in agricultural work and animal grazing activities, which expose them to mycetoma-causative agents in endemic areas. Older individuals, particularly those aged more than 60 years, appeared to be at lower risk, presumably because this age group is less likely to be involved in farming activities. Alternatively, this group may include individuals who have been exposed to the causative organisms over time but did not develop disease due to effective host immune defences. Sero-epidemiological studies support this possibility, and host genes may play a role in determining resistance to developing mycetoma after infection with causative organisms [34].

As expected, livestock ownership was a decisive risk factor for mycetoma. There are several possible mechanisms by which people who raise animals may be at increased risk of mycetoma. People living in rural areas tend to make animal enclosures from the wood of thorny trees and may be at risk of thorn pricks during the construction and maintenance of these structures [96]. As well as being in close contact with the environment, they are likely to be at higher risk of ticks, which are highly prevalent in domestic animals in Eastern Sennar Locality and recently hypothesised to play a role in the transmission of mycetoma causative agents to humans [54]. The evidence for this route of transmission is not conclusive, however. While the DNA of mycetoma-causative organisms has been isolated from ticks, this does not prove they are involved in transmission.

I found that unmarried individuals' odds of contracting mycetoma is four times compared to their married counterparts. This is likely to reflect the social stigma of mycetoma, which may mean affected individuals are less likely to get married, or more likely to get divorced if they contract the disease after marriage. In rural communities, early marriage is often considered mandatory and social pressure is put on young adults to get married by early teen years. If they fail to get married early, this pressure can cause phycological distress in addition to the stress and depression occurring as a result of such a stigmatizing disease [105].

Interestingly, there was no significant difference between literate and illiterate populations and increased odds for mycetoma in desk employees, contradicting previous reports in the literature that mycetoma is commonly prevalent among poor communities with low education levels [90]. This may be explained by the fact that in mycetoma-endemic areas, most individuals share the same social and economic activities and behaviour irrespective of their educational level due to economic constraints. Another factor may be that schools in rural areas are limited to certain areas, and students often have to walk for long distances, putting them at higher risk of contact with mycetoma-causative organisms. I also observed that housewives and desk employees had increased odds of mycetoma; usually, housewives are responsible for the cooking, and hence they walk long distances to collect wood to use as fuel. Furthermore, housewives work in farming, especially in harvesting season, which may mean that they have a similar level of exposure.

In this population-based case-control study on mycetoma, the first of its kind at this scale, the high number of participants enabled precise estimates for the strength of association of various risk factors with the disease. The methods used for confirmation of the disease, including clinical examination of all individuals, followed by ultrasound examination for confirmation, also meant that cases and controls were classified with high accuracy. One limitation of this study was that some suspect cases were lost to follow-up and were not included in the comparative analysis. However, I have no reason to believe that the absence of these individuals was due to their disease status or other systematic factors, so consider this data to be missing at random.

The results of this study could be applied to inform the future control of mycetoma. Efforts to raise awareness among clinicians of mycetoma risk factors - particularly age, history of trauma, and ownership of animals - could promote earlier diagnosis and treatment of mycetoma in patients presenting swellings or wounds in endemic regions of Sudan. These factors could also inform the design of health awareness campaigns in communities at risk, educate the population about activities that may put them at risk of the disease and encourage them to present early to health facilities if they experience early signs. Finally, this study adds further evidence for the substantial social impact of the disease and the stigma associated with it, which should not be overlooked in assessments of its global burden.

Chapter Four: Modelling the spatial distribution of mycetoma in Sudan

4.1. Overview

This chapter aimed to identify the environmental niche predictors of fungal and bacterial mycetoma (eumycetoma and actinomycetoma, respectively) in Sudan and identify areas of the country where these niche predictors are met. A combination of regression and machine learning techniques were used to model the relationships between mycetoma occurrence in Sudan and the primary environmental predictors. Demographic and clinical data from patients seen at the MRC, University of Khartoum, Sudan, between 1991 and 2018 who presented with confirmed mycetoma were included in this study. Suitability maps resulting from this work are intended to guide health authorities, academic institutes, and organisations in planning national scale surveys for early case detection and management, which is required for better patient management and the development of prevention and control programmes for mycetoma.

4.2. Introduction

Mycetoma is a neglected tropical disease that has numerous health and socioeconomic impacts on patients and communities [75, 78, 95]. Mycetoma is reported worldwide, but it is endemic in tropical and subtropical regions [25, 106]. Mycetoma endemic areas are characterised by moderate aridity, low humidity, and a short rainy season [4, 47, 53]. Sudan is considered one of the endemic regions for mycetoma among a list of other countries [107, 108]. Eumycetoma is frequently reported in Africa and India, whereas actinomycetoma is more predominant in Asia and Central and South America [104, 109].

Most of the epidemiological characteristics of mycetoma disease are uncertain. The route of infection, the incubation period, and the factors that determine susceptibility and resistance to disease are largely unknown [27, 96]. Likewise, its incidence, prevalence, and burden have not been appropriately estimated at global or national levels. This lack of data is one reason for the absence of control and prevention programmes targeting the disease [96, 110].

Although the infection pathway is unknown, bacteria and fungi causing mycetoma are thought to penetrate through skin lesions and wounds [111]. It is hypothesized that thorns from trees and shrubs of the Acacia genus are a significant cause of wounds through which mycetoma infectious agents can infect humans. In most mycetoma endemic countries, thorny trees and shrubs such as Acacia species and other species from the pea family (Family: Fabaceae) are present. Many Acacia species bear sharp thorns that protect their leaves from herbivorous animals and which in the ground pose a threat to individuals walking barefoot or in open shoes such as sandals [112]. Other potential environmental risk factors that have been identified include hygiene practices and proximity to animals [96]. Many individuals living in rural areas have poor access to improved water and sanitation facilities, which impedes good hygiene and could be one of the factors allowing the organism to establish infection in the context of direct and sustained contact with soil, animals, and their dung [54]. Many mycetoma-causative organisms have been found in dung-enriched soil; hence dung could also provide a natural habitat for mycetoma-causative agents [27]. Despite the long history of mycetoma in Sudan and efforts to fight it, the incidence, prevalence and geographical distribution remain unclear [53]. In the literature mycetoma patients in Sudan are often poor and illiterate, and contracting mycetoma adds to their socio-economic burden.

The distribution of mycetoma has previously been modelled using ENM techniques, which suggested central Sudan be suitable for mycetoma occurrence due to evident overlap of Acacia distribution and mycetoma cases. However, in this study, only 44 mycetoma confirmed cases were used, and they were distributed 12ºS and 19ºN [53]. In this work, I aim to update these models by constructing models using a large occurrence dataset, separated by type of mycetoma (actinomycetoma vs eumycetoma), using a more comprehensive set of environmental predictors, and with a range of modelling algorithms. The present study was conducted at the MRC, The University of Khartoum, the only WHO collaborating centre on mycetoma globally [26]. The MRC was established in 1991 to tackle the burden of mycetoma in Sudan with well-trained dedicated staff for the diagnosis and management of mycetoma patients attending from all the states of Sudan. More than 9,500 patients have been seen and managed at the centre.

4.3. Materials and Methods

4.3.1. Data Source

The data included in this study were extracted from the patient database at the MRC. The study included only patients from Sudan who were seen at the center during 1991-2018. The variables of interest included the patients' demographic characteristics, details of their clinical presentation and diagnosis, and their location of origin using patients' addresses recorded within the database then referenced to get the geographical coordinates referred to below as mycetoma occurrence locations. The diagnosis of mycetoma and its classification into eumycetoma and actinomycetoma were based on a careful clinical interview and examination, grain culture, cytological examination of aspirates from mycetoma lesions histopathological examination of surgical biopsy samples. Molecular diagnosis using conventional Polymerase chain reactions (PCR) using Pan fungal/bacterial primers and species-specific primers was introduced in 2017.

4.3.2. Environmental predictors and variable selection

4.3.2.1. Candidate predictors

Since some determinants of mycetoma occurrence remain unelucidated, I started with a wide range of candidate predictors to avoid the exclusion of relevant predictors. I used correlation analysis followed by principal components analysis (PCA), a technique used to reduce the dimensionality of large datasets [113].

Continuous gridded datasets (rasters) of 52 environmental variables relevant to the mycetoma ecological niche were assembled. These raster datasets included precipitation and temperature, soil composition and pH, livestock distributions, proximity to water sources, elevation and related topographical variables, the predicted distributions of *Acacia* species and other thorny vegetation, measures of atmospheric moisture availability determine potential vegetative growth and an index of vegetation coverage. Previous studies have indicated that these variables are associated with mycetoma occurrence in Sudan and elsewhere [7, 26, 53, 114].

Climate variables such as minimum, maximum, and mean temperature and precipitation levels were obtained from the WorldClim database (version 2.0), a repository of climatic indicators based on long-term data collection from weather stations [109]. In addition, I obtained from the Consortium for Spatial Information (CGIAR CSI) estimates of aridity (rainfall deficit) and potential evapotranspiration (PET, an indicator of atmospheric demand for moisture) [115], both modelled by using WorldClim datasets and a dataset of modelled elevation, based on data collected by the Shuttle Radar Topography Mission (SRTM) [116].

I compiled soil composition and pH datasets from the International Soil Reference and Information Centre (ISRIC)-World Soil Information project [117]. Raster datasets of predicted livestock distributions (cattle, chicken, sheep and goat), modelled by collaborators from the International Livestock Research Institute (ILRI), the Food and Agriculture Organization of the United Nations (FAO) and the Université Libre de Bruxelles (ULB-LUBIES), were obtained from the Gridded Livestock of the World (GLW) version 2 database [118].Then I downloaded spatial data on waterbodies and waterways from the OpenStreetMap project (OSM) through the *Geofabrik* platform and produced continuous surfaces of straight-line (Euclidean) distance in km to each [119]. The

Enhanced Vegetation Index (EVI) dataset was generated using satellite imagery data from the MODIS (Moderate Resolution Imaging Spectroradiometer) satellite from the Earth Explorer NASA site [120]. Full details of these data sources are shown in (Appendix 7, Table 1).

Finally, I modelled the distributions of 11 species of thorny trees which are considered potential vehicles for the inoculation of mycetoma-causative organisms using occurrence records downloaded from the Global Biodiversity Information Facility [53] along with a suite of environmental variables. Full details of the modelling of these thorny plant species are given in a supplemental file (Appendix 7).

Each of these gridded datasets was resampled to 1 km^2 resolution using bilinear interpolation and clipped and aligned to the outline of Sudan. Bilinear interpolation is a method of estimating a value for a cell based on four neighboring, cells creating a smoother surface than other resampling techniques, such as nearest neighbor resampling. Bilinear interpolation is appropriate for continuous datasets- including all of those included as covariates, apart from the thorn variety dataset, which did not require resampling as it was generated at 1km2. The WorldClim datasets, the elevation dataset (from which the wetness index and flow accumulation were derived), potential evapotranspiration and aridity index were downloaded at a resolution of 30 arc-seconds (slightly below 1km2 in the study area) in the WGS coordinate reference system (CRS). These were projected to the pseudomercator CRS and resampled up to 1km2. The soil variables were downloaded at 250m and resampled up to 1km2. The livestock density rasters were obtained at a resolution of 3 arc-minutes (approximately 5km2 in the study area), projected to the pseudomercator CRS and resampled down to 1km2. All other datasets were downloaded or generated at a resolution of 1km2. . Raster processing was done using the *raster* package in R v3.3.2, and the final map layouts were created with ArcGIS 10.5 software (ESRI Inc., Redlands CA, USA).

4.3.2.2. Variable Selection

Initially, the correlation coefficients was calculated between all candidate predictors across Sudan and identified groups of predictors correlated with a Pearson's correlation coefficient >0.8.

I then extracted values of each of the compiled raster datasets at each of the mycetoma occurrence locations to produce a matrix of covariates (a table showing the x and y coordinates of the occurrences, and at each of these locations, the values of all candidate covariates). And then I ran PCA separately for actinomycetoma and eumycetoma to identify the most relevant predictors of the distribution. In this process, the original variables are reduced into a set of "components" (or axes), which are linear combinations of variables and uncorrelated. The components are ordered by the fraction of the total dataset variance they contribute, and a value summarises the variance contribution of each component termed an eigenvalue. I identified the original variable with the most substantial influence from the minimum set of components that collectively contributed at least 80% of the total variation in occurrence locations (measured via the factor loading). I ensured that no variables were correlated with another included variable. This process resulted in a selection of uncorrelated variables that collectively contributed the most significant variance to the occurrence locations. I used the *prcomp* package in R to implement this analysis.

4.3.2.3. Ensemble modelling

A separate ensemble distribution models for actinomycetoma and eumycetoma was built, using the occurrence records and the suite of selected covariates for each. The ensembles were constructed from four algorithms from the BIOMOD package: Generalized Boosted Regression Model (GBM), Random Forest (RF), Generalized Linear Models (GLM) and Generalized Additive Models (GAM) [121]. I used the default parameters set by the biomod2 R package for each model. These algorithms are all classified as presence-absence models, meaning that they require both occurrence and absence points to determine environmental suitability, absence points being locations confirmed by surveys to be clear of the outcome under investigation. To account for the lack of absence points within the dataset, I generated "pseudoabsence" points, representing areas presumably unsuitable for mycetoma. Using pseudoabsence points to represent areas presumed to be unsuitable for a species is a well-established approach in species distribution modelling. Pseudoabsence (PA) points or (background points) are not to be considered absences, but a comparator class which is more practical to generate compared to true absence, which is difficult and costly to establish with certainty, and rather represent the available environment in the studied area. They will be used to compare the observed used environment (represented by the presence points) against what the available environmental conditions in the study area, or- as in this case- in areas less likely to be suitable. Usually obtaining true absences point is difficult and exhausting and hence using this technique is the practical option. I used the Surface Range Envelop (SRE) model, a presence only model which predicts suitability in an "envelope" representing the area containing values between the 2.5th and 97.5th percentiles of the selected predictor variables and used the area outside of this envelope for PA selection[121]. I also used geographical restriction to limit PA selection to more than 5km from the nearest occurrence point, and to within a defined geographical extent of the occurrence range. Restricting PA points to a certain distance from occurrence points has been recommended by previous authors as way to provide an ecologically meaningful definition of the study range[122]. To characterize the spatial structure, I used principal component analysis (PCA) to examine change in the influence of the most important environmental predictors over increasing geographical area[122]. I generated 1,000 random points within a buffer area of 50km radius around occurrence points and identified the factors which collectively contributed at least 70% of the variance in the first principal component. I repeated this process with buffer areas increasing in radius by 50km, until the contribution of the identified factors decreased or stopped increasing. The buffer size at this point was set as the geographical extent of the study. This method was applied to the both eumycetoma and actinomycetoma cases to set the geographical extent for the two datasets, and was found to be 150km for both types. It should be noted that there are other possible approaches to generating PA points which would be expected to impact model results, and the impact of this could have been assessed more thoroughly. However, I believe the approach used is supported by existing theory from species distribution models and known environmental risk factors for the disease. [123, 124]. I implemented pseudoabsence selection within the BIOMOD framework, using the "surface range envelope" approach to define the area of assumed unsuitability. The envelope is estimated through a presence-only suitability model [125], which identifies the range of locations at which the values of the chosen environmental covariates are within a specified range (here between the 5th and 95th percentiles) of the covariate values at the occurrence locations [121]. A set of pseudoabsence samples (here, a set of 5 samples), equal in size to the number of occurrence points, is randomly extracted from outside this envelope each time the model is run. Each algorithm was run 50 times with a random sample of 80% of the data points used for model training and the remaining 20% reserved for evaluation. The mean accurate skill statistic (TSS), the mean proportion correctly classified (PCC) which is equivalent to Kappa and the mean area under the curve (AUC) of the receiver operating characteristic (ROC) were used to evaluate the performance of each algorithm.

4.3.2.4. Variables of final models

For eumycetoma, aridity index, soil calcium concentration, wetness index, mean diurnal temperature, distance to the nearest river, presence of cattle, goats, and chickens, and predicted occurrence of Acacia mellifera and Faidherbia albida trees in the area were included. While for actinomycetoma, aridity index, distance to the nearest river, distance to the nearest water body (pond or lake), wetness index, soil sodium and iron concentrations, presence of cattle and sheep in the area, mean diurnal temperature and mean temperature in the coldest year quarter.

All AUC > 0.8 were compiled into the final ensemble distribution model. The predicted suitability value was the mean of the included models within the ensemble, weighted by their relative AUC. Upper and lower suitability limits were estimated by calculating confidence intervals around the ensemble mean suitability value for each cell within the grid. The final results were obtained by combining predicted probabilities from each of the models in the ensemble. The suitability index was generated from the weighted mean probability, with the contribution of each model weighted according to its performance based on evaluation statistics. The binary threshold was set by finding an optimal cut-off, maximizing the sensitivity and specificity of the ensemble model based on the receiver operating characteristic. Areas with weighted mean suitability above this cut-off were classified as suitable. Variable contribution plots were produced to show the relative contribution of each variable to the model, along with marginal effect plots to show the response of both mycetoma types to changes in each modelled covariate.

4.4. Results

4.4.1. Dataset of mycetoma occurrence

The modelled data included 7812 unique points after excluding patients from outside Sudan and those lacking diagnostic or geographical information. The patients were seen at the MRC from 1991 to 2018, and they came from all the states of Sudan. The study included 5513 patients (79%) with confirmed eumycetoma and 1470 patients (21%) with actinomycetoma (Figure 4.1). Most of the Mycetoma patients were from Al Jazirah State (34.4%) and Khartoum State (14.5%) (Table 4.1).

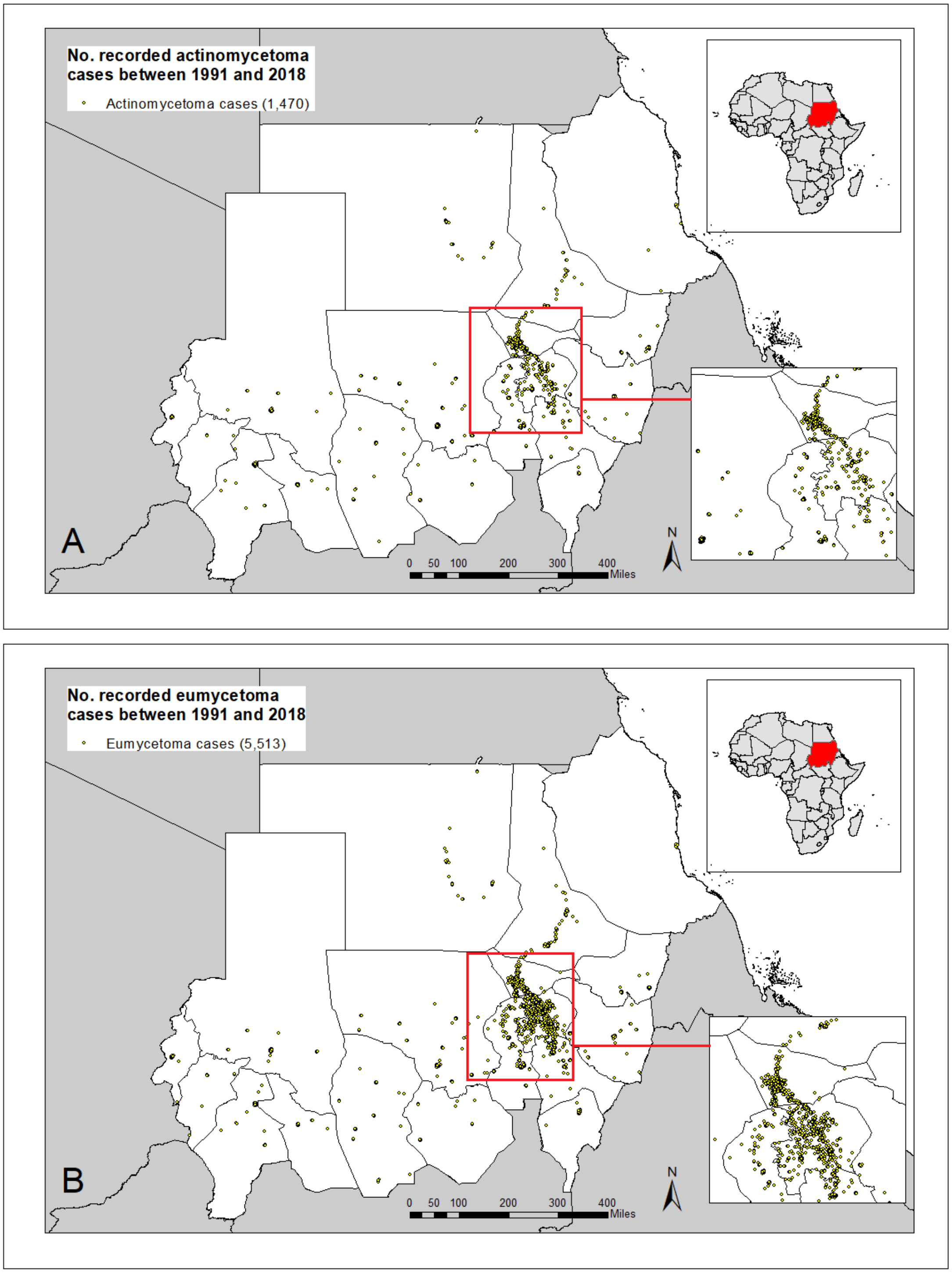

Figure 4.1 Records of mycetoma cases in Sudan (1991-2018), (A) is the records for the actinomycetoma cases and (B) is the records for eumycetoma cases, yellow dots represents the cases. Mycetoma cases are distributed along the River Nile, focusing on central Sudan, and cases are distributed sporadically in the western part of Sudan

Table 4.1 Distribution by State of mycetoma patients from Sudan seen at the MRC in the period 1991 – 2018

State	Eumycetoma	Actinomycetoma	Total
Al Jazirah	2163	234	2397 (34.4%)
Khartoum	730	280	1010 (14.5%)
White Nile	753	101	854 (12.2%)
North Kordofan	479	341	820 (11.7%)
Sennar	562	69	631 (9%)
River Nile	128	59	187 (2.7%)
South Darfur	118	62	180 (2.6%)
North Darfur	97	73	170 (2.4%)
Al-Qadarif	108	42	150 (2.1%)
Kassala	95	40	135 (1.9%)
South Kordofan	80	46	126 (1.8%)
West Darfur	44	36	80 (1.1%)
Northern	45	29	74 (1.1%)
Blue Nile	38	10	48 (0.7%)
East Darfur	34	15	49 (0.7%)
West Kordofan	19	12	31 (0.4%)
Red Sea	13	13	26 (0.4%)
Central Darfur	7	8	15 (0.2%)
Total	5513	1470	6970

4.4.2. Environmental predictors of eumycetoma occurrence in Sudan

The PCA for eumycetoma occurrence in Sudan identified ten variables that characterised the environmental conditions at the occurrence locations. These were aridity index, soil calcium concentration, wetness index, mean diurnal temperature, distance to the nearest river, presence of cattle, goats, and chickens, and predicted occurrence of *Acacia mellifera* and *Faidherbia albida* trees in the area. Two variables- the predicted density of cattle and chickens- were excluded after the initial modelling step as each contributed less than 1% of the variability in the response variable (Figure 4.2).

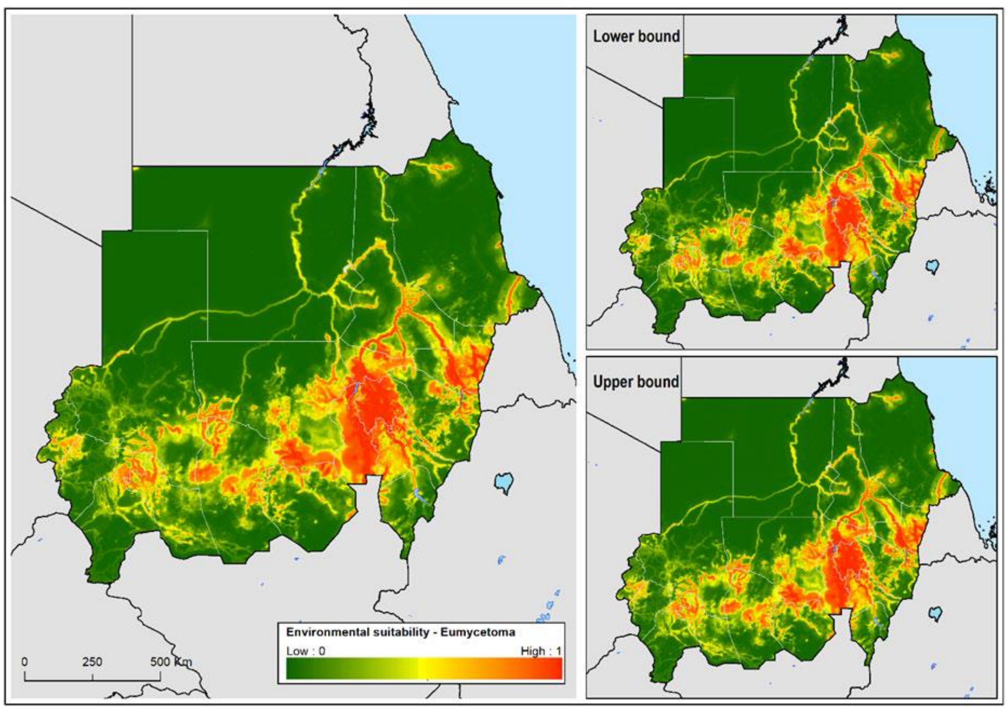

Figure 4.2 Predicted environmental suitability for eumycetoma in Sudan. Eumycetoma is predicted to occur along the River Nile and its tributaries with a hot spot on the Central and southeastern part of Sudan.

All models for eumycetoma occurrence performed well with an ensemble ROC score of 0.993, the sensitivity of 96.475 % and specificity of 94.476%. The mean TSS was 0.909, and the mean kappa score was 0.898 (Table 4.2).

Table 4.2 Validation metrics for ensemble models for eumycetoma and actinomycetoma suitability

		Weighted Mean	**Lower CI**	**Upper CI**
Eumycetoma	TSS	0.909	0.909	0.911
	ROC	0.993	0.993	0.993
	Kappa	0.898	0.897	0.898
Actinomycetoma	TSS	0.921	0.922	0.922
	ROC	0.995	0.995	0.995
	Kappa	0.903	0.903	0.902

Across the RF models, the distance to the nearest river was the strongest environmental predictor of suitability for eumycetoma occurrence, and the suitability decreased with increased distance to the nearest river. The diversity of thorny trees, represented by the number of species predicted to be present, was the second most crucial predictor of suitability for eumycetoma occurrence, with a greater diversity of thorny trees associated with a higher probability of occurrence of the disease. I found the suitability for the eumycetoma to be higher in areas with a lower probability of occurrence of *Acacia mellifera* and arid areas. Soil calcium concentration suitable for eumycetoma occurrence is between 3000 and 11000 mg/kg while mean diurnal temperature range and the probability of occurrence contributed to a lesser extent and goats' density was the minor contributor. (Appendix 7 figure 1).

4.4.3. Environmental predictors of actinomycetoma occurrence in Sudan

The PCA for actinomycetoma occurrence also revealed ten variables as potential environmental predictors. The variables were aridity index, distance to the nearest river, distance to the nearest water body (pond or lake), wetness index, soil sodium and iron concentrations, presence of cattle and sheep in the area, mean diurnal temperature, mean temperature in the coldest year quarter. After the primary analysis, four variables; distance to the nearest river, distance to the nearest water body (pond or lake), mean temperature in the coldest year quarter, and variety in thorny trees. (Figure 4.3)

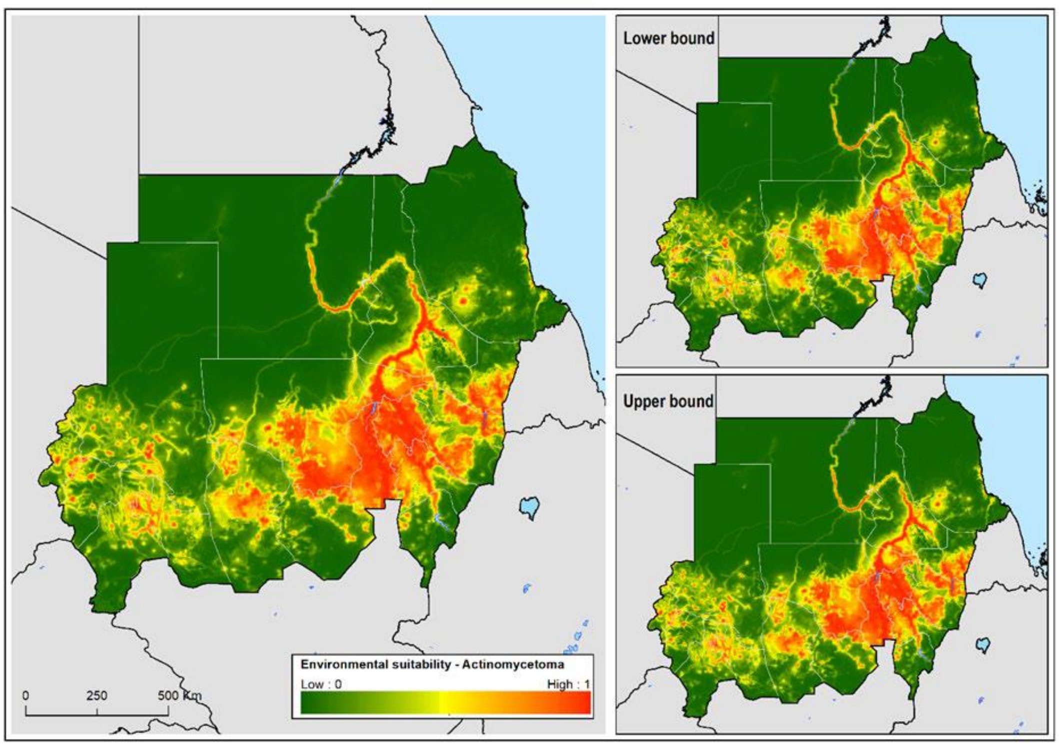

Figure 4.3 Predicted environmental suitability for actinomycetoma in Sudan. Actinomycetoma is predicted to occur along the River Nile and its tributaries with a hot spot in the central and south-eastern part of Sudan with sporadic occurrence in the western part of Sudan.

All models for actinomycetoma occurrence performed well, with a ROC score of 0.995, a sensitivity of 95.445% and specificity of 96.709%. The mean TSS was 0.921, and the mean kappa was 0.903 (Table 4.2).

Across the RF models, distance to the nearest water body was the major contributor to actinomycetoma occurrence, followed by the distance to the nearest river. At the same time, the mean temperature during the coldest quarter in a range of 18-25ºC contributed to the predicted suitability for actinomycetoma occurrence by 13%. Arid areas with a low concentration of soil sodium contributed to almost 11 %. The diversity of thorny trees in an area showed no difference between areas with no diversity of thorny trees and areas with a diverse group of thorny trees. (Appendix7, figure 3).

4.4.4. Predicted suitability for mycetoma occurrence in Sudan

Both eumycetoma and actinomycetoma were predicted to occur around Sudan's central and south-eastern parts and along the Nile River valley and its tributaries. The central states of Sudan, Al Jazirah, White Nile, Khartoum, and Sennar State had the most significant areas predicted suitable for eumycetoma. The predicted distribution of actinomycetoma was slightly more comprehensive than that of eumycetoma, with more patchy suitability predicted in the western states of Sudan (Appendix 7, figure 5 and figure 6). The overlapping areas for actinomycetoma and eumycetoma risk are illustrated in Figure 4.4.

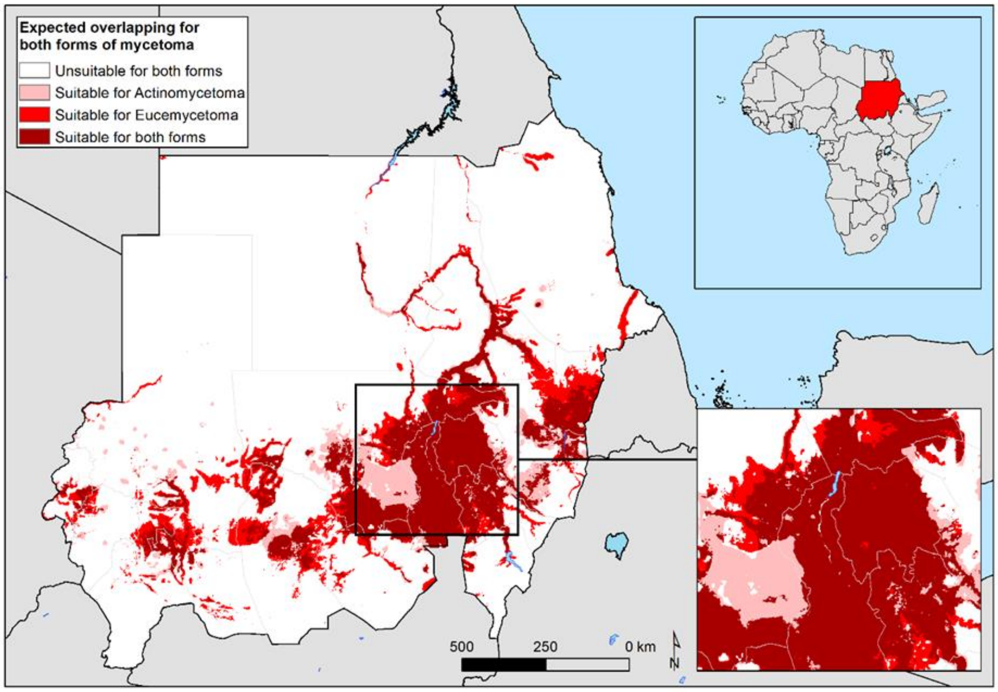

Figure 4.4 Overlaps of actinomycetoma and eumycetoma environmental suitability in Sudan. The map shows central Sudan to be the most suitable area for both types of mycetoma. suitability based on optimal cut-off of 0.60 for actinomycetoma and 0.39 for eumycetoma.

4.5. Discussion

I have applied ecological niche modelling techniques to identify environmental variables associated with mycetoma in Sudan. Models were run separately for eumycetoma and actinomycetoma and gave similar results in the predicted distribution. Both types were predicted along the River Nile and its tributaries, mainly around the central and south-eastern parts of Sudan covering Khartoum, Al Jazirah and the White Nile States, the northern part of Sennar State and the eastern part of North Kordofan State, which are known to have a high burden of cases [69]. The models also predicted the occurrence of eumycetoma in the north and eastern parts of the country, where cases have not previously been recorded, and this might indicate underreporting or misdiagnosis of cases from these areas. Such areas must be considered priorities for future surveillance activities and to offer targeted training of medical personnel for early detection and better diagnosis of mycetoma.

This study showed that the suitability for mycetoma occurrence was widespread in Khartoum State. This may be attributed to the high influx of people from rural endemic areas to the state due to various socioeconomic difficulties, often secondary to having the disease. Also, Khartoum residents regularly visit their families in endemic regions and contract the infection during visits. Furthermore, much of the state beyond the city is undeveloped rural land harbouring ecological and socio-demographic characteristics associated with mycetoma.

As previously mentioned, mycetoma is distributed globally in a belt form, and areas within the belt are dry with annual rainfall between 50 and 1000 mm and high temperatures [126]. Given the recognized influence of temperature on the survival of mycetoma-causative organisms [7], it is not surprising that temperature-related variables had a significant impact on the suitability of both types of mycetoma. Our results indicate that environments with mean temperatures in the coldest year quarter between 20ºC and 25°C are most suitable for actinomycetoma-causative organisms. Daily variation in temperature was a strong predictor of suitability for eumycetoma. It has been reported that fungal organisms that cause eumycetoma cannot survive in environments where the daily range in temperature is more than 15°C on average. This could be explained because the organisms do not live in regions with extreme temperature variations [127]. According to the results, mycetoma seems to be predicted in arid areas; as mentioned previously in the literature, mycetoma prevails in reasonably arid areas with a short rainy season of 4–6 months [7].

Analysis of the MRC data showed that most of the patients reside in locations close to water sources, and the models predicted the same pattern. This might be explained by the presence of favourable environmental conditions for organism survival also by the higher density of population around water sources.

To our knowledge, the influence of the soil mineral components on the growth of mycetoma-causative organisms has not been studied thoroughly. Our findings suggested that soil calcium and sodium concentrations contribute to the disease occurrence suitability. In histopathological studies, calcium, among other elements, was found in abundance in the cell wall of Madurella mycetomatis. It is crucial in forming the cement substance, a hard-brown material containing melanin, heavy metal ions, proteins, and lipids. Cement is a fungal protective mechanism, acting as a sink for harmful unpaired electrons providing the cell walls with structural rigidity and storing water and ions to prevent desiccation [128]. This may indicate that calcium could be an element in the organism's virulence by contributing to the myelinization and sclerosis of the grain [127]. In a recent cross-sectional study conducted in Mexico, cases were prevalent among three soil types with a high calcium level [114]. This observation warrants further laboratory studies to explore the possible role of these soil elements in the pathogens' survival.

I found that environments with a more significant number of species of Acacia trees were more suitable for eumycetoma, supporting previous evidence that had suggested a role for skin penetration by thorns in the transmission of the disease [53]. In my study and contrast to the study conducted by samy and his colleagues in 2014, the distribution of Acacia trees is tested, and the role of diversity of these trees in mycetoma occurrence in both types [53]. However, areas with either no thorny trees or areas with more than five species of thorny trees were both suitable for actinomycetoma occurrence. In this study, eumycetoma, but not actinomycetoma, occurrence seems to be predicted by the diversity of thorny trees. The observation could explain that eumycetoma-causative organisms live near the thorns. This study showed no strong association between the individual species types and mycetoma occurrence. These observations on the association between disease occurrence and individual tree species, their botanical properties, and other parameters need further investigation. Such studies are essential to gain more in-depth knowledge about disease transmission and the life cycle of the causative microorganisms to develop ways to interrupt them.

The results presented entail various potential sources of error beyond the uncertainty captured by the range of predictions. Some of this results from the predictor variables used and the ways in which these were handled and processed. For example, the climatic predictors from WorldClim are based on average conditions from 1970-2000 which might not be reflective of contemporary climate. Error from these datasets would also be incorporated into suitability models for thorny trees which included them as predictors. Additionally, the soil characteristic variables from ISRIC are based on interpolation of measured values, but given sparse data from the north of Sudan, predicted values may be unreliable here. The livestock density surfaces also originate from predictive models, employing Random Forest to downscale sub-national census estimates of livestock densities to finer spatial resolution, which are also subject to the influence of spatial predictors, which may include confounding and circular effects. The livestock rasters were also resampled to a finer resolution than they were produced at, which can lead to erroneous results by introducing false accuracy, and making assumptions about the values within the smaller cells.

Additionally, the models were intended to represent overall disease occurrence status and environmental suitability over the timespan of case detection. However, many of the environmental factors included would have varied over this period (28 years), leading to possible changes in environmental suitability and endemicity which have not been captured in the models.

The outcome "suitability" is also limited in terms of interpretability. Although other locations considered to be suitable might be more likely to be endemic, these results are not able to explain why that is. They leave many unanswered questions about the reservoirs and transmission of mycetoma causative organisms.

The internal validation statistics indicated that all models within the ensemble performed well. However, the generalisability of the models cannot be evaluated without comparison to new datasets. It is possible that some of the models developed are overfitted to the data; that is that they model associations with noise or random error in the epidemiological data and might be prone to erroneous predictions. The use of an ensemble of different model types (generalised boosted regression, general additive model, random forest, generalised linear model) is one way in which this risk has been reduced. Ensemble modelling avoids the shortcomings of a single algorithm to a certain extent and increases the accuracy of the model's prediction by decreasing variance and bias, hence minimising the risk of overfitting. But this comes with a cost as the increase of computational time and expertise.

In conclusion, the studied models have indicated that arid areas proximal to water sources, soil with low concentrations of calcium and sodium, and a variety of thorny tree species provide the most suitable environment for mycetoma.

This work included environmental probable risk factors that have not been studied previously, which could guide the implementation of preventive interventions and control strategies in the affected areas. Until there is robust evidence on disease transmission and the life cycle of the microorganisms involved, limiting the use of thorny trees in building animal enclosures and houses may be advisable. However, such simple and effective interventions may not align with long-standing community members to ensure community acceptance. Protective measures such as footwear should be encouraged in people who are in direct contact with these trees.

This study also considered the suitable environmental indicators across the whole of Sudan with updated MRC database records data using a wide range of environmental risk factors. The results could help and guide planning for national and international scale surveys for mycetoma identification and design targeted training for medical staff on early detection and diagnosis. Moreover, they could aid in designing national and global mycetoma advocacy and awareness programmes leading to early active case detection with early appropriate patient management in areas that are highly endemic for mycetoma.

Chapter Five: Estimating the burden of mycetoma in Sudan for the period 1991-2018 using a model-based geostatistical approach

5.1. Overview

This chapter used the results of the prediction of suitable areas for mycetoma occurrence in the previous chapter. Estimating the population at risk and disease burden is critical for delivering targeted and equitable prevention and treatment services, planning control and elimination programs, and implementing tailored case finding and surveillance. These indicators are typically obtained through routine disease surveillance (secondary data sources), house-to-house case searches or large-scale surveys. Nowadays, geospatial modelling techniques for predicting the distribution and burden of diseases have proven critical to producing reliable estimates, especially in low-income countries with limited resources. I used this approach, combining a large dataset of mycetoma cases recorded by the MRC in Sudan over 28 years (1991-2018) with a suite of environmental and sanitation-related datasets into a geostatistical framework to produce estimates of the disease burden across the country. Incidence estimates were generated separately for the bacterial (actinomycetoma) and fungal (eumycetoma) forms of mycetoma.

5.2. Introduction

Mycetoma is widespread in tropical and subtropical regions favouring arid areas with low humidity and a short rainy season [53, 96, 129]. It has been reported from Africa, South and Central America, and Asia [103]. Sudan is one of the highly endemic countries for mycetoma with a massive impact on patients, the community, and the health system. Most mycetoma cases in Sudan (approximately 70%) are caused by the fungal form (eumycetoma), and the primary causative agent is *Madurella mycetomatis* [45, 91]. The epidemiological features of mycetoma are not well described. The incidence and prevalence of the disease are globally underestimated, and most of the reported cases are based on hospital records and short prevalence surveys conducted locally [10, 110]. Since the middle of the last century, much effort has been made to estimate the mycetoma burden in Sudan accurately. Abbott's study in 1952 estimated the prevalence of mycetoma to be 4.6 per 100,000 inhabitants based on a cohort of 1,231 mycetoma patients admitted to hospitals throughout the country [84]. More recently, in 2014, a large meta-analysis conducted by van de Sande et al. estimated that the mycetoma prevalence in Sudan was 1.81 cases per 100,000 inhabitants, although the authors acknowledged this could be much higher in some villages [34].

- Estimating the population at risk and disease burden is critical for delivering targeted and equitable prevention and treatment services, planning control and elimination programs and implementing tailored case-finding and surveillance. Estimates of incidence and population at risk are typically obtained through routine disease surveillance (secondary data sources), house-to-house case searches or large-scale surveys. For diseases of low prevalence such as mycetoma, routinely collected data is not reliable enough to produce estimates of the disease burden since it underestimates their actual impact and distribution. On the other hand, although house-to-house and large-scale surveys can deliver more accurate estimates, they tend to be unfeasible because of their high cost and logistic needs. Nowadays, geospatial modelling techniques for predicting the distribution and burden of diseases have proven critical to producing reliable estimates, especially in low-income countries with limited resources [130-133]. In this study, I have combined a large dataset of mycetoma cases recorded by the MRC in Sudan over 28 years (1991-2018) with a suite of environmental and sanitation-related datasets in a geostatistical framework to produce estimates of the disease burden across the country. Incidence estimates were generated separately for the bacterial (actinomycetoma) and fungal (eumycetoma) forms of mycetoma.

5.3. Materials and Methods

5.3.1. Records of mycetoma cases

The data included in this study were extracted from the patient database compiled by the MRC at Soba University Hospital in Khartoum (Sudan) from 1991-to 2018. This centre was established in 1991 to manage mycetoma patients attending from all states of Sudan, and more than 10,000 cases have been registered at the centre since it opened [134].

The variables extracted from the database included patients' demographic characteristics, details of their clinical presentation and diagnosis, and their location of origin using patients' addresses, which were then used to find the most accurate location, namely geographical coordinates, for all the recorded mycetoma cases (Figure 5.1).

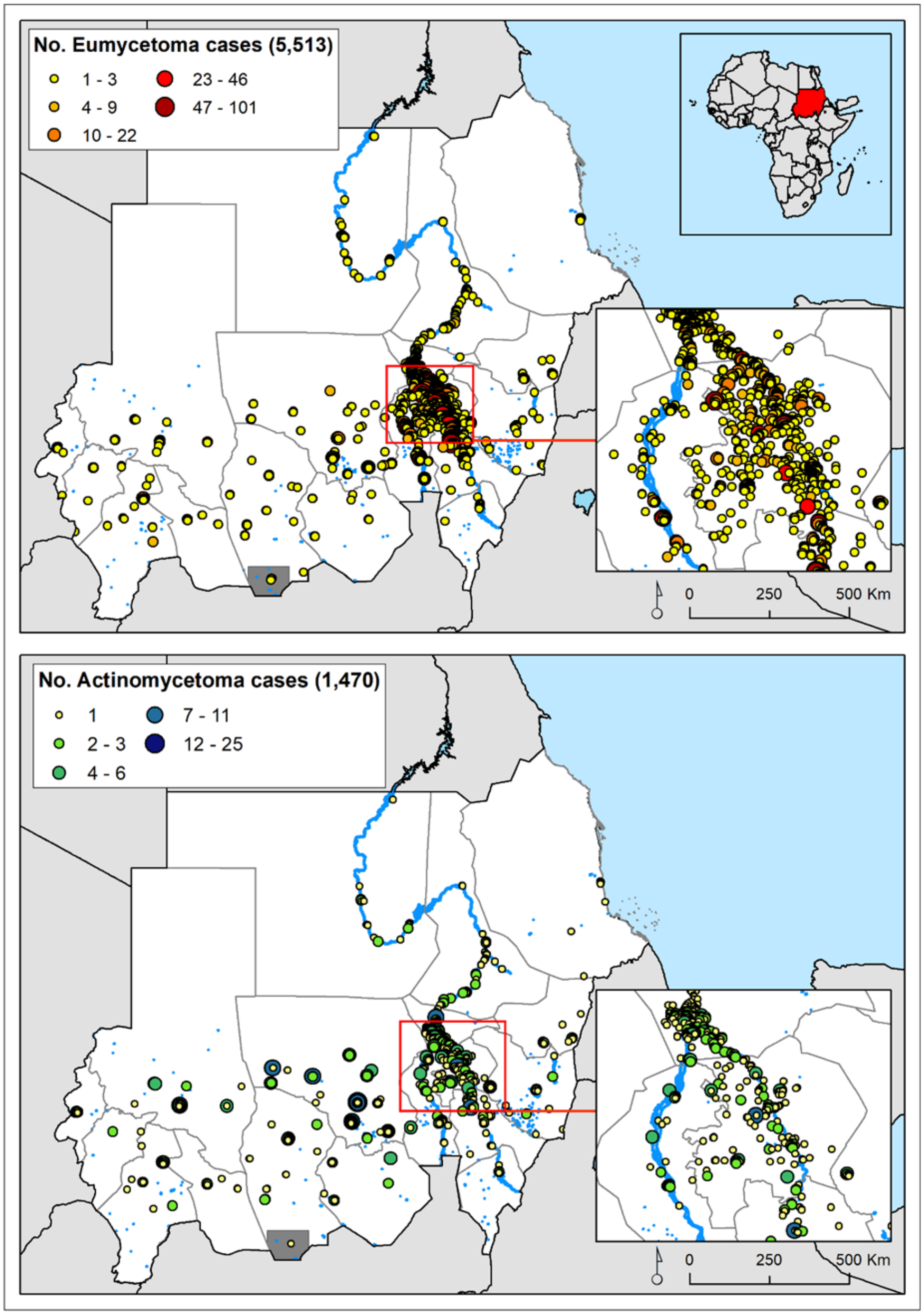

Figure 5.1 Distribution of eumycetoma and actinomycetoma cases recorded by the MRC from 1991 to 2018. The upper figure shows the distribution of eumycetoma including a map of Africa with Sudan highlighted in red in the right upper corner, and a zoomed screenshot of the central part of Sudan where cases are clustered, and the lower figure shows the distribution of actinomycetoma with a zoomed screenshot of the central part of Sudan where cases are clustered.

5.3.2. Explanatory variables

Geostatistical models based on the counts of actinomycetoma and eumycetoma cases were constructed separately using two independent variables potentially associated with the occurrence and distribution of mycetoma: environmental suitability and an indicator of poor sanitation conditions including access to water and sanitation (waste disposal) [127]. I used gridded surfaces of predicted environmental suitability for both types of mycetoma as modelled previously in environmental suitability paper in chapter four (Figures 5.2) [96].

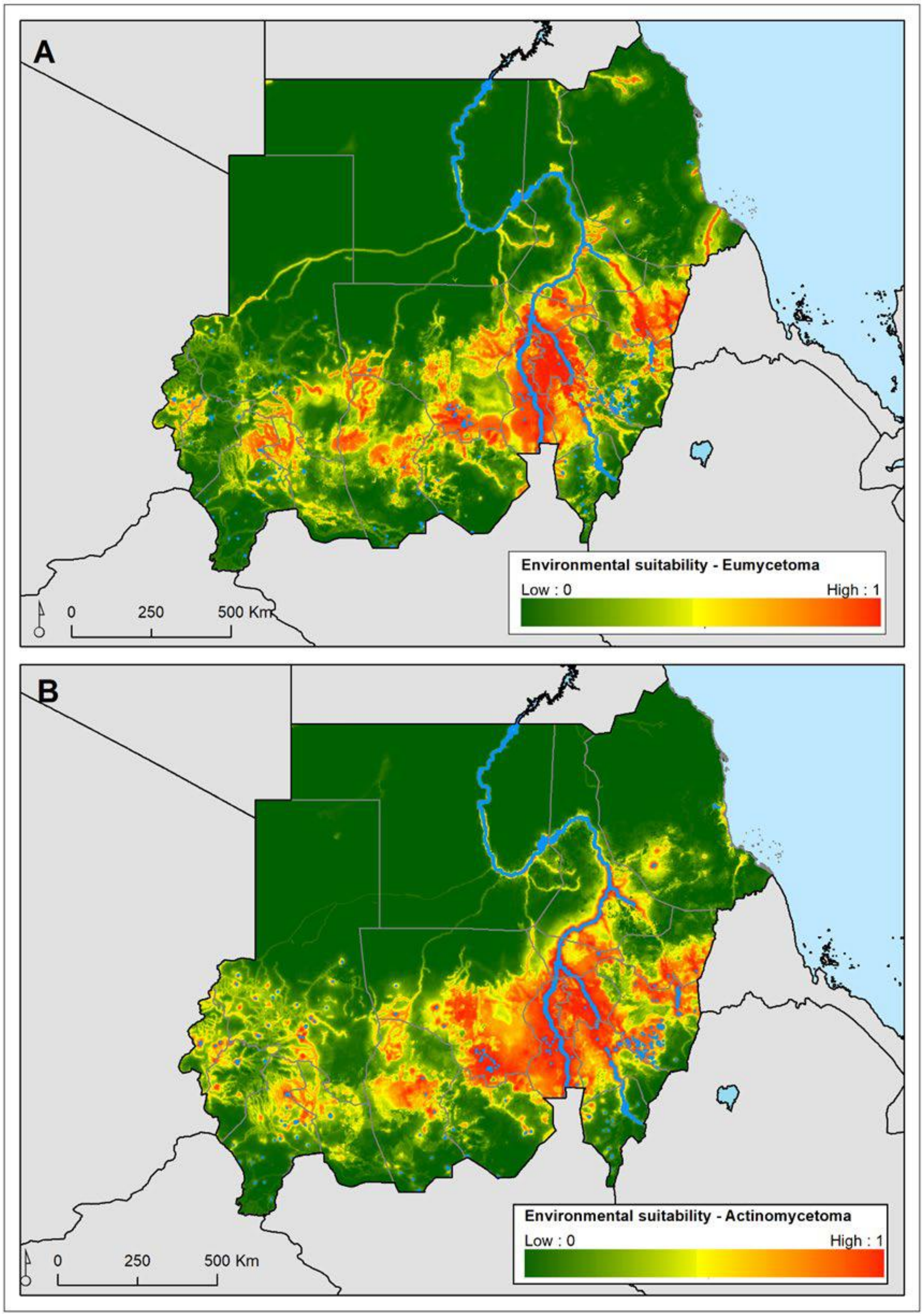

Figure 5.2 Maps of environmental suitability for eumycetoma (A) and actinomycetoma (B). Upper bound of 95% credible interval was used as covariate for the construction of the geospatial Poisson models of mycetoma incidence for the period 1991 – 2018.

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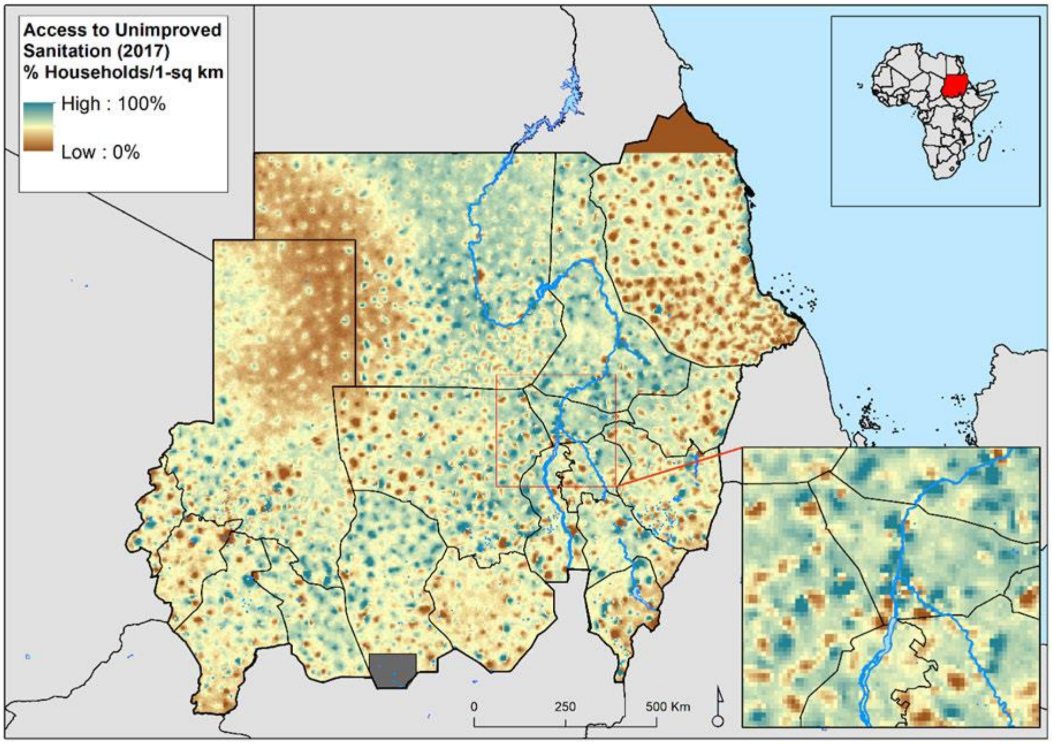

Figure 5.3 Estimated percentage of households accessing unimproved sanitation by 2017. This gridded map displaying the estimate percentage of households using unimproved sanitation is based on a Bayesian geostatistical model developed using a suite of environmental and socio-economic data and data from 600 sources across more than 88 low-income and middle-income countries (LMICs).

The records of mycetoma cases and modelled environmental suitability and accessibility to unimproved sanitation were combined within a geostatistical framework. The records of geolocated mycetoma cases were aggregated on a spatial grid of 1 sq-km resolution across the country. Thus, I modelled counts of mycetoma cases per 1 km2 for 1991-2018 and took the estimated gridded population at the exact spatial resolution for 2020 into account. Due to the lack of reliable census data for all the populated areas across Sudan, I obtained gridded continuous estimates of the total population for 2020 from the WorldPop project [135, 136]. Because of the patchy distribution of the population in Sudan, I opted for using the constrained version of modelled estimates at 100 metres resolution [137, 138]. This method only generates estimates within areas containing built settlements (Figure 5.4). I aggregated the population estimates to a grid of 1 sq-km resolution to match the geographical aggregation for mycetoma cases and the covariates.

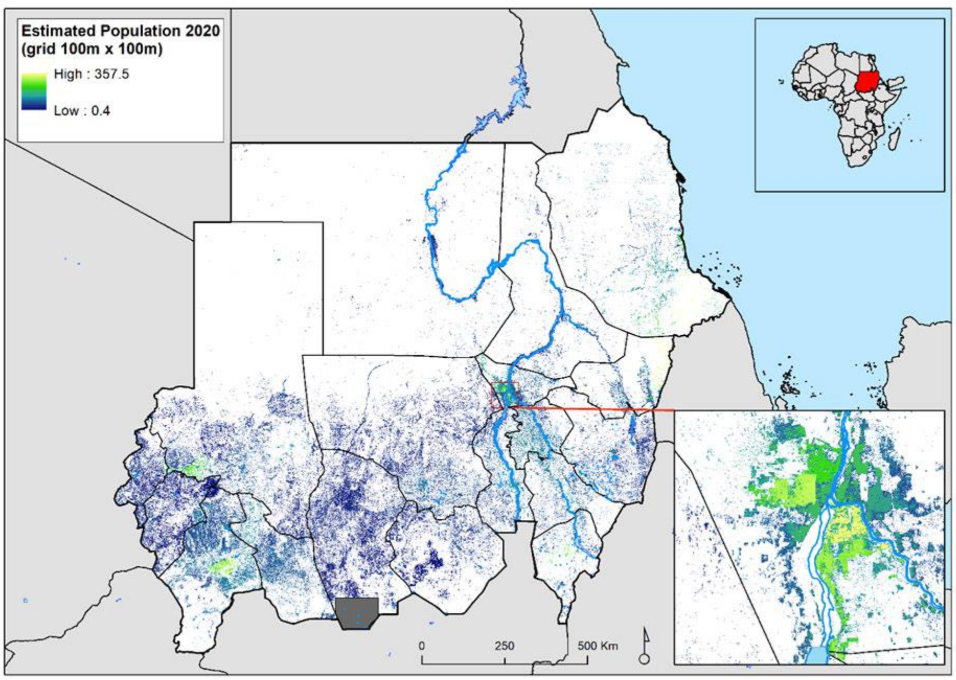

Figure 5.4 Map displaying estimated total population for Sudan in 2020 based on constrained Methods. This method generates estimation only within areas mapped as containing built settlement.

I developed geostatistical models to predict the incidence of actinomycetoma and eumycetoma in areas considered environmentally suitable for the two mycetoma forms, as delineated by a previous modelling exercise across Sudan in chapter four (Figures 5.5 & 5.6) [96].

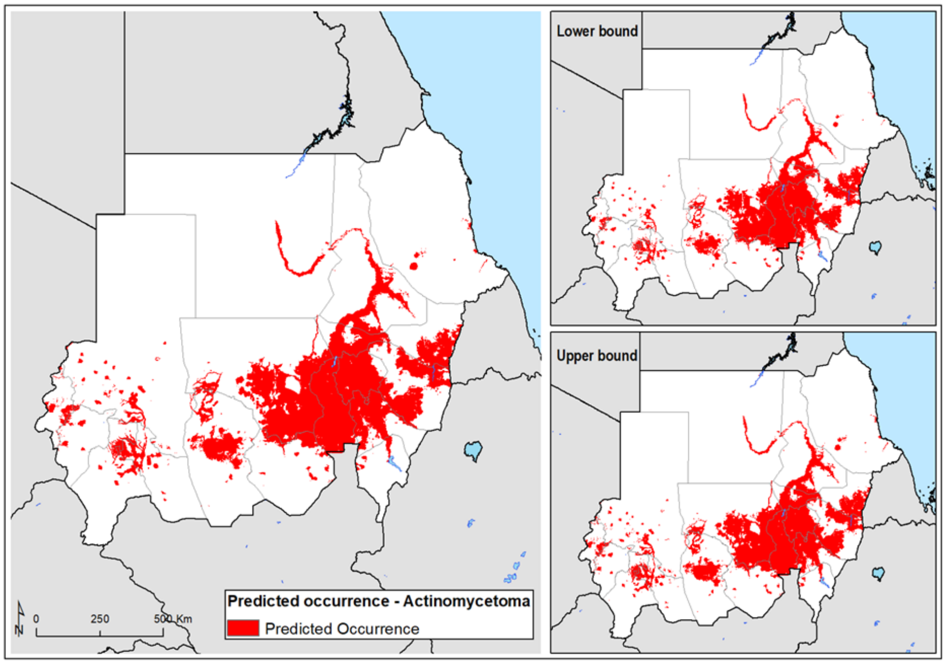

Figure 5.5 Predicted occurrence of actinomycetoma form of mycetoma and uncertainty range across Sudan. This binary maps were generated from the predicted environmental suitability based on a cut-off above which the occurrence of actinomycetoma is highly likely. Optimal threshold was fitted to get better trade-off between sensitivity, specificity and proportion correctly classified (PCC).

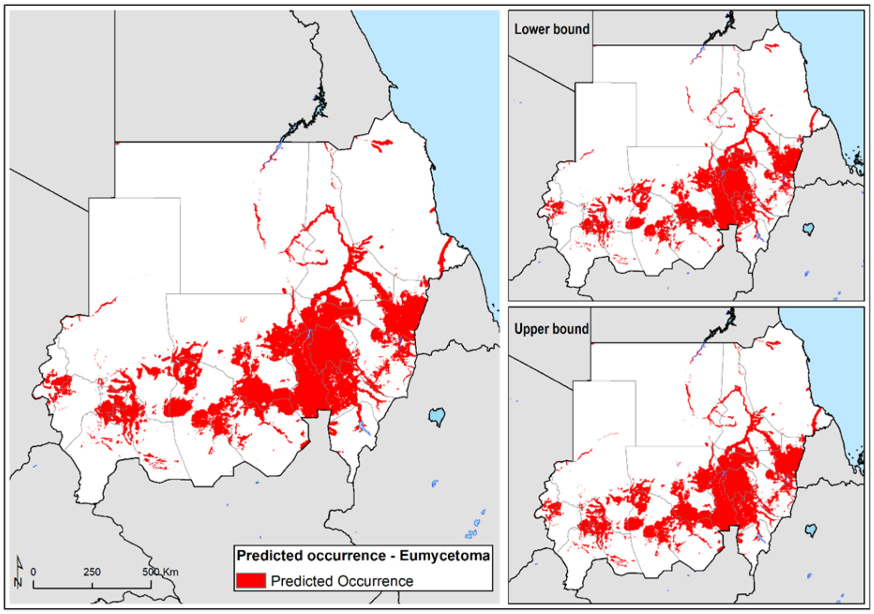

Figure 5.6 Predicted occurrence of eumycetoma form of mycetoma and uncertainty range across Sudan. This binary maps were generated from the predicted environmental suitability based on a cut-off above which the occurrence of eumycetoma is highly likely. Optimal threshold was fitted to get better trade-off between sensitivity, specificity and proportion correctly classified (PCC).

I let mycetoma risk depend on the predicted environmental suitability and household level utilization of unimproved sanitation values obtained in the previous step. I included spatial random effects to account for spatial variation in mycetoma incidence between locations of origin that the explanatory variables and independent random effects were not explained to account for potential overdispersion. For a quick exploratory analysis, the records of eumycetoma and actinomycetoma cases confirmed between 1991 and 2018 by the MRC in Sudan were aggregated by district and then estimated the relative risk, also known as standardized incidence ratio (SIR), for each district taking the distribution of cases and population into account, as follows:

$$SIR_i = \frac{O_i}{E_i}$$

where O_i is the observed number of cases in the period 1991 - 2018, $E_i = rP_i$ is the expected number of cases, P_i is the population and $r = \frac{\sum_{i=1}^{n} O_i}{\sum_{i=1}^{n} P_i}$ is the overall incidence ratio.

Let Yi denote the number of confirmed mycetoma cases at location xi from 1991 to 2018 over a total population m (exposure) at each location as determined by 2020 population estimates. I then assume that, conditionally on a zero-mean spatial Gaussian process S(x), the Yi observations are generated from a Poisson distribution with mean mλ, where m is an offset accounting for the exposure population as per demographics estimates for 2020. A canonical log link is used, thus the linear predictor assumes the form

$$\log\{\lambda(xi)m(xi)\}= \beta0+ \beta1EnvSuit(xi)+ \beta2UnimpSanitation(xi)+ S(xi)$$

where the explanatory in the above equation is the modelled environmental suitability and estimates (percentage) of households using unimproved sanitation at location xi.

I model the Gaussian process S(x) using an isotropic and stationary exponential covariance function given by

$$Cov\{S(x),S(x')\} = \sigma2exp\{-\|x-x'\|/\phi\}$$

Where $\|x-x'\|$is the Euclidean distance between x and x', $\sigma2$ is the variance of S(x) and ϕ is a scale parameter that regulates how fast the spatial correlation decays to zero for increasing distance.

To check the validity of the adopted exponential correlation function for the spatial random effects S(x), I carried out the following Monte Carlo algorithm, I started with Simulation of a Poisson geostatistical dataset at observed locations xi by plugging-in the maximum likelihood estimates from the fitted model then estimating the unstructured random effects Zi from a non-spatial Poisson mixed model obtained by setting S(x) =0 for all locations x and use the estimates for Zi from the previous step to compute the empirical variogram. The previous steps were repeated 10,000 times and use the resulting 1000 variograms to compute the 95% tolerance bandwidth under the hypothesis that the analysed data were generated by the fitted model. If the empirical variogram from the original data, obtained as in the second step, lies within 95% bandwidth, I then conclude that I do not find evidence against the assumption of an exponential correlation function for S(x)[139].

The analysis was carried out using the R package PrevMap v 1.5.3 [137], which implements geostatistical model parameter estimation and spatial prediction using Monte Carlo Maximum Likelihood [138]. This model was applied to produce predictions of the incidence of eumycetoma and actinomycetoma since 1991 at 1km2 spatial resolution and probability maps of exceeding a 50 per 10,000 people and 5 per 10,000 people incidence thresholds for eumycetoma and actinomycetoma, respectively. I checked the validity of the assumed covariance model for the spatial correlation using the Monte Carlo algorithm and empirical semi-variogram as described in the supplemental file. Additionally, maps of 95% confidence intervals for the number of cases were generated for each 1 sq-km grid location.

I used the raster dataset (gridded map) with the population estimates for 2020 downloaded from the WorldPop project to compute the potentially affected population since 1991. An output raster dataset computing the estimated number of eumycetoma and actinomycetoma cases per grid cell was obtained by multiplying the 1km2 raster dataset of predictive incidence with the corresponding grided map with the total population estimated per 1 sq-km pixel for built-up areas. The same procedure was used to estimate the uncertainty range of the affected population using the gridded maps of 95% confidence interval (CI) for predicted incidence. These raster datasets were then used to extract the aggregate number of people with mycetoma and uncertainty range by administrative area (district and states).

5.4. Results

5.4.1. Mycetoma records: general description

The modelled data included 7,812 unique points obtained from patients seen at the MRC in the period 1991- 2018, and they came from all eighteen states of Sudan. The study included 5,513 patients (79%) with confirmed eumycetoma and 1,470 patients (21%) with actinomycetoma. Most of the mycetoma patients were from Al Jazirah State (34.4%) and Khartoum State (14.5%) (Figure 5.1). Further details of the patients' geographical distribution have been published elsewhere [96].

5.4.2. Predicted incidence of mycetoma and burden estimation

The relative risk of eumycetoma was over 4-fold higher in the districts around the Khartoum area, most of them in the state of Al Jazirah. In contrast, those at higher risk of actinomycetoma were from the North Kurdufan state (Figure 5.7). The geostatistical models confirmed this heterogeneous and distinct distribution of the estimated incidence of eumycetoma and actinomycetoma across Sudan (Figures 5.8 & 5.9).

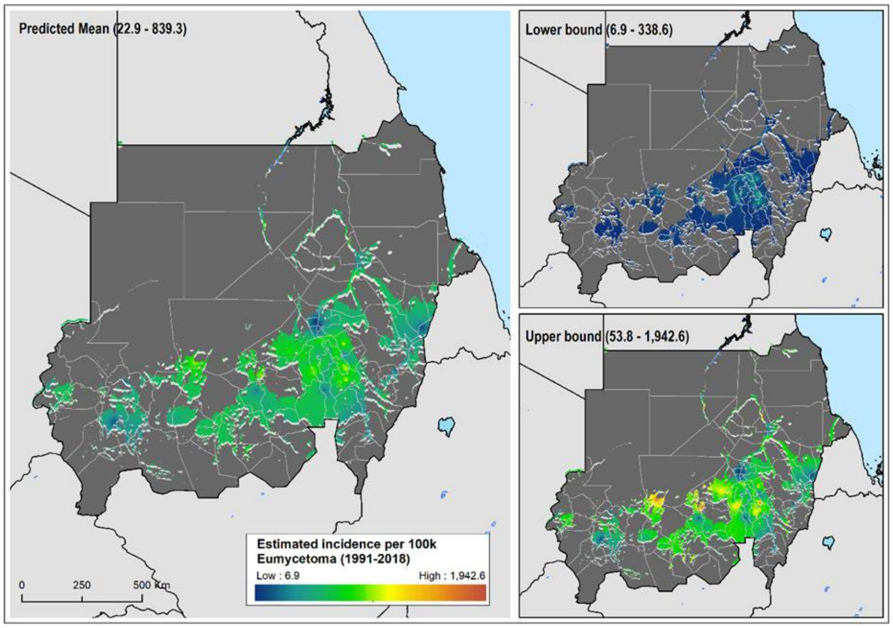

Figure 5.7 Predicted mycetoma (eumycetoma form) incidence per 100,000 inhabitants since 1991; mean predicted incidence and lower and upper 95% CI bounds. Areas considered environmentally unsuitable for eumycetoma as predicted by the environmental model have been excluded.

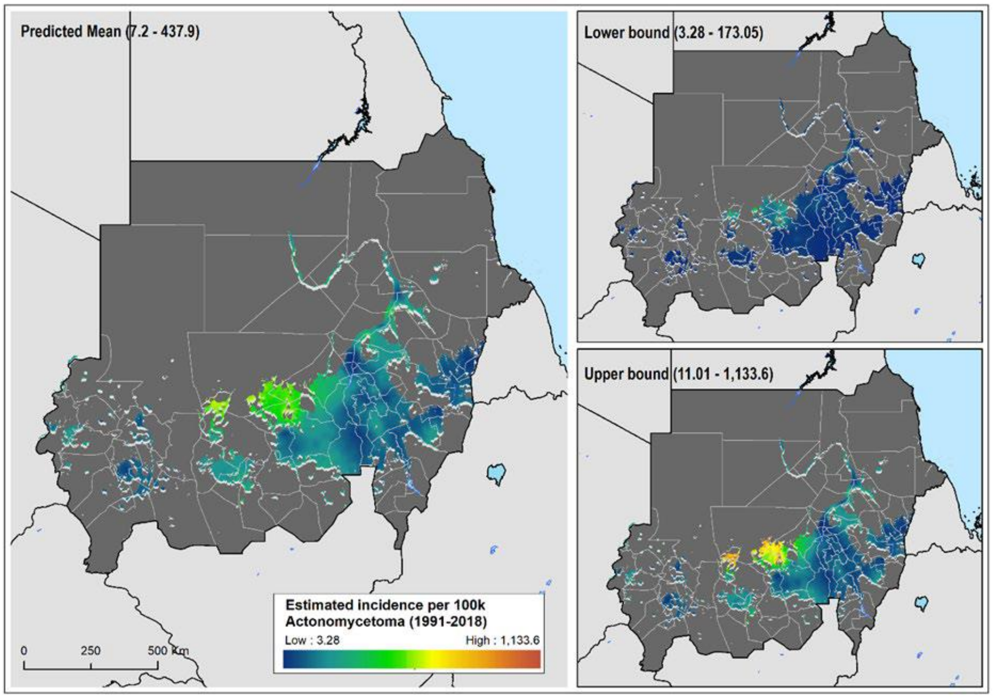

Figure 5.8 Predicted mycetoma (actinomycetoma form) incidence per 100,000 inhabitants since 1991; mean predicted incidence and lower and upper 95% CI bounds. Areas considered environmentally unsuitable for actinomycetoma as predicted by the environmental model have been excluded.

For eumycetoma, these higher-risk areas were smaller and scattered across Al Jazirah, Khartoum, White Nile, and Sennar states, while for actinomycetoma, a higher risk for infection was shown across the rural districts of North and West Kurdufan. The data also clearly showed the exceedance probability of an incidence rate of 50 per 10,000 inhabitants and 5 per 10,000 inhabitants for eumycetoma and actinomycetoma, respectively (Figure 5.8).

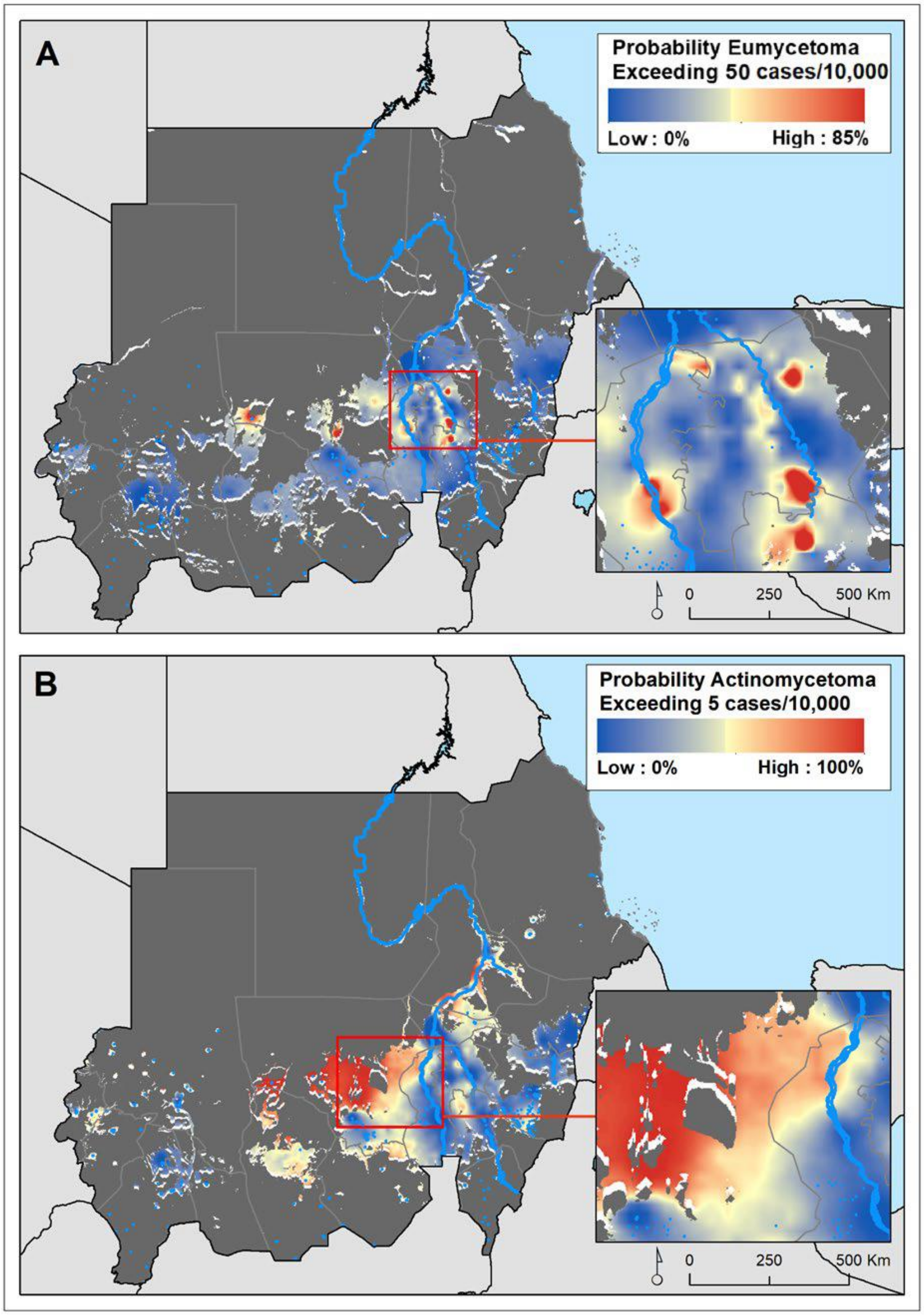

Figure 5.9 Maps display the probability of exceeding 50 cases and 5 cases per 1,000 inhabitants for eumycetoma (A) and actinomycetoma (B), respectively, since 1991 in Sudan.

Nationally, I estimated 63,825 people (95%CI: 13,693 to 197,369) suffered from mycetoma since 1991 in Sudan (Tables 5.1 & 5.2): 51,541 people (95%CI: 9,893 – 166,073) with eumycetoma and 12,284 people (95%CI: 3,800 – 31,296) with actinomycetoma. Five regions (Al Jazirah, White Nile, North Kurdufan, Khartoum and Sennar) would have contributed over 66% of the total number of cases expected in 1991-2018. The greatest proportion (22%) of people affected by any form of mycetoma resided in the Al Jazirah state, although when differentiating by type of mycetoma, most actinomycetoma cases would have occurred in the North Kurdufan state. The remaining thirteen states were predicted to have had less than 1,000 cases of mycetoma during the study period (Figure 5.10). Tables 1 and 2 in appendix 8 provides district-level estimates of the number of expected eumycetoma and actinomycetoma cases, respectively.

Table 5.1 Estimation of actinomycetoma cases by state in Sudan since 1991

State	Area predicted suitable (sq-km)	Estimated Actinomycetoma Cases		
		No.	95% CI	
			Lower Bound	Upper Bound
Al Jazirah	22,995	1,801	719	3,794
Al Qadarif	33,525	489	129	1,341
Blue Nile	2,341	118	33	318
Central Darfur	2,850	89	16	289
East Darfur	5,661	184	45	543
Kassala	16,028	367	103	978
Khartoum	15,910	1,351	580	2,882
North Darfur	9,765	449	113	1,291
North Kurdufan	69,740	2,826	747	7,714
Northern	4,934	527	139	1,392
Red Sea	2,549	123	32	352
River Nile	23,565	869	260	2,220
Sennar	25,218	729	247	1,722
South Darfur	8,896	357	91	1,014
South Kurdufan	9,498	161	33	496
West Darfur	4,368	143	40	393
West Kurdufan	22,912	545	117	1,650
White Nile	42,463	1,156	356	2,907
Total	**323,218**	**12,284**	**3,800**	**31,296**

Table 5.2 Estimation of eumycetoma cases by state in Sudan since 1991

State	Area predicted suitable (sq-km)	Estimated Eumycetoma Cases		
		No.	95% CI	
			Lower Bound	Upper Bound
Al Jazirah	22,307	12,263	2,979	34,452
Al Qadarif	21,786	1,910	297	6,689
Blue Nile	4,030	543	88	1,854
Central Darfur	3,894	255	31	970
East Darfur	12,964	1,046	129	3,984
Kassala	28,963	3,205	430	11,799
Khartoum	18,113	5,139	1,231	14,837
North Darfur	18,683	1,163	158	4,266
North Kurdufan	52,628	4,680	696	16,582
Northern	6,696	989	159	3,396
Red Sea	8,101	792	102	2,999
River Nile	27,403	2,731	464	9,176
Sennar	28,373	5,046	1,110	15,271
South Darfur	13,169	1,547	224	5,599
South Kurdufan	16,397	713	96	2,621
West Darfur	5,983	506	73	1,834
West Kurdufan	34,959	1,850	238	6,910
White Nile	40,855	7,163	1,388	22,834
Total	**365,304**	**51,541**	**9,893**	**166,073**

Tables 3 and 4 in appendix 8 report the Monte Carlo maximum likelihood estimates for the eumycetoma and actinomycetoma models. The point estimate of the scale parameter (ϕ) for the eumycetoma model was 60.71 km and 87.24 for the actinomycetoma model, indicating that the spatial correlation decayed to a value of 0.05 at a distance log20 x 60.71 ≈ 181.85 km and log20 x 60.71 ≈ 261.69 km for eumycetoma and actinomycetoma, respectively. In terms of validating the geostatistical models fitted, the variogram-based procedure conducted using the data simulated from the models led us to conclude that the observed data are compatible with the assumptions of an exponential correlation function and that the underlying spatial structure has been accounted for by the spatial fixed and random effects (Appendix 8, Figures 1 &2).

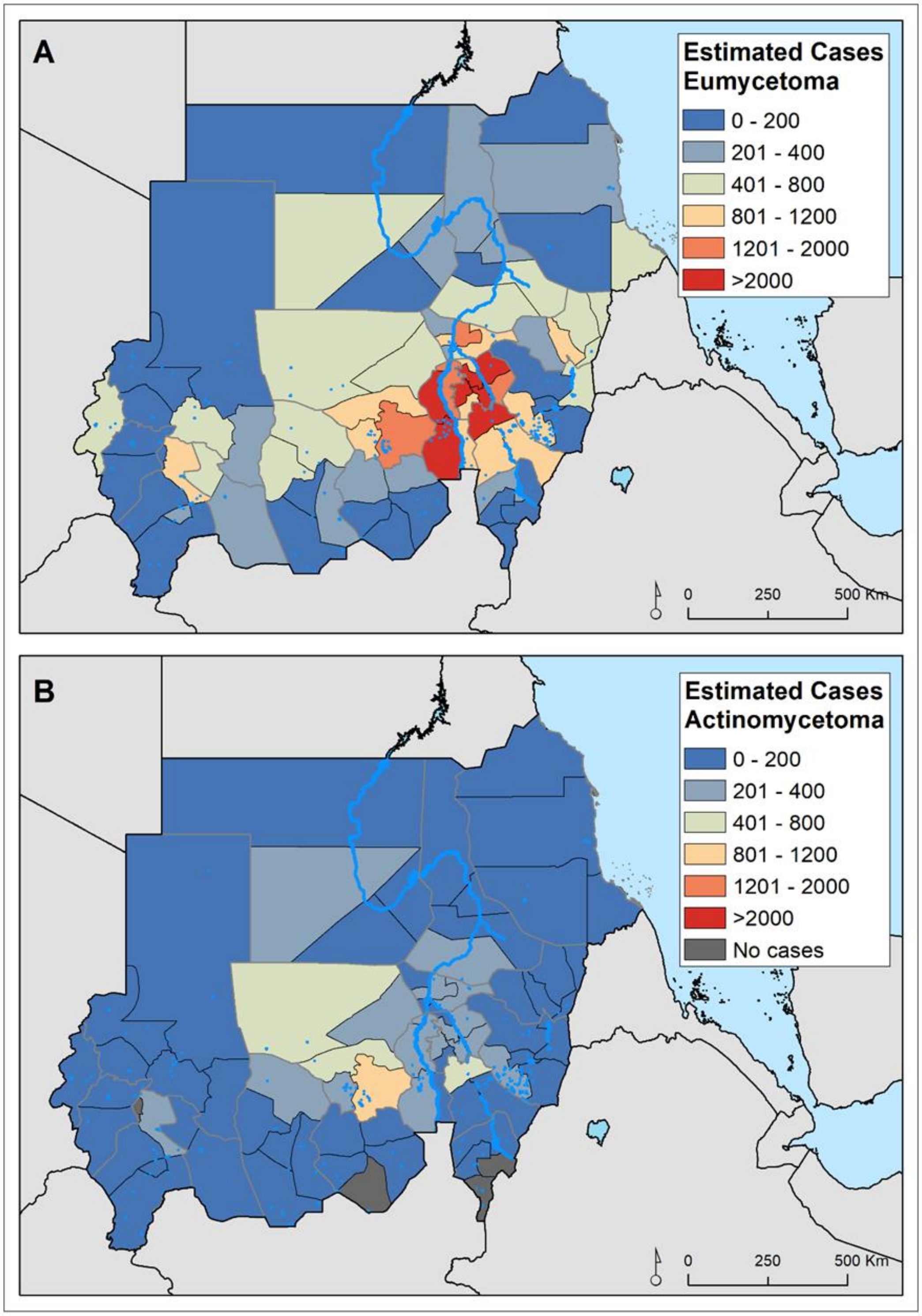

Figure 5.10 Estimated number of people that have suffered from eumycetoma (A) and actinomycetoma (B) since 1991 as predicted by the fitted Bayesian-based geostatistical models.

5.5. Discussion

To our knowledge, this is the first analysis to use geostatistical modelling to estimate the burden of mycetoma. The novelty of the current analysis is that I combined mycetoma case data with a suite of environmental factors to estimate the burden of mycetoma across Sudan. I have also provided the associated uncertainty interval to identify areas where further data collection is required to improve the estimates. Given the difference in aetiology and potential risk factors for each type of mycetoma, I have fitted separate models for eumycetoma and actinomycetoma. This analysis can serve as a framework to estimate the global burden of mycetoma.

The geostatistical models fitted based on the eumycetoma and actinomycetoma cases recorded by the MRC in Sudan between 1991 and 2018 have shown a spatially heterogeneous and distinct distribution of both mycetoma forms across this endemic country. According to my predictions, most of the eumycetoma cases would have occurred around the Khartoum and Al Jazirah, and most of the actinomycetoma cases would have concentrated in the rural North and West Kurdufan states. It has also shown that the incidence of mycetoma in Sudan, so far mostly estimated through hospital records and local prevalence surveys [25, 84], is likely to be sizeably underestimated.

Overall, the estimated incidence of eumycetoma is four times higher than actinomycetoma in Sudan, across all states. This agrees with what has been reported through clinical observations and reports where the number of eumycetoma dominates [84]. Nonetheless, there is no difference in the proportions of areas suitable for the occurrence of both mycetoma forms. One sixth (17%) and 19% of the landmass of Sudan is suitable for the occurrence of actinomycetoma and eumycetoma, respectively. For both types of mycetoma high environmental suitability was predicted in North Kurdufan and White Nile states. Al Jazirah and North Kurdufan states showed high estimates of mycetoma cases, which can be attributed to the nature of the economic activities of the population residing in those areas. Most people there are sustained by arable farming or animal grazing, which increase the risk of contact with mycetoma causative organisms. Al Jazirah state had the biggest agriculture scheme in Sudan, and North Kurdufan state is one of the biggest states in the production of gum Arabic

The localities with higher eumycetoma incidence estimates include North Jazirah, Khartoum Bahri, Um Rawaba, Sennar and Ad Douiem. These localities have large populations that mainly depend on farming as an occupation. For actinomycetoma, North Kurdufan state accounted for most of the cases and again, Um Rawaba had the highest estimates.

This modelling approach is not without limitations. First, I must assume there might be underlying geographical and temporal biases in the recorded number of mycetoma cases in the country, as all the records were collected by the MRC, located in Khartoum, since its inception in 1991. From the exploratory analysis of this dataset presented elsewhere [9], the number of mycetoma cases confirmed by the MRC has steadily increased since 1991, and the vast majority are coming from rural and peri-urban areas near the Khartoum area (Figure 1). To account for this potential geographical bias, I adjusted the number of cases by the estimated population density in 2020, although there remains some uncertainty on where the infection may have occurred due to most cases being diagnosed in advance stages of the disease. Neither was it possible to account for any existing temporal variation due to the uncertainty on when the infection occurred; severe cases are more likely to be diagnosed in health facilities. Second, I did not account for individual or household-related risk factors such as work and social activities, which may also have driven the distribution and intensity of transmission [10]. I, however, included an estimate of poor sanitation coverage, assuming a higher risk in more deprived areas with limited access to protected sanitation.

My findings here have several implications for clinical and public health practices. The analysis indicated that only slightly less than a quarter of the landmass of Sudan is suitable for the occurrence of mycetoma. Nonetheless, the cases are ubiquitous across all states of Sudan. This implies that clinicians working in Sudan should have a high index of suspicion for mycetoma for people presenting with swelling and sinuses discharging grains with a history of trauma. Expanding diagnostic and treatment services to at least high burden states is required to address the existing cases.

On the other hand, public health interventions focusing on prevention should focus on states and areas suitable for the occurrence of mycetoma. To prevent this disabling disease, geographically targeted public health education and social mobilization are required. Mycetoma can be prevented by encouraging safe farming practices like wearing shoes that decrease the chance of trauma, organizing interaction with animals and building ‘human-friendly’ animal cages that avoid the use of thorny tree branches. The burden presented here warrants a mycetoma control program in Sudan, which should coordinate the treatment and prevention of the disease.

In conclusion, the risk of mycetoma in Sudan is exceptionally high in certain restricted areas, but cases are ubiquitous across all states. Both prevention and treatment services are required to address the burden, and such work provides a guide for future control and prevention programs for mycetoma, highly endemic areas are targeted, and resources are directed to areas with high demand. Moreover, medical personnel will have high levels of suspicion for mycetoma in areas with high burden and cases will be adequately diagnosed and managed within their communities without seeking medical treatment in centralized medical facilities, reducing the financial burden mycetoma patients. Specialized mycetoma management centres can be established in communities where mycetoma is endemic, providing early case detection and management of mycetoma patients

Chapter Six: Discussion

6.1. Overview

This thesis used existing data from the database of the MRC and new data generated from a community survey in an endemic area for mycetoma in Sudan. The data obtained from the study was intended to determine the clinical and epidemiological characteristics and risk factors for mycetoma. At the same time, the data obtained from the archive of the MRC were used to feed prediction models to determine areas suitable for mycetoma occurrence and the population at risk. The results of this thesis identified areas ideal for mycetoma and the people at risk of contracting the disease. Moreover, clinical and epidemiological features and risk factors for mycetoma at a community level were recognised. My thesis' findings will contribute to the development of mycetoma management guidelines for rural areas on earlier diagnosis and appropriate case management towards reducing morbidity from this devastating disease. Acknowledging the mycetoma risk factors and practices that increase the odds of the disease occurrence will help design prevention measures. Educating communities and health care practitioners about these risk factors and appropriate practices to avoid contracting the disease will also be important in prevention strategies. It also provided maps with clusters of mycetoma in Sudan, which provides a guide for future surveillance. Thus, the overall results of this study will help establish a prevention and control program for mycetoma.

6.2. Principle findings

Chapter two explored the clinical, and epidemiological characteristics and contributing socioeconomic factors to mycetoma. Most of the literature related to the clinical features of mycetoma covered cases diagnosed in hospitals. In this study, community-based research was performed in an endemic state for mycetoma in Sudan to determine the clinical, epidemiological characteristics of mycetoma in 60 villages, with data collected at three different levels: community, household, and individual. Moreover, the data were used to determine the characterisation of the economic factors contributing to the development of mycetoma. The overall prevalence of mycetoma in Sennar state is 0.87%. This estimated prevalence is the first of its kind to be determined using data obtained through a community study in more than one village. In 2014 Fahal et al. surveyed an endemic town in White Nile state called Al-Anadolus and estimated mycetoma prevalence to be 1.45%. The prevalence was high because the study was conducted in a village known to have an increased number of mycetoma cases [12]. From the data collected in the survey in this study it was found that cases were highly prevalent around the central and north-eastern study area with a cluster in Doba administrative unit (1.14%) while the lowest was in Elreif Elshargi (0.15%). This could be driven by the practices of the population in the Doba, which include poor hygiene and sanitation practices and the nature of occupation for most residents that render them susceptible to mycetoma [14]. Mycetoma is most common among males aged 16-45 years, who are usually involved in farming activities. Furthermore, several socio-economic parameters were found to be associated with mycetoma including agricultural activities, limited water supply, poor sanitation, lack of accessible health care, and financial costs to families. In combination with the natural occurrence of mycetoma pathogens, weather conditions (road blocks during rainy season) and remoteness (affordability of transport fees), these factors may contribute to a low cure rate and poor outcomes for mycetoma in Sudan.

In chapter three, I analysed the data from the Eastern Sennar Locality survey to perform a case-control study. Patients with confirmed mycetoma were compared to community- and sex-matched controls to identify determinants of risk in the study population. The risk for mycetoma was doubled in individuals who experienced previous trauma such as a thorn or sharp object injury, despite previous analyses of mycetoma cases that showed a history of trauma in only a few patients.

Trauma has long been thought to play a role in the development of mycetoma, and the work presented in this thesis supports this and the discrepancy with previous studies probably reflects that the trauma may be minor and pass unnoticed by the patient [14]. The case-control design I employed, included 359 mycetoma patients and 1436 community-matched controls, allowed us to conclusively demonstrate a significant difference in the history of trauma between these two groups. The odds were also increased in the age group 16-30 years and individuals who own livestock. Additionally, the unmarried population had higher odds for mycetoma which was four times that of married individuals. Interestingly, there was no significant difference between literate and illiterate populations, and I found an increased odds for mycetoma in desk employees, contradicting previous reports in the literature that mycetoma is commonly prevalent among poor communities with low education levels [90]. And this finding can be explained by the fact that individuals living in endemic areas share the same habits and practices such as walking barefooted and work in farming practices in farming seasons and hence, are all facing the same risk of mycetoma

In chapter four, I employed the ecological niche modelling (ENM) techniques to predict the mycetoma distribution in Sudan using the data of the MRC database. Models were run separately for eumycetoma and actinomycetoma and gave similar results in the predicted distribution. The models indicated that arid areas proximal to water sources soil with low calcium and sodium concentrations provide the most suitable environment for mycetoma. While for the variety of thorny trees species, eumycetoma suitable areas have greater diversity, on the other hand, areas defined as suitable for actinomycetoma have either no thorny trees or more than five species of thorny trees and this indicate that thorny trees do not have a role in the actinomycetoma transmission as it does for eumycetoma but still further studies are needed. These environmental characteristics of suitable areas for mycetoma occurrence in Sudan indicated that mycetoma is predicted along the River Nile and its tributaries, mainly around Sudan's central and south-eastern parts. They cover Khartoum, Al Jazirah and the White Nile States, the northern part of Sennar State and the eastern part of North Kordofan State, which have a high burden of cases.

In chapter five, I used the results of the ecological niche modelling analysis, which combines a large dataset of mycetoma cases recorded by the MRC over 28 years (1991-2018) with a suite of environmental and sanitation-related datasets in a geostatistical framework to produce estimates of the disease burden across the country. According to our predictions, most of the eumycetoma cases would have occurred around the Khartoum and Al Jazirah, and most of the actinomycetoma cases would have concentrated in the rural North and West Kurdufan states. Overall, the estimated incidence of eumycetoma is four times higher than actinomycetoma in Sudan and across the states. The localities with higher eumycetoma incidence estimates include North Jazirah, Khartoum Bahri, Um Rawaba, Sennar and Ad Douiem.

6.3. Limitations

For chapters two and three, the diagnosis of mycetoma was a limitation as it is usually based on clinical presentation, typical radiological findings and microbiological identification of the causative organisms (e.g., using microscopy and cytological, histopathological, immunohistochemical and molecular techniques). In the current study, the diagnosis was based on a combination of clinical and ultrasound examinations since mycetoma grains, and the accompanying inflammatory granulomata have a characteristic ultrasonic appearance. Given that microbiological identification of the mycetoma-causative pathogen was not possible due to the lack of available facilities locally, there was a possibility I excluded actual cases of mycetoma in individuals who presented with swelling or sinus formation but had negative ultrasound examinations. Another limitation was that some suspect cases were lost to follow-up and were not included in the comparative analysis.

However, I have no reason to believe that the absence of these individuals was due to their disease status or other systematic factors, so considered this data to be missing at random.

For chapters four and five, I must assume there might be underlying geographical and temporal biases in the recorded number of mycetoma cases in the country, as all the records were collected by the, which is located in Khartoum. From the exploratory analysis of this dataset, the number of mycetoma cases confirmed by the MRC has steadily increased since 1991, and the vast majority are coming from rural and peri-urban areas near the Khartoum area. To account for this potential geographical bias, I adjusted the number of cases by the estimated population density in 2020, although there remains some uncertainty on where the infection may have occurred due to most cases being diagnosed in advanced stages of the disease. Neither was it possible to account for any existing temporal variation due to the uncertainty on when the infection occurred; severe cases are more likely to be diagnosed in health facilities. Second, I did not account for individual or household-related risk factors such as work and social activities, which may also have driven the distribution and intensity of transmission. I , however, included an estimate of poor sanitation coverage, assuming a higher risk in more deprived areas with limited access to protected sanitation.

6.4. Conclusion

This thesis provides an accurate prevalence of mycetoma in an endemic area in Sudan using extensive community data. It revealed an equal sex ratio which contradicts most previous reports. Other findings are in line with those reported previously. Further studies are needed to determine mycetoma prevalence in Sudan more widely and bridge the gaps in our understanding of the epidemiology of mycetoma, which is vital to design evidence-based control and prevention programmes. Furthermore, these surveys are also helpful in early case detection and treatment, health education, disease awareness, and advocacy, reducing the disease burden and improving the disease prognosis. However, implementing surveys in rural areas in Sudan could be difficult, particularly during the rainy seasons, and proper team training, good facilities and collaboration between different stakeholders are all required. The case-control study results with this high number of participants enabled precise estimates for the strength of association of various risk factors with the disease. The results of this study could be applied to inform the future control of mycetoma. Efforts to raise awareness among clinicians of mycetoma risk factors- particularly age, history of trauma, and ownership of animals- could promote earlier diagnosis and treatment of mycetoma in patients presenting swellings or wounds in endemic regions of Sudan. These factors could also inform the design of health awareness campaigns in communities at risk, educate the population about activities that may put them at risk of the disease and encourage them to present early to health facilities if they experience early signs. Finally, this study adds further evidence for the substantial social impact of the disease and the stigma associated with it, which should not be overlooked in assessments of its global burden.

The modelling analysis and burden estimation results will guide both prevention and treatment services. Such work provides direction for future control and prevention programmes for mycetoma, highly endemic areas are targeted, and resources are directed to areas with high demand. Moreover, medical personnel will have high levels of suspicion for mycetoma in areas with high burden and cases will be adequately diagnosed and managed within their communities without seeking medical treatment in centralized medical facilities, reducing the financial burden mycetoma patients. Specialized mycetoma management centres can be established in communities where mycetoma is endemic, providing early case detection and management of mycetoma patients could help and guide planning for national and international scale surveys for mycetoma identification and design targeted training for medical staff on early detection and diagnosis. Moreover, they could aid in designing national and global mycetoma advocacy and awareness programmes leading to early active case detection with early appropriate patient management in areas that are highly endemic for mycetoma.

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Appendix 1

Mapping the spatial distribution of mycetoma protocol

Purpose of the document:

This document was developed in the early stages of the PhD and submitted to the ethics approval committee and used as the field manual for logistics and training. This document includes a background about the problem and a research plan in addition to standard operating procedures for all the research activities.

Mapping the spatial distribution of mycetoma in Sennar State, Sudan, 2018

Joint project between

Mycetoma Research Centre, Khartoum, Sudan

Brighton and Sussex Medical School, Brighton, UK

Sennar State Ministry of Health, Sennar, Sudan

Table of Contents

Summary

Mycetoma is a chronic, progressive and debilitating neglected tropical disease that ultimately destroys both the bodies and lives of those it affects and worsens poverty in some of the world's poorest people. Sudan is home to approximately one third of all Mycetoma cases in the world and has the highest global burden of the fungal form of the disease. It is difficult to treat, prevent and control Mycetoma and these are currently held back by a lack of information about risk factors and the disease distribution.

The study will include a household survey to explore the disease prevalence, socioeconomic demographic aspects, to detect the possible environmental risk factors and the mode of transmissions of Mycetoma.

Sixty villages, with a total of 56,100 individuals, from 11,216 households will be covered in Eastern Sennar locality, by house to house survey for early case detection. Then cases will be confirmed by Ultrasound examination, and then followed by generation of distribution maps. The socioeconomic disease impact on patients, families and health authorities will be determined. The studied villages environmental characteristics to determine the disease risk factors and hopefully the predisposing factors and infection transmission route. The obtained data will be available to program planners and policy makers to plan a Mycetoma control. This work will be conducted in collaboration with Ministry of Health, Sennar State and NIHR Unit, with Brighton and Sussex Medical School, UK.

Background:

Mycetoma is a chronic, specific, granulomatous, progressive and disfiguring inflammatory disease. It is caused by true fungi or by certain bacteria and hence it is usually classified into eumycetoma and actinomycetoma respectively. [1, 2] *Madurella mycetomatis* is the commonest eumycetoma causative agent, while *Streptomyces somaliensis* and *Nocardia brasiliensis* are the common causative organisms for actinomycetoma. [3, 4] The triad of a painless subcutaneous mass, sinuses formation and purulent or seropurulent discharge that contains grains is pathognomonic of mycetoma. [5, 6] It usually spreads to involve the skin and the deep structures, resulting in destruction, deformity and loss of function, occasionally it can be fatal. The foot and hand are the most frequently affected sites accounting for 82% of cases. In endemic areas other parts of the body may be involved such as the knee, arm, leg, head and neck, thigh and perineum. No age is exempted in mycetoma; however, it occurs more frequently in young adult men in the age range 20–40 years and almost 30% of reported patients were young students. [7] whereas incubation period in mycetoma is still unknown due to the difficulty in establishing the time of initial infection.

The true incidence and prevalence of mycetoma world-wide are not precisely known. [8, 9, 10] It is interesting to note that most of the reported mycetoma data are on hospitalised patients with advanced disease. [13,14] This is attributed to the nature of mycetoma, which is usually painless, the patients' low socioeconomic status and poor health education level, lack of health facilities in endemic areas and the geographic distance from medical centres. [15, 16]

The worldwide distribution of mycetoma varies extensively. It is endemic in many tropical and subtropical regions and prevails in what is known as the 'mycetoma belt', which stretches in a band between the latitudes of 15°S to 30°N. [9] The belt includes Sudan, Somalia, Senegal, India, Yemen, Mexico, Venezuela, Columbia and Argentina. [17–23] However, mycetoma has been reported in many temperate regions as well. [24,25] There are reports of mycetoma from the USA, Sri Lanka, Germany, Egypt, Turkey, Philippines, Japan, Lebanon, Thailand, Saudi Arabia, Tunisia and Iran, (Fig 1). [26 – 30]

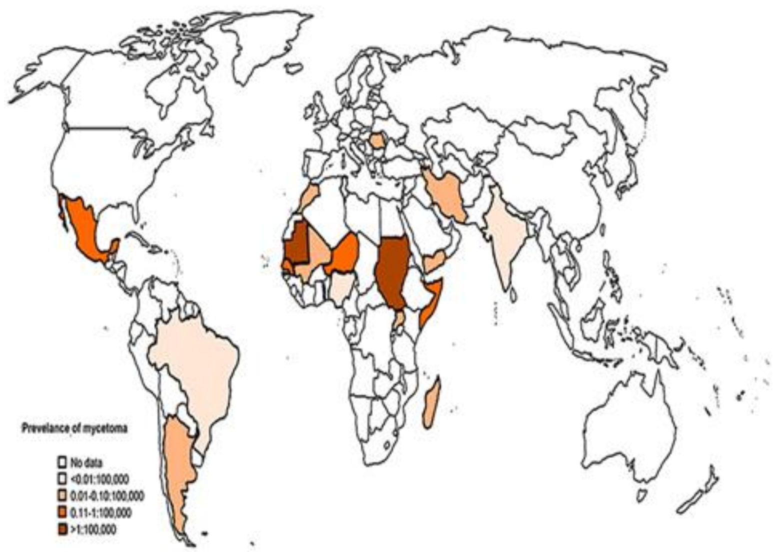


Fig 1: A map showing the mycetoma prevalence worldwide, adapted from van de Sande WW. Global burden of human mycetoma: a systematic review and meta-analysis [49]

Globally, only three large epidemiological studies have been performed to estimate the disease prevalence. These studies were reported by Abbott in Sudan during the period 1952–1955, by Lopez Martinez and colleagues in Mexico between 1956 and 1985 and Fahal and associates in Sudan in 2015. [11, 12, 31] Abbott's study included 1231 mycetoma patients who had been admitted to hospitals throughout the country over a period of 2.5 years. The estimated prevalence was 4.6 cases per 100,000 inhabitants. Lopez Martinez reported on 2015 mycetoma cases from 14 dermatological centres throughout Mexico over a period of 30 years and the estimated prevalence was 0.6 cases per 100,000 inhabitants. [11, 12] All of the three studies were facility based. There are no community based published studies conducted to estimate the prevalence, distribution and epidemiology of mycetoma.

Sudan is considered the mycetoma homeland; the history of mycetoma in Sudan is long and interesting. Treatment by cautery and/or amputation was practised during the time of the Mahdeya (1885-1899). However, the first documented report of a case of mycetoma in Sudan was published by Belfour in 1904. [(11)] It was noted that, the disease was common among Northern Sudanese that the foot was affected most and the commonest type of mycetoma was the black grain variety. These findings which were documented more than a century ago are still valid. Since then many studies have been carried out in the Sudan on mycetoma. [(32)]

In the early 1970's, the Sudanese medical community responded to the mycetoma threat by establishing The Khartoum North Mycetoma Clinic, for patient medical care and research.

Following that, The Mycetoma Research Centre (MRC) was established in 1991 under the umbrella of the University of Khartoum. The centre is recognized globally as a world leader and an authoritative advisor on mycetoma management and research and it is a WHO Collaborating Centre on Mycetoma.

Diagnosis

In mycetoma it is vital to identify the causative organism and the disease extent along the different tissue planes. Cytological smears taken by fine needle aspiration (FNA) from the lesion are essential to identify the causative organism and tissue reaction. Surgical biopsies taken under general or spinal anaesthesia are important to obtain grains for culture, molecular identification and histopathological identification of the causative organism and the tissue reaction. The polymerase chain reaction (PCR) is currently an important tool to identify the causative agent to the species level.

A battery of imaging techniques are available to determine the disease spread including the conventional X-ray, ultrasound, MRI and CT scan examinations. In all these modalities mycetoma has a characteristic appearance. They are used for mycetoma diagnosis and for follow up of patients on treatment and they have prognostic indicators. [32-40]

Management

The treatment depends mainly on the aetiological agent, the site and the extent of the disease. Until recently, the only available treatment for mycetoma was amputation or multiple mutilating disfiguring surgical excisions. Combined medical treatment in the form of antifungals for eumycetoma and antibiotics for actinomycetoma and various surgical excisions is the gold standard in mycetoma.

Reports on medical treatment in eumycetoma are scarce and inadequate. Over the years the treatment of eumycetoma is based on personal clinical experience and on the results of sporadic case reports, rather than controlled clinical trials. In general, extensive surgical excision or amputation of the affected part combined with long term antifungal treatment is the treatment of choice. [41, 42]

Actinomycetoma is amenable to medical treatment with antibiotics and other chemotherapeutic agents. Combined drug therapy is always preferred to a single drug to avoid drug resistance and for disease eradication. [42, 43] Cure is possible, although a prolonged period of treatment is needed. Combined medical and surgical treatment accelerates healing and reduces the chance of relapse; however, a good number of patients respond to medical treatment alone. [44, 45]

Medical treatment for both types of mycetoma must continue until the patient is clinically, ultrasonically and cytologically cured. Recurrence is more common after an incomplete or irregular course of medical treatment. With drug non-compliance, there is a good chance for the organism to develop drug resistance.

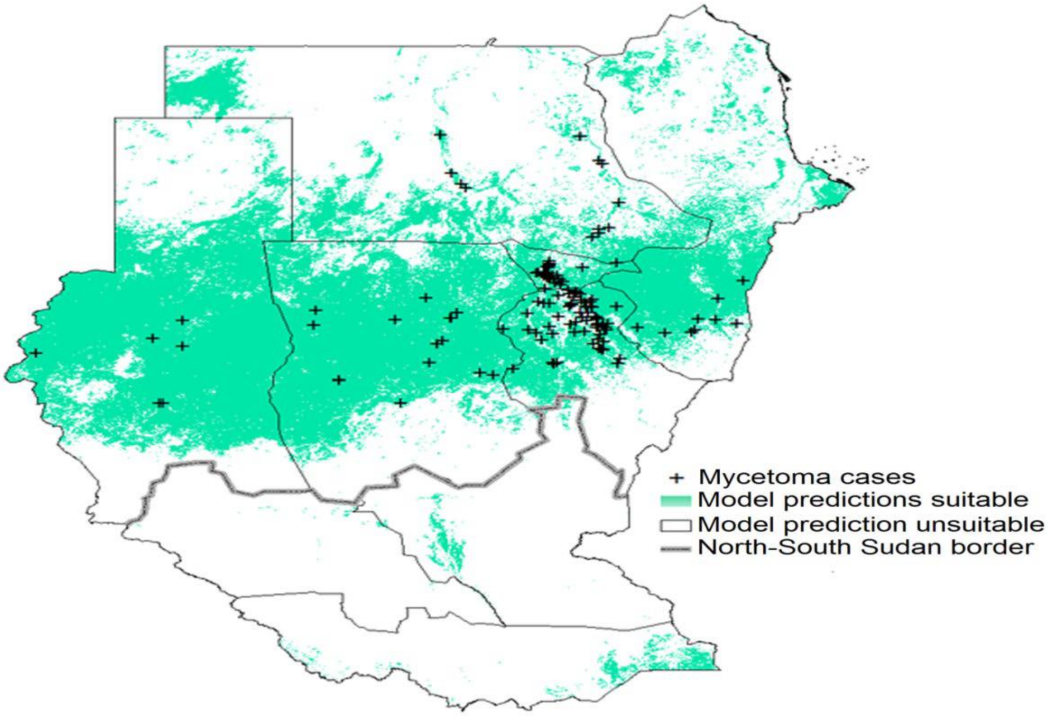
Prevention

Currently there are no controls or preventive programmes for mycetoma worldwide. But there is an urgent need to develop such programmes. However, populations in endemic areas should be advised to wear protective shoes during outdoor activities, to avoid thorn pricks, to maintain general hygiene including cleaning sites of superficial injury and to keep animals in special separate areas. In addition, health education and raising awareness, early case detection and management may provide the best means of reducing the high morbidity and complications of mycetoma.

Mycetoma Environmental Risk Factors

The presence of Madurella species DNA on Acacia thorns has suggested that infection results from inoculation by infected thorn pricks. (46) An ecological niche model was constructed to estimate the potential distribution of mycetoma in important endemic areas such as Sudan, assess risk factors associated with mycetoma infections and test Acacia-mycetoma associations based on overlap of the ecological niche of mycetoma infections with Acacia trees. This model was able to predict 149 of 158 of those additional occurrence points. The model was significantly more successful in anticipating these independent data than random expectations (Fig 2). (47) In addition, cattle dung may play a significant role in the ecology of mycetoma on the observation that *M. Mycetomatis* was phylogenetically closely related to dung-inhabiting fungi. (48)

Fig. 2: Adapted from Samy AM, van de Sande WW, Fahal AH, Peterson AT. Mapping the potential risk of mycetoma infection in Sudan and South Sudan using ecological niche modelling. (48)



The study justification

Mycetoma is one of the neglected tropical diseases. It is endemic in many tropical and subtropical regions across the world and Sudan is suspected to have the highest endemicity. The disease usually affects lower limbs but it can be anywhere else in the body. Mycetoma is contracted through a minor trauma creating a portal for organism entry. Environment in communities where disease is endemic, favors disease occurrence. Most of the population practice outdoor activities such as farming barefooted which make them more susceptible to trauma.

The affected patients are usually young adults of low socio-economic status leading to serious economic and social consequences. Most patients present late with advanced disease and serious complications due to the painless nature of the disease, low socioeconomic status, low health education and lack of health facilities in endemic areas.

Still the basic epidemiological characteristics, including incidence and prevalence of the disease remain unknown. Mycetoma is one of the diseases that if discovered in early stages, cure may be possible. Therefore, early detection is the key point in disease management which cannot be established without first acknowledging the true burden of the disease and its distribution. Therefore, mapping of mycetoma is a prerequisite for establishing a national control program in all endemic areas.

For all of the above mentioned areas with a high burden of mycetoma need to be identified to design appropriate evidence based interventions to promote early disease detection, proper patient management and disease control and elimination.

Study Aim and objectives:

Study Aim:

The study aims to determine the epidemiology, spatial distribution and associated factors that predispose people to mycetoma in Sennar State, Sudan.

Study Objectives:

The study objectives are to determine:

- The spatial distribution of mycetoma in Sennar State, Sudan.
- The prevalence of mycetoma in the studied areas.
- The ecologic, environmental and socioeconomic factors that influence the spatial distribution of mycetoma.
- The population at risk.

Study Question

What is the spatial distribution of Mycetoma in Sennar state?

Study area and population

The study will be conducted in Sennar State, Sudan, one of the most highly endemic states in the Sudan, (Fig. 3). The selection of this state was based on the Mycetoma Research Centre records which showed the high prevalence of mycetoma in Sennar, White Nile and Al Gaziera states. Since no recent studies have been conducted to estimate the prevalence of mycetoma the study will be conducted in two phases; pilot phase and expansion phase.

The pilot phase will be conducted in eastern Sennar locality, Sennar state. This phase will be done to assist in development of an appropriate sampling technique and to estimate the sample size for the full scale study. An expansion phase will follow in which the sample size will be appropriately established and a wider scale study implemented to cover the remaining localities in Sennar state and the other mycetoma endemic states.

Sennar state which lies in a rich savannah region between latitude 12.5°–14.7° N and longitude 32.9°–35.4° S in central eastern Sudan. It shares borders with Gezira state in the north, White Nile and Upper Nile states in the west, Gadarif state in the east and Blue Nile state and Ethiopia in the south. The summer extends from March to May, with average daily temperatures of 32–40° C and relative humidity of 25%. The rainy season starts early in June and continues until September. Winter starts in October with average daily temperatures of 20–25 °C. Most of the resident in that area work in close contact with the filed and animals as most of them work in agriculture and herdsmen (51)

Pilot phase:

The study will be conducted in Eastern Sennar locality, Sennar State which is situated in the south east part of Sudan (Fig. 4). Eastern Sennar Locality is subdivided into five administrative units, (Table1). A regional Mycetoma management Centre (WAMRC) was established at Wad Onsa village, Eastern Sennar locality which is one of the most highly endemic localities in Sennar State.

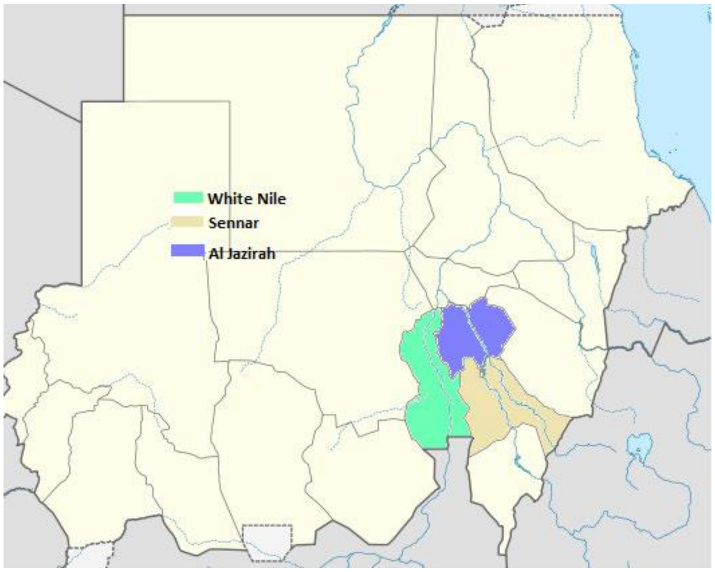


Fig. 3: A map of Sudan showing the three mycetoma highly endemic state

Fig. 4: A map showing Sennar state

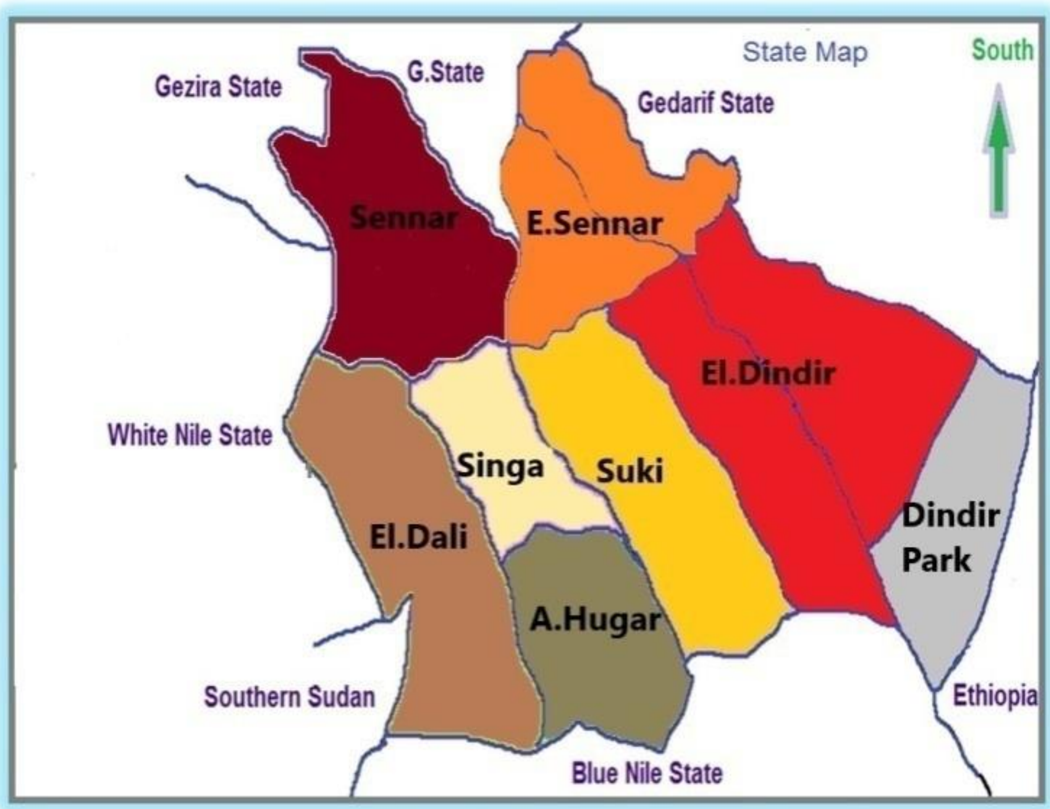


Fig.5 A map of Sennar State localities

Table (1): showing the total number of administrative units, villages and total population residing Eastern Sennar locality, Sennar State (www.cbs.gov.sd)

State	**Locality/ district**	**Administrative Unit**	**Villages No.**	**Population**
Sennar	**Eastern Sennar**	Dooba	**292 villages**	**353,196 Inhabitants**
		Wad Al Abbas		
		Wad Taktook		
		Alreef Alsharqi		
		Wad Onsa		

Materials and methods

Study design:

A community-based descriptive cross sectional study will be employed.

Source population:

The source population includes all individuals living in Eastern Sennar locality during the data collection period.

Study population:

Total population living in eastern Sennar locality who fulfil the inclusion criteria.

Inclusion criteria

- All individuals who live in the area regardless of age and sex will be included.

Exclusion criteria

- Has not provided consent
- Individuals who are not from the village targeted

sampling strategy:

Assuming a total Eastern Sennar population of 353,196, sampling 60 villages of 935 people, would generate prevalence estimates for each outcome at a precision below 0.03%, assuming a prevalence estimate of 5 cases per 10,000, a design effect of 2.5 and a community participation rate of 80%. Which was bases on the standard sample size formula and a finite population correction factor:

$$n = \frac{DEFF \times z^2 \times P(1-P)}{e^2 \times R}$$

Where:

DEFF = the design effect

z = standard normal deviate corresponding to 95% confidence intervals

P = expected prevalence

R = community participation rate

e = precision

and a finite population correction factor of:

$$n_{adj} = \frac{n \times N}{n + (N - 1)}$$

The rationale for this assumption is based on the prevalence of mycetoma of 5 cases per 10,000 populations, with the average population size of the villages with 935 people, and the population of Eastern Sennar locality 353,196 which in total will cover 56,100 individuals, from 11,216 households. Important to note is that the prevalence in one of the studies conducted in one village in White Nile was found to be 14.5/1000 but this village is one of high prevalence. Therefore 5/10000(which is a highly conservative estimate) has been used to calculate the sample size. The villages will be randomly selected.

Study participants:

After village leaders are informed with the study and they have given their consent, all households will be visited. Informed written consents from heads of household will be obtained prior to conducting the survey.

Diagnosis of mycetoma patients:

Individuals with a swelling of any part of the body, that is painless, +/- sinuses, +/- grains discharge are considered suspect, but still need to be diagnosed using ultra-sonographic (US) findings which are presence of pocket of fluid containing echogenic grains, then surgical excision to confirm diagnosis. Frequently, mycetoma is mis-diagnosed as lipoma, foreign body granuloma or some other dermatological disease. This mandates good training of the survey team in detecting mycetoma and differentiating it from other probable diseases. Due to the painless nature of mycetoma, diagnosis is often missed so clinical examination of individuals should be thorough.

Case definition:

- **Suspected case**

  Any individual with a swelling in any part of the body, who is living in mycetoma endemic area

- **Probable cases**

  Any individual with a swelling in any part of the body or sinus formation and +/- grain discharge.

- **Confirmed case**

  Any individual with a swelling in any part of the body or sinus formation and +/- grain discharge that is evident by ultrasound examination in a form of Pocket of fluids containing echogenic grains.

Data collection

Data collection will be done using electronic questionnaires, it starts with demographic data (Age, Gender, Marital status, Educational level and Occupation) to describe the characteristic of the targeted population. Then clinical data related to mycetoma will be collected, as onset of the disease, site of the lesion, history of trauma and family history.

For the behavioral practices, shoes wearing and direct contact with animals and thorny trees will be addressed. While health economic aspect will be covered by asking about the health services received specially for mycetoma and the economic burden to these services.

The survey team

In each village a field worker will be selected and a coordinator in each of administrative units will be appointed. All of them as well as all health assistants and nurses in the locality will be included in the survey team. The team will be trained in early mycetoma case detection and on using the electronic questionnaire prior to the study. The Mycetoma Coordinator will be responsible for the overall technical leadership, management and liaison, as well as the overall compliance with the protocol. The team members will be responsible for locating the targeted villages and making the necessary pre-survey arrangements including community sensitization and mobilization.

Pre-survey Logistics:

The Mycetoma Coordinator, a field logistic coordinator will be appointed and will be responsible for the survey logistics. That will include team transport between villages, transport of suspected cases to Wad Onsa Mycetoma Center (WOMC), and other supplies.

A training manual will be prepared and a training workshop will be conducted. The training includes survey methods, clinical examination, identification of mycetoma lesions and the use of portable Geographical Positioning System devices. Pre-survey team training will be conducted to explain to them the survey objectives, early case detection, and referral of suspected cases.

Initial training will be given in Sennar with the aim of sensitizing regional leaders, explaining the objectives of the survey, and identifying and training potential survey team members.

After the training, a pre-test of field procedures, including the use of tablets for data entry, identification of mycetoma lesions and use of portable GPS devices, will be conducted. Based on the pre-test results, problems will be identified and corrected. In addition, the training will help the coordinators to select and make initial plans. Following this, coordinators will organize training for data collectors in their respective areas. The Standard Training Guide will be the source document for all training. The actual field data collection date will be selected with input from coordinators, taking into account the heavy rainy season that can potentially hinder data collection and make some remote villages inaccessible.

The teams, consisting of the coordinator, the interviewer and the village guide, will visit targeted villages before the actual day of the survey to accomplish the following tasks: to inform local leaders and community representatives about the objectives of the survey and get permission, to record geo-coordinates using a GPS device, to select a suitable day for the survey in consultation with the community members, and to record travel time and directions to the villages. The team will supervise data collection, ensuring adherence to protocol and responding rapidly to problems encountered. There will be continuous communication with coordinators through mobile phones, so that problems arising during data collection are communicated for prompt action.

All forms completed using the tablet will be manually checked for completeness and validity, then all the data will be sent to the main server. Each questionnaire form has a automatically generated unique ID, but for linking the data from the questionnaires with the Ultrasound result a barcode technique will be used. A barcode will be attached to each referral form of the suspected cases, will be read by the tablets and eventually will be read at the center after confirmation of patient status.

The team will work closely with the Ministry of Health throughout the project, which will benefit from use of resources including vehicles and personnel from local health offices.

Survey process:

The teams will enter the targeted households, a brief introduction will be given to the head and other members of the household about the team and purpose of the study. Then written consent will be taken from the head of the household. The team will start with the household questionnaire which includes questions about the demographics of the household members, educational level, occupation, history of trauma and practice of shoe wearing by directly asking the head of the household.

Subsequently, a clinical examination will be conducted on all members of the household. If any member is suspected to have mycetoma, an individual questionnaire will be completed for the targeted individual. In the individual questionnaire, the data collected will include; disease symptoms, family history, contact with the environment and actions taken towards the disease. Geographical coordinates will be collected as well as photos of the lesions. If the household was empty during the survey, the team will visit it a second time on the same day before labeling it as absent, the same applies for any household member absent during diagnosis if he or she was suspected of having mycetoma. The suspected case will have the referral form filled on their behalf with attachment of barcode number, so they can be referred for confirmation of diagnosis.

After the survey process finishes in each village the total number of suspected cases will be known, then in coordination with the village health focal person, transport arrangements will be made from village to the WOMC and back to the village.

Global Geographical Positioning System (GPS)locality:

Community GPS coordinates will be collected using a Handheld Global Geographical Positioning System from the central point in the village. GPS coordinates will put onto a map using GIS software, to display the distribution of mycetoma in eastern Sennar locality

Data entry and analysis:

Data on the MRC data server will be transferred into an Excel form which can be easily imported by different statistical package. Data verification, cleaning and analysis will be done using Statistical Package for Social Sciences, SPSS version 23, or above, for MS Windows (SPSS, Chicago, IL, USA) and ARC-GIS software packages. Appropriate statistical tests will be used for the data analysis.

Ethical considerations:

Ethical approval will be obtained from the Mycetoma Research Centre, IRB Committee, Sudan and from the BSMS Research Governance and Ethics Committee. Potential participants will have the purpose of the study explained to them in their local language at the time of recruitment. A verbal consent will be taken at the village level after explaining the survey purpose and process. informed consent will be obtained from head of the household before starting the survey process which explain the what will be done for the suspected cases. Consent will be taken for the adults or minors as their guardian(s)will consent for them. The consent will be obtained using Arabic language.

Participants with confirmed Mycetoma will receive medical treatment and will undergo surgery and be followed up until they are cured in WOMC or the MRC.

Expected outputs from the study

1. A map of the spatial distribution of Mycetoma in eastern Sennar locality, defining its endemicity.
2. Estimated prevalence of the disease in eastern Sennar locality which will guide the sampling strategy for the remaining localities in Sennar state.

Study limitations and source of bias:

One of the main limitations is linking the data generated from the households in the field with the confirmatory test results, from the ultrasound examination. In this regard, a barcode will be used to link the questionnaire from the suspected case with the result at referral center. Another limitation is the validity of the data collected by the health assistants, for that matter proper training in case detection and examination of suspected patients. Following the training each participant (health assistant) should undergo an assessment test for the training and at least 70% are needed to progress to the survey stage.

Significance of the study

The results of the study will benefit the local and federal health authorities and other stakeholders to develop an efficient and cost effective mycetoma elimination programme by precisely locating endemic areas and affected and at risk communities. Furthermore, this will guide the sampling strategy in the expansion phase.

Dissemination of Results and Ensuring the Implementation

The operational research will provide important data to provide key knowledge and reduce the information gaps that currently hinder the development of Mycetoma elimination strategies in Sudan. After completion of the data analysis, the results will be disseminated to all partners in the form of publications.

Study team members and activities:

<table>
<tr><th>Team member</th><th>Key responsibilities</th></tr>
<tr><td>Coordinators</td><td>Community
Communicate with community leaders to obtain support and consent for mapping
Point of contact for community during mapping
Field Team
Lead the field team to conduct mapping surveys
Oversee surveys and supervise field team activities, ensuring all team members are following the mapping protocol
Monitor all field data collection
Manage time of field activities and time spent on each mapping s
Data collection
Collect GPS points for mapping site
Other
Ensure transport of referred patients to WOMC</td></tr>
<tr><td>Health Assistant</td><td>Completion of survey questionnaire.
Conducting physical examination for all household members.
Refer suspected cases to WOMC for confirmation.</td></tr>
</table>

Field workers	• Obtain informed consent from the household head. • Sensitize and inform the local community about mycetoma and the mapping survey • Motivate community members to participate in the survey
Clinicians	• To perform clinical examination • To perform surgical excision
Radiologist	• Performing US examination to confirm mycetoma diagnosis.
Drivers	• Safely transport all field team members and equipment from be-tween mapping sites
Statistician/data analyst	• Statistician to oversee the data analysis

Appendix 2

Household and individual questionnaires

Household information panel	**HH**
HH1. Village name and number Name ________________________ ___ ___	**HH2**. Household number: ___ ___ ___
HH3. Day / Month / Year of interview: ___ ___ /___ ___/ 2018	**READ BARCODE**
HH4. Result of household interview: Completed/ .... 01 No household member or no competent respondent at home at time of visit........ 02 Entire household absent for extended period of time .... 03 Refused........ 04 Other (*specify*)________________________ 96	

List of household members **HL**

First, please tell me the name of each person who usually lives here, starting with the head of the household.

List the head of the household in line 01. List all household members (HL2), their relationship to the household head (HL3), and their sex (HL4)

THEN ASK: Are there any others who live here, even if they are not at home now? IF YES, COMPLETE LISTING FOR QUESTIONS HL2-HL4. THEN, ASK QUESTIONS STARTING WITH HL5 FOR EACH PERSON AT A TIME.

HL1. *Line no.*	**HL2.** ***Name***	**HL3.** **What is the relationship of (*name*) to the head of household?**	**HL4.** **Gender** 1 Male 2 Female		**HL5.** **Age** *Record in completed years. If age is 95 or above, record '95'.*	**HL6.** **Marital status** 1 Unmarried 2 Married 3 Divorced 4 Widow	**HL7.** **Education level** 1 Under the age of school 2 Illiterate 3 Khalwa 4 Preschool 5 Primary 6 Secondary 7 University or higher 8 Currently involved in a degree	**HL8.** **Occupation** 1 Under the age of work 2 Student 3 Farmer 4 Shepherd 5 Housewife 6 Mechanic 7 Merchant 8 Employee 9Unemployed 10 other, ……………….	**HL9.** **Does (*name*) have Mycetoma?** 1 Yes 2 No 8 Do not know (DK)	**HL10.** **Does(name) wear shoes/ Slippers?** 1 At work only 2 At home only 3 Both work and home 4 Not at all	**HL11.** **Is there any history of trauma?** 1 Yes 2 No 3 Not sure
Line	Name	Relation*	M	F	Age				Y N DK		Y N NS
01		**0 1**	1	2	__ __	1 2 3 4	1 2 3 4 5 6 7	1 2 3 4 5 6 7 8 9 10	1 2 8	1 2 3 4	1 2 3
02		___ ___	1	2	__ __	1 2 3 4	1 2 3 4 5 6 7	1 2 3 4 5 6 7 8 9 10	1 2 8	1 2 3 4	1 2 3
03		___ ___	1	2	__ __	1 2 3 4	1 2 3 4 5 6 7	1 2 3 4 5 6 7 8 9 10	1 2 8	1 2 3 4	1 2 3
04		___ ___	1	2	__ __	1 2 3 4	1 2 3 4 5 6 7	1 2 3 4 5 6 7 8 9 10	1 2 8	1 2 3 4	1 2 3
05		___ ___	1	2	__ __	1 2 3 4	1 2 3 4 5 6 7	1 2 3 4 5 6 7 8 9 10	1 2 8	1 2 3 4	1 2 3
06		___ ___	1	2	__ __	1 2 3 4	1 2 3 4 5 6 7	1 2 3 4 5 6 7 8 9 10	1 2 8	1 2 3 4	1 2 3
07		___ ___	1	2	__ __	1 2 3 4	1 2 3 4 5 6 7	1 2 3 4 5 6 7 8 9 10	1 2 8	1 2 3 4	1 2 3
08		___ ___	1	2	__ __	1 2 3 4	1 2 3 4 5 6 7	1 2 3 4 5 6 7 8 9 10	1 2 8	1 2 3 4	1 2 3
09		___ ___	1	2	__ __	1 2 3 4	1 2 3 4 5 6 7	1 2 3 4 5 6 7 8 9 10	1 2 8	1 2 3 4	1 2 3
10		___ ___	1	2	__ __	1 2 3 4	1 2 3 4 5 6 7	1 2 3 4 5 6 7 8 9 10	1 2 8	1 2 3 4	1 2 3

Household characteristics		**HC**
HC1. How many rooms in this household are used for sleeping?	Number of rooms.....................................__ __	
***HC2**. Main material of the dwelling floor.* *Record observation.*	Earth/ soil.. 01 Sand.. 02 Dung.. 03 Brick.. 04 Cement.. 05 Ceramic... 06 Other (*specify*) ______________________ 96	
HC3. *Main material of the roof.* *Record observation.*	Traditional roof عرش بلدي .... 01 Plastic cover.. 02 Wood cover.. 03 Cardboard/ Cartoon .... 04 Zinc .... 05 Concrete.. 06 Other (*specify*) ______________________ 96	
HC4. *Main material of the exterior walls.* *Record observation.*	No walls.. 01 Branches of trees and wood .... 02 Mud and animal dung جالوص..................... 03 Stone.. 04 Red brick... 05 Concrete.. 06 Other (*specify*) ______________________ 96	
HC5. What type of fuel does your household <u>mainly</u> use for cooking?	Wood .... 01 Gas .... 02 Coal .... 03 Animal dung .... 04 No food cooked in household.....................05 Other (*specify*) ______________________ 96	 02⇨HC7 03⇨HC7 04⇨HC7 05⇨HC7 96⇨HC7
HC6. What are the types of wood that is used for cock-ing? *Circle all that apply*	Mesquite ....A Sidir...B Sonot ....C Taror..D Heglig .... E Sunflower.. F Dhaseer...G Talih ....H Gasab .... I Other (*specify*) ________________________ X	

HC7. Does your household have: [A] Electricity? [B] A radio? [C] A television? [D] A non-mobile telephone? [E] A refrigerator? [F] A washing machine	Yes Electricity .... 1 Radio .... 1 Television .... 1 Non-mobile telephone .... 1 Refrigerator .... 1 Washing machine 1	
HC8. Does any member of your household own: [A] A watch? [B] A mobile telephone? [C] A bicycle? [D] A motorcycle? [E] An animal-drawn cart? [F] A car or truck? [G] Raksha?	Yes Watch .... 1 Mobile telephone .... 1 Bicycle .... 1 Motorcycle .... 1 Animal-drawn cart .... 1 Car / Truck .... 1 Raksha 1	
HC9. Do you or someone living in this household own this dwelling?	Own .... Rent .... Other *(specify)* ____________	
HC10. Does any member of this household own any land that is used for agriculture?	Yes........ No........	2⇨HC12
HC11. Does this household own any live-stock, herds, other farm animals, or poul-try?	Yes........ No........	2⇨HC14
HC12. How many of the following animals does this household have? [A] cow(s)? [B] Goats? [C] Sheep? [D] Camels? [E] donkeys? [F] Horses? [G] Chickens? [H] Pigeons? [I] Dogs? [J] Cats?	*If none, record "00". If 95 or more, record "95".* *If unknown, record "98".* Cows........ ___ Goats........ ___ Sheep .... ___ Camels .... ___ Donkeys .... ___ Horses .... ___ Chicken .... ___ Pigeons .... ___ Dogs .... ___ Cats........ ___	
HC13. Does this household have an animal shed inside the house? Add	Yes........ No........	
HC14. Does this household have an animal shed outside the house?	Yes........ No........	2⇨HC16

Water and Sanitation — WS

WS1. What is the <u>main</u> source of drinking water for members of your household?	Piped water .... 01 Tube Well, Borehole.... 02 Protected well.... 03 Unprotected well .... 04 Tank station.... 05 Tanker-truck.... 06 Cart with small tank .... 07 Canal.... 08 Nile.... 09 Other (*specify*) ______ 96	
WS2. Do you do anything to the water to make it safer to drink?	Yes .... 1 No .... 2 Do not know 3	2⇨WS4 8⇨WS4
WS2.1. What do you usually do to make the water safer to drink? *Probe:* Anything else? *Record all items mentioned.*	Boil .... A Add bleach / chlorine .... B Strain it through a cloth .... C Use water filter (ceramic, sand etc.).... D Alum .... E Let it stand and settle.... F Other (*specify*) ______ X Do not know .... Z	
WS3. What kind of toilet facility do members of your household usually use?	Flush toilet.... 1 Pit latrine .... 2 Ventilated Improved Pit latrine (VIP) .... 3 No facility, Bush, Field .... 4 Other (*specify*) ______ 96	
WS4. How do you get rid of your household waste?	Burning .... 1 Burying .... 2 Throw in special place outside the home.... 3 Other (*specify*) ______ 96	

HH 5. *Thank the respondent for his/her cooperation and check the List of Household Members:*

☐ *A separate Individual Questionnaire has been issued for the suspected cases in the List of Household Members (HL12).*

INDIVIDUAL'S INFORMATION PANEL	IN
This questionnaire is to be administered only to suspected Mycetoma cases .	
IN1. Village name and number Name ________________________ ___ __	**Read barcode**
IN5.Interviewer's name and number: Name_____________________________ ___ ___	**IN6**. Day/Month/Year of interview: ___ ___ /___ ___ / 2018
IN7. Result of individual's interview	Completed....................01 Not at home....................02 Refused....................03 Partly completed....................04 Incapacitated....................05 Other (*specify*)______________________ 96

Individual's background		IB
1. **IB1**. How old are you? *2.*	Age (in completed years) .... __ __	
3. **IB2**. Have you ever attended school or pre-school?	Yes....................1 No....................2	2⇨IB4
4. **IB3**. What is the highest level of school you attended? 5. 6.	Preschool....................0 Primary....................1 Secondary....................2 Higher....................3	
7. **IB4**. Where were you born? in this village or outside the village?	Outside this village....................1 In this village....................2	2⇨IB6
8. **IB5**. Where were you born outside the village? *9. Write the names in the space provided*	State ______________________ Locality ______________________ City of village______________________	
10. **IB6**. Have you lived outside your place of birth?	Yes....................1 No....................2	2⇨Next module (EF)
11. **IB7**. Where did you live outside your place of birth? *12. Write the names in the space provided*	State ______________________ Locality ______________________ City of village______________________	
13. **IB8**. How many years did you live outside your place of birth? 14. *Enter 00 if less than 1 year*	Years outside place of birth............... ____ ____	

Environmental factors		EF
15. **EF1**. Do you or a member in this household own a farm?	Yes ... 1 No ... 2	
16. **EF2**. Do you practice agriculture? 17.	Yes ... 1 No ... 2	 2⇨EF11
18. **EF3**. Do you practice agriculture regularly or irregularly?	Regularly during the whole year ... 1 Irregularly ... 2	
19. IEF4. For how many years did you practice agriculture? *enter 00 if less than 1 year*	Years practiced agriculture... ____ ____	
20. EF5. Which types of agriculture do you practice, manual or mechanical?	Manual ... 1 Mechanical ... 2	
21. EF6. Which type of crops do you cultivate?	Sorghum ... 1 Wheat ... 2 Sunflower ... 3 Other (*specify*) ______ 6	
22. EF7. Is there any type of trees around the farm you work in?	Yes ... 1 No ... 2 Don't Know ... 8	 2⇨EF9 8⇨EF9
23. **EF8.** What are the name(s) of these trees? 24. *25. Circle all that apply*	مسكيت ... A سنط ... B سدر ... C هجليج (لالوب) ... D طلح ... E لعوت ... F Other (*specify*) ______ X	
26. EF9. Are there animals in the farm you work on**?**	Yes ... 1 No ... 2	 2⇨EF11
27. EF10. Which animals are there in the farm you work on**?** **28.** *29. Circle all that apply*	Cows ... A Goats ... B Sheep ... C Camels ... D Donkeys ... E Horses ... F Chickens ... G Pigeons ... H Dogs ... I	
30. **EF11.** Do you practice animal grazing? **31.** **32.**	Yes ... 1 No ... 2	 2⇨Next module
33. **EF12.** Which animals do you graze? **34.** **35.** *Circle all that apply*	Cows ... A Goats ... B Sheep ... C Camels ... D Donkeys ... E Horses ... F Chickens ... G Pigeons ... H	

Clinical features and disease related data		CL
36. CL1. For how many years did you have Mycetoma? *enter 00 if less than 1 year*	Years had Mycetoma ....____ ____	
37. CL2. At which age did you first have Mycetoma?	Age had Mycetoma..........................____ ____	
38. **CL3**. Where do you think you got the Mycetoma infection, in this village or outside of the village?	Outside this village .... 1 In this village 2	 2⇨CL6
39. **CL4**. In which place did you get the Mycetoma infection? *40. Write the names in the space provided*	State ________________________________ Locality ______________________________ City of village__________________________	
41. CL5. In which season did you get the Mycetoma infection?	Summer.. 1 Winter .... 2 Autumn .... 3 Don't know... 8	
42. CL6. Did you have a wound before the progress of your Mycetoma lesion(s)?	Yes... 1 No .... 2 Don't know... 8	 2⇨CL9 2⇨CL9
43. CL7. What is the cause of the wound?	Thorn... 1 Wood.. 2 Sharp instrument .... 3 Other (*specify*) ___________________________ 6	
44. CL8. How many Mycetoma lesion(s) do you currently have in your body?	Number of lesions............................____ ____	
45. **CL9.** Where are the site(s) of the lesion(s) in your body? **46.** **47.** *Circle all that apply*	Hand... A Face.. B Neck .... C Head.. D Knee...E Foot...F Pubic .... G Other (*specify*) ___________________________X	
48. CL10. Is there any history of Mycetoma in your family?	Yes... 1 No 2	 2⇨Next module
49. CL11. What is your relationship to the affected person(s)?	Father .... 01 Mother... 02 Brother .... 03 Sister .... 04 Grandfather.. 05 Grandmother.. 06 Other (*specify*) 96	

Health CARE	HC	
PC1. In the last year have you visited medical specialists because of your mycetoma?	Yes…………………………1 No…………………………..2	2⇨HC5
PC2. If yes, what type of healthcare professionals did you visit?	Doctor /Number of visits ………………….01 Nurse/Number of visits ……………..........02 Medical assistant /Number of visits……….03 Pharmacy Number of visits……………….04 Laboratory Number of visits………………05 Other (specify) Number of visits…………96	
PC3. In the last year have you paid for visiting medical specialists?	Yes…………………………1 No…………………………..2	
PC4. How much did you pay for the medical services you received in SDG?	…………. SDG	
PC5. Did you travel to visit medical specialists?	Yes…………………………1 No…………………………..2	2⇨PC9
PC6. How long did you have to travel to visit medical specialists?	Hours of travel……………..	
PC7. In the last year how much you spent on transport to visit medical specialists?	…………. SDG	
PC8. Did somebody (household member, friend or relative) accompany you on your visit to the medical specialists?	Yes…………………………1 No…………………………..2	
PC9. Have you been admitted to the hospital because of your mycetoma?	Yes…………………………1 No…………………………..2	2⇨PC17
PC10. In the last year how many times have you been admitted to the hospital because of your mycetoma?	Number of times……………	
PC11. In the last year, how much have you paid for your stays at the hospital?	Total amount over a year ………….	
PC12. How long did you have to travel to the hospital?	Hours of travel……………..	
PC13. In the last year how much have you spent on transport when you travelled to the hospital?	Total amount over a year………….	
PC14. Did somebody (household member, friend or relative) accompany you when you travelled to the hospital?	Yes…………………………1 No…………………………..2	
PC15. Did you or your accompanying person stay overnight when you went to the hospital?	Yes…………………………1 No…………………………..2	
PC16. How much have you and your accompanying person paid for the whole stay?	Total amount over a year………….	
PC17. In the past year, how much have you spent on medication to treat your mycetoma?		

	Total amount over a year…………	
OTHER EXPENCES	OE	
OE1. Did you visit a traditional healer in the past year?	Yes…………………………1 No…………………………..2	2⇨OE3
OE2. In the past year, how much have you spent on visiting traditional healers because of your mycetoma?	Total amount over a year…………	
OE3. In the past year have your borrowed any money from family, relatives or community?	Yes…………………………1 No…………………………..2	2⇨WP1
OE4. In the past year, how much money have you borrowed from family, relatives or community?	Total amount over a year…………	
WORK PRODUCTIVITY	WP	
WP1. Did your mycetoma affect your daily activity?	Yes…………………………1 No…………………………..2	2⇨END INTERVIEW
WP2. In the past year, how many days were you completely unable to work or go to school because of mycetoma?	Total amount over a year…………	
WP3. In the past year, how many days have you had adults helping you with your household work?	Total amount over a year…………	
WP4. In the past year, how many days have you had school age children helping you with your household work?	Total amount over a year…………	

Appendix 3 – Paper 1 Clinical epidemiological characteristics of mycetoma in Eastern Sennar locality, Sennar State, Sudan.

PLOS NEGLECTED TROPICAL DISEASES

(award number 16/136/ RESEARCH ARTICLE

Clinical epidemiological characteristics of mycetoma in Eastern Sennar locality, Sennar State, Sudan

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Abstract

Mycetoma epidemiological features remain uncharacterised. Few studies have been conducted in a community-based setting to explore the epidemiological features and risk factors for mycetoma in Sudan. To bridge this gap, this study was conducted in Eastern Sennar
Locality, Sennar State, Sudan, to report the clinical, epidemiological characteristics of mycetoma patients and the disease burden in the state.

We used cluster sampling; sixty villages were randomly selected across the locality's five administrative units, and a household-to-household survey was conducted. We collected data using pre-designed questionnaires at the community, household, and individual levels.
We performed descriptive analyses of the data and produced prevalence maps using ArcGIS 10.5 ([ESRI] Inc., Redlands CA, USA).

A total of 41,176 individuals were surveyed, and 359 mycetoma patients were identified. The overall prevalence of mycetoma was 0.87% (95%CI = 0.78–0.97%), the prevalence among males was 0.83% (95%CI = 0.71–0.96%), and females 0.92% (95% CI = 0.79– 1.06%). Individuals in the age group 31–45 years had the highest prevalence among the different age groups (1.52%, 95% CI = 1.23–1.86%). The prevalence map showed patients clustered within the central and north-eastern part of the locality, while villages in the southwestern part had few or no cases.

In conclusion, this clinical epidemiological study is pioneering and shows that mycetoma is prevalent in certain parts of Sudan. This data obtained will support the design of measures to reduce the disease burden in the state. The survey procedures and protocols can be adopted for further studies in Sudan and beyond.

groups. Married individuals had a higher prevalence when compared to unmarried individuals. Prevalence among those who could not read or write was 1.24% compared to 0.87% in those who could. History of trauma increased the prevalence to 1.63%, while individuals with no history of trauma had a prevalence of 0.70%. There were nine villages with no cases and an average of seven cases per village across the other 51 villages (range 1–39). The prevalence map showed patients clustered within the central and north-eastern part of the locality, while villages in the south-western part had few or no cases. The disease prevalence reported here may be more accurate as it was generated from a large study population. Further epidemiological studies are needed to determine mycetoma prevalence in Sudan and bridge the gaps in our understanding of the epidemiology of mycetoma.

Author summary

Mycetoma is a neglected tropical disease documented in many countries around the world, yet few community-based studies have been performed investigating the disease burden, clinical epidemiological features, and risk factors. The overall prevalence of mycetoma in the study area was 0.87%, and the prevalence of mycetoma among males and females was almost the same. Individuals in the age group 31–54 years had the highest prevalence among all age

29). The grant was awarded to MN and is being carried out in partnership between BSMS in the UK and the MRC, University of Khartoum in Sudan. The views expressed in this publication are those of the author(s) and not necessarily those of the NIHR or the Department of Health and Social Care. KD is supported by the Wellcome Trust [grant number 201900/Z/16/Z] as part of his International Intermediate Fellowship. RH,KD,MN,AH and SB received financial support from NIHR. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

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Introduction

Mycetoma is a neglected tropical disease (NTD) that is widely endemic in tropical and subtropical regions [1]. It usually presents mostly in the feet with painless soft tissue swelling associated with multiple sinus tracts formation and discharge of grains [2]. Mycetoma is caused by bacterial and fungal organisms, namely actinomycetoma and eumycetoma respectively, it spreads to the skin, deep structures, and bone, leading to deformities and disability [3,4]. It is speculated that the organisms are introduced to the body through a minor wound caused by sharp objects such as thorns, particularly acacia tree thorns [5]. Mycetoma affects poor people in remote rural communities, especially those working directly with the environment and animals such as farmers who tend to crop and livestock and shepherds[6]. Young male adults are a commonly affected group [7,8].

Diagnosis is usually made by the distinctive clinical presentation of mycetoma followed by different imaging techniques to confirm the disease presence and extent [9,10]. A biopsy is taken from the lesion site for histopathological examination or fine-needle aspirates for cytological examination to identify the causative organism. Recently, molecular identification techniques were introduced [11]. Management of mycetoma depends on the type of disease. Eumycetoma is treated with a combination of medical treatment and surgical excision, while actinomycetoma cases respond well to medical therapy alone[12].

The epidemiological features associated with mycetoma are not well described. The disease prevalence and incidence are still unknown worldwide. In Sudan, there are few studies reporting prevalence and incidence [13]. Although mycetoma was first reported in 1842, Abbott's first attempt to determine its prevalence was in 1956. He studied 1321 mycetoma cases from different parts of Sudan, and he reported a disease prevalence of 0.51% among hospital patients seen in Khartoum during a study period of 36 months. He reported higher prevalence in Atbara, Ed Dueim, and Wad Madani cities (within central Sudan states) with estimates of 0.92%, 0.93% and 1.18%, respectively [14]. In 2014, Fahal and colleagues conducted a study in a village in White Nile State, Sudan, to determine the burden of mycetoma, and reported a prevalence of 1.45% [15].

Only a few community-based studies have investigated the disease burden, clinical epidemiological features and risk factors for mycetoma. This study uses data collected from a large survey in Eastern Sennar Locality, Sennar State, to investigate the disease epidemiological characteristics.

Methods and materials

Ethical statement

Ethics approval for this study was obtained from the Mycetoma Research Centre, Khartoum, Sudan IRB (Approval no. SUH 11/12/2018) and from the BSMS Research Governance and Ethics Committee (ER/BSMS435/1). Written informed consent was obtained from each adult patient and parents or guardians of the population under 18 years old. Confirmed mycetoma cases were referred for management at Wad Onsa Regional Mycetoma Centre or the Mycetoma Research Centre (MRC).

Study setting

Eastern Sennar Locality is one of seven localities in Sennar State. This state is situated in the southeast part of Sudan. Jazeera State in the north borders it, the Blue Nile State in the south, Al-Gadaref State in the east and the White Nile State & the Upper Nile State of South Sudan in the west [16]. Eastern Sennar Locality is subdivided into five administrative units: Wad Alabbas, Wad Onsa, Wad Taktok, Elreif Elshargi and Doba. (Fig 1A and 1B)

Sampling strategy and participant selection

A cross-sectional community-based study was conducted. Cluster sampling was used to select sixty villages randomly within the five administrative units of Eastern Sennar Locality.

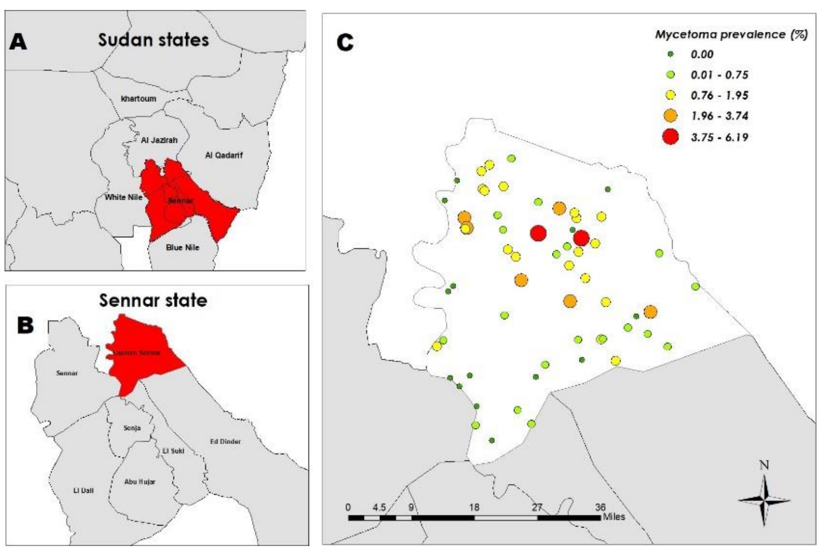

Fig 1. Geographical distribution of mycetoma in the sixty villages in Eastern Sennar Locality, Sennar State, with a total population of 41,176 individuals. Base map of Sudan link: https://www.diva-gis.org/datadown. A-Sudan states map with Sennar State in red. B-Sennar State Localities with Eastern Sennar Locality in red. C-Geographical distribution of mycetoma in Eastern Sennar locality.
https://doi.org/10.1371/journal.pntd.0009847.g001

Total coverage of these villages with a household-to-household survey was conducted. Data were collected using pre-designed validated questionnaires at three levels: community, household, and individual. All individuals living in the selected villages who provided written informed consent were included in the study.

The survey team consisted of a coordinator responsible for organising the survey activities and communication with village leaders and a medical doctor responsible for obtaining the written consent, performing the clinical examination and interviewing individuals within the household. Also, the team had a village guide who was responsible for the facilitation of movement within the village and communications with villagers. The teams visited the villages before the actual day of the survey, where the local leaders and community representatives were informed of the survey objectives, then teams identified a suitable day and time for the survey process and recorded geo-coordinates of the village using a GPS device. All households were then visited. Informed written consent was obtained from each household's head to conduct the survey. All individuals residing in the study area were screened for mycetoma by careful clinical examination after verbal consent.

Diagnosis of mycetoma patients

All individuals with swelling involving any part of the body or sinus formation with or without grains were classified as suspected mycetoma cases. All suspected cases were referred to Wad Onsa Mycetoma Satellite Centre, where they were clinically examined, and an expert radiologist performed lesional ultrasound (US) examinations to ascertain a mycetoma diagnosis. Confirmed mycetoma patients were defined as individuals with swelling in any part of the body with or without sinus formation, multiple sinuses with or without grain discharge that was evident by ultrasound examination in the form of a pocket of fluid containing echogenic grains [17].

Data collection was done using electronic questionnaires, and it included suspected cases' demographic data (age, gender, marital status, educational level, and occupation) to describe the characteristics of the targeted population. The clinical data related to mycetoma collected were onset of the disease, lesion site, history of trauma and family history. For the behavioral practices, data on shoe-wearing and direct contact with animals and thorny trees were collected in form of practicing arable farming which deals with cultivating crops and animal grazing. Animal grazing is considered a farming activity where domestic livestock are allowed outdoors to roam around and consume wild vegetations. The data were collected by trained medical doctors who received training on the data collection process and consent form administration.

Data analysis

Data were sent directly to a server at the Mycetoma Research Centre, Data Centre, Khartoum and visualised using Microsoft Excel (Microsoft Corp., Redmond, WA). Data verification, cleaning and analysis were done using Statistical Package for Social Sciences, SPSS 25 (SPSS, Chicago, IL, USA). Descriptive statistical analysis was performed to calculate the overall prevalence and to determine the individual data variables. Maps for mycetoma prevalence were developed using ArcGIS 10.5 ([ESRI] Inc., Redlands CA, USA).

Results

A total of 41,176 individuals were surveyed, from which 515 suspected mycetoma cases were detected. Of these, 359 patients (69.7%) proved to have mycetoma, the diagnosis was not confirmed in 133 (25.8%), and 23 cases did not attend for confirmation of the diagnosis. (Fig 2)

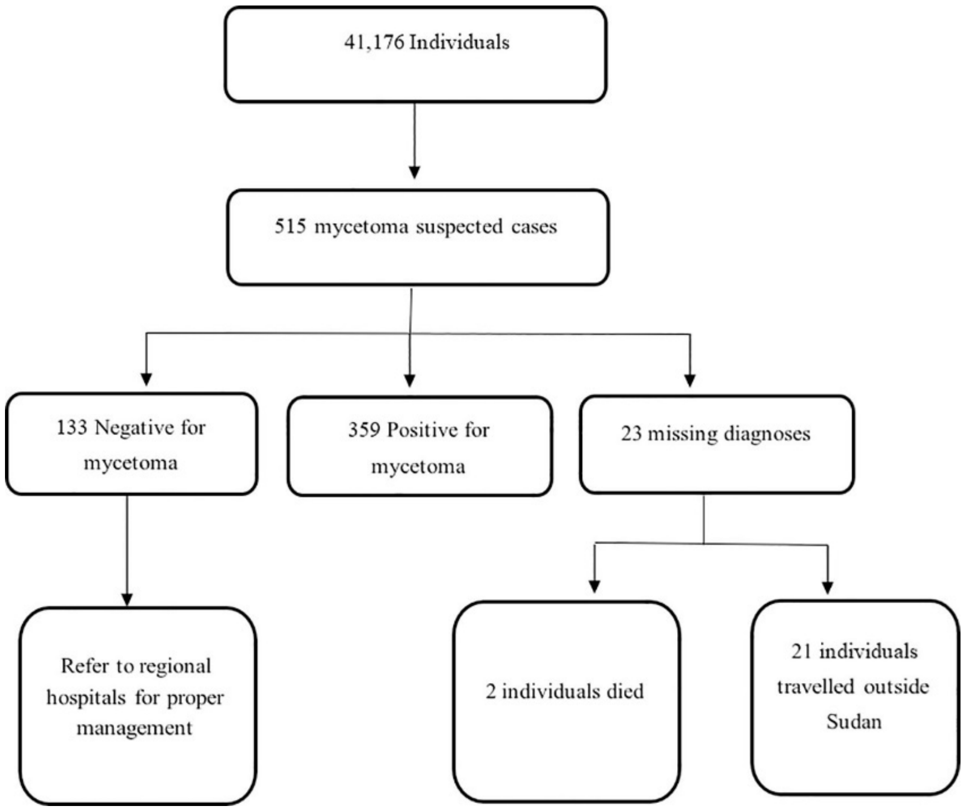

Fig 2. Flow diagram of the total population covered in the survey and the suspected cases identified after clinical examination. It also describes the category of the patients after ultrasound examination.

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The median age of the participants was 17 years (interquartile range [IQR], 8 to 34). The maleto female ratio of the respondents in the population of the study was 1.0:1.0. The sex ratio of the cases was male to female; 0.9:1.0. Only 36.2% experienced pain, and 35.7% had a family history of mycetoma. Students in the age group of 10–20 years were the commonest and constituted 19.2% of mycetoma cases, followed by farmers and shepherds (17.5%). (Table 1)

Most of the lesions were on the lower extremities (68.2%), followed by the upper extremities (26.2%), and 1.7% of patients had multiple lesions at different sites. Females had more lesions in the upper extremities than males with a percentage of (58.5%). (Fig 3)

Most of the patients (80.8%) had swelling, 38.4% had sinuses, 33.9% had discharging sinuses only following previous surgical excisions, 32% had grains discharge, and 97.4% of the grains were black.

The study showed that most houses, 334 (93%), had soil or sand floors, and most of them (83%) had roofs made of traditional material such as tree branches and palm leaves. Surrounding walls, when present, were made of mud and animal dung (29.8%), red bricks and concrete (27.7%) or tree branches (8.6%), and 33.7% had no surrounding walls.

More than half (51%) of the mycetoma patients owned animals, and 34.8% raised animals within the household. The study showed most of the cases (64.9%) practised arable farming while only 29.5% practised animal grazing (rearing).

The overall mycetoma prevalence was 0.87% (95% CI = 0.78–0.97%), the prevalence among males was 0.83% (95%CI = 0.71–0.96%), and among females was 0.92% (95% CI = 0.79– 1.06%). The mycetoma prevalence was highest in the age group 31–45 years (1.52%, 95%

Table 1. The demographic features of mycetoma cases seen in Eastern Sennar Locality, Sennar State, Sudan. (N = 359 mycetoma cases and 41,176 surveyed individuals).

Variable	No. (%)
Visible swelling	
Yes	290 (80.8%)
No	69 (19.2%)
Visible sinuses	
Yes	138 (38.4%)
No	221(61.6%)
Discharge	
Grains	115 (32%)
Fluid, pus, blood	7 (1.9%)
No discharge	237 (66%)
Grains	
Black	112 (97.4%)
White	3 (2.6%)
Site of the lesion	
Upper extremities	94 (26.2%)
Lower extremities	245 (68.2%)
Head, neck, trunk, back and perineal area	14 (3.9%)
Multiple sites	5 (1.7%)
Pain	
Yes	130 (36.2)
No	229 (63.8%)
Family History	

Yes	128 (35.7%)
No	231 (64.3%)

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CI = 1.23–1.86%) followed by the age group 16–30 years (1.11%, 95% CI = 0.93–1.33%). Most of the males with mycetoma were in the younger age groups compared to females. (Fig 4) Married and illiterate individuals had a higher mycetoma prevalence (1.34%, 95% CI = 1.16–1.53%) and (1.24%, 95% CI = 1.04–1.48%) respectively. Prevalence was higher among individuals with a history of trauma (1.63%, 95% CI = 1.36–1.95%). Wearing shoes did not affect the mycetoma prevalence as individuals who wore shoes most of the time had a prevalence of 0.94% (95% CI = 0.84–1.06), while individuals who wore shoes either at home or work only had a prevalence of 0.74% (95% CI = 0.50–1.08). (Table 2)

Geographical distribution of mycetoma

The study included sixty villages distributed among all the administrative units of the locality.

Doba had the highest prevalence of mycetoma among all the administrative units (1.14%), and

Elreif Elshargi had the lowest (0.15%). The highest village prevalence was recorded in Awlad El-Tai village (6.2%), followed by Wad Yagoub (4.9%), and the lowest prevalence was estimated in Kasab Garbi (0.11%). There were nine villages with no cases and an average of seven cases per village across the other 51 surveyed villages (range 1–39). The mycetoma prevalence map showed cases clustered within the central and north-eastern part of the locality, while the south-western part had few or no cases. (Fig 1) In the figure, more than nine villages have a prevalence of 0.00, and that was because eight villages recorded only one case.

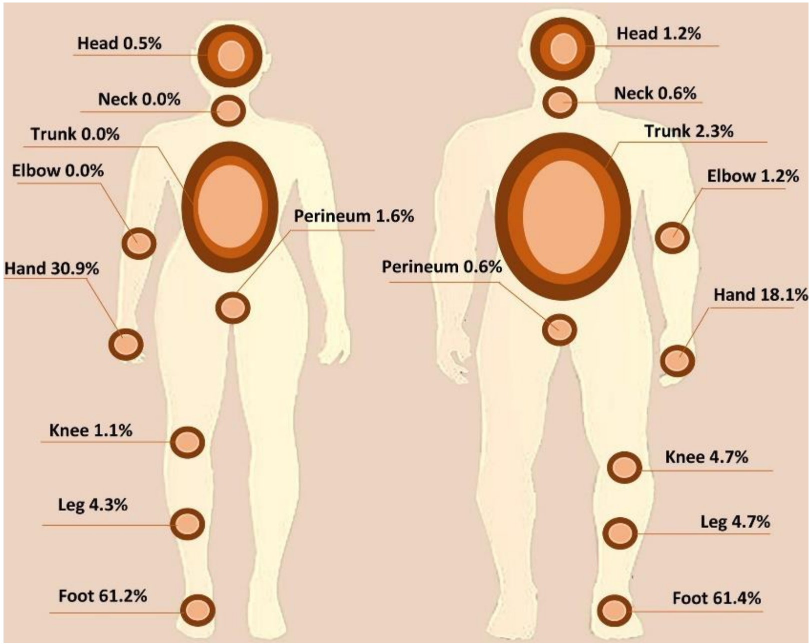

Fig 3. Physical distribution of mycetoma lesions for female (left side) and male (right side) cases. The feet constituted more than 60% of the sites of the lesion in both males and females. The fewest lesions were recorded on the neck and perineum for males and on the neck, trunk, and elbows.

https://doi.org/10.1371/journal.pntd.0009847.g003

Discussion

Mycetoma is one of the neglected tropical diseases that is increasingly recognised by the international scientific and funding communities. Most of its epidemiological characteristics are an enigma [18]. Globally, its incidence and prevalence are not well known. Furthermore, the infection route, incubation period and factors contributing to susceptibility and resistance to mycetoma are not well documented [19]. This is due to a lack of international attention, research funding, and interested institutes to work on mycetoma, leading to a scarcity of data on the disease's basic epidemiological features and its seriousness and magnitude, promoting the negligence cycle. Furthermore, due to the patients' low socio-economic and health education levels, the painless and slow-progressing nature of the disease, the lack of health facilities in endemic regions and the patients' inability to reach central hospitals for management, they tend to present late with advanced disease [3]. Hence, most of the mycetoma epidemiological characteristics were obtained from case reports and a series of hospital patients with advanced disease, representing the tip of the iceberg. The present study is distinctive as it is community-

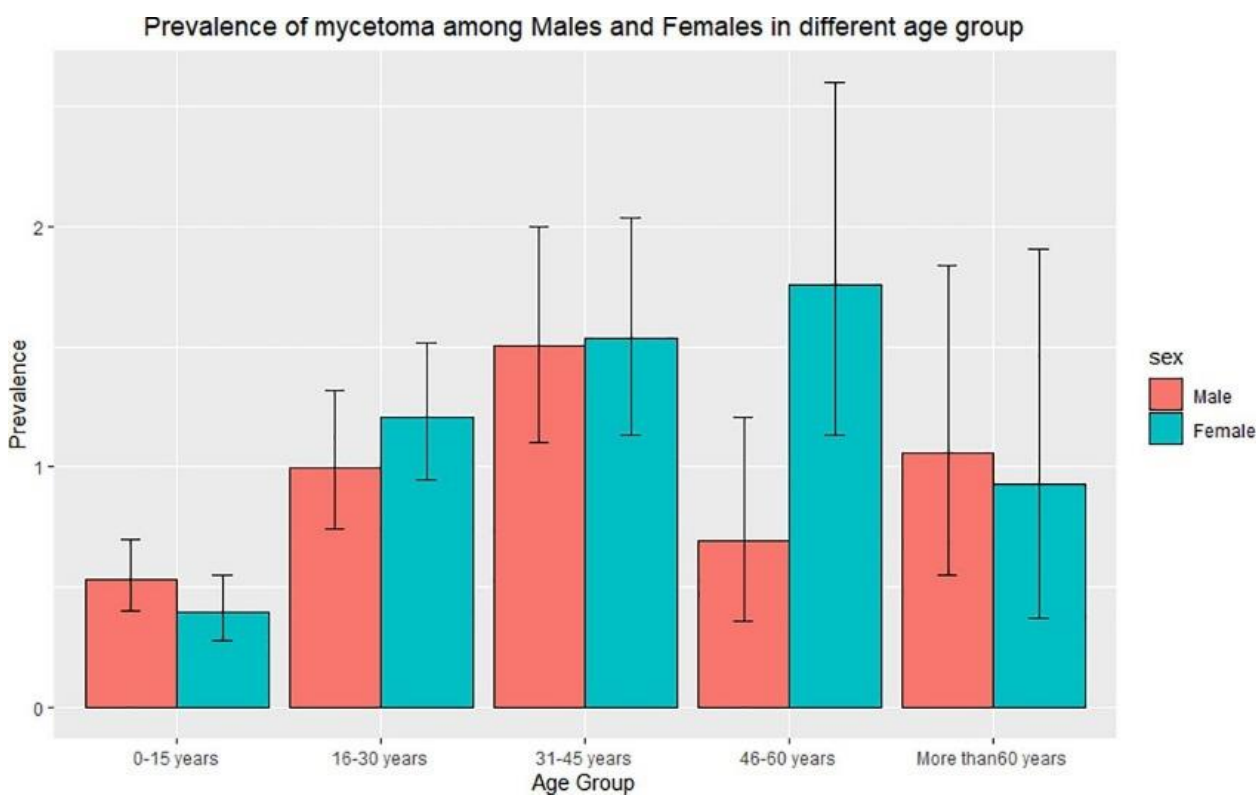

Fig 4. Bar plot of the prevalence of mycetoma among different age groups according to gender. Males had a higher prevalence among the age groups 0–15 years and more than 60 years, and females recorded a higher prevalence among the other age groups.

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based, and multi-level data were collected to determine the mycetoma clinical epidemiological characteristics in the study area.

This study documented a mycetoma prevalence of 0.87% in the studied locality, higher than previous Sudan estimates [20]. Abbott studied individuals who managed to reach health facilities for diagnosis and reported a disease prevalence of 0.51% among hospital patients seen in Khartoum during a study period of 36 months. In addition to higher prevalence in Atbara, Ed Dueim, and Wad Madani cities (within central Sudan states) with estimates of 0.92%, 0.93% and 1.18%, respectively and still the reported prevalence could have underestimated the actual burden of disease [14]. The reported prevalence (1.45%) by Fahal and associates in 2014 is higher than that reported in the present study. That could be attributed to the high endemicity of mycetoma in the village where that survey was conducted [15].

Van de Sande in 2014 conducted a systemic review in an attempt to determine the global burden of mycetoma, reviewing 8,763 cases from different countries around the world, and estimated the prevalence for endemic countries such as Sudan and Mexico to lie between

0.0015 and 0.018 cases per 1000 inhabitants [20].

Previous studies suggested that the distribution of mycetoma is affected by environmental and climate factors [21]. A modelling study predicted mycetoma occurrence in central and south-eastern states of Sudan and along the Nile river and indicated that arid areas proximal to water sources, soil with low concentrations of calcium and sodium and areas with a variety of thorny tree species provided the most suitable environment for the occurrence of mycetoma in Sudan [22].

Our results showed that mycetoma is prevalent in the central and north-eastern locality, with Doba administrative unit having the highest prevalence recorded. The local environmental and sanitation conditions and may explain this geographical distribution [8,17]. People in those areas mainly work in farming and with animals, are exposed to organisms residing in

Table 2. Prevalence mycetoma patients (N = 359) among the studied individuals (41,176) in Eastern Sennar locality, Sennar State, Sudan.

Variable	Cases	Population	Prevalence %(95%CI)
Gender			
Male	172	20753	0.83 (0.71–0.96)
Female	187	20423	0.92 (0.79–1.06)
Age group			
0–15 years	90	19227	0.47 (0.38–0.57)
16–30 years	121	10859	1.11 (0.93–1.33)
31–45 years	93	6111	1.52 (1.23–1.86)
46–60 years	36	3094	1.16 (0.82–1.61)
>60 years	19	1885	1.01 (0.61–1.57)
Marital Status			

Currently married	198	14830	1.34 (1.16–1.53)
Currently unmarried	161	26346	0.61 (0.52–0.71)
Education			
Literate	208	23805	0.87 (0.76–1.00)
Illiterate	124	9976	1.24 (1.04–1.48)
Underage of school	27	7395	0.37 (0.25–0.53)
History of trauma			
Yes	123	7527	1.63 (1.36–1.95)
No	236	33649	0.70 (0.61–0.80)
Wearing shoes/ slippers			
Both work and home	264	28027	0.94 (0.84–1.06)
At work or home only	27	3647	0.74 (0.50–1.08)
Not at all	68	9502	0.72 (0.56–0.91)

CI = Confidence interval

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soil and lack access to good sanitation. These factors are all likely to promote susceptibility to mycetoma.

Male predominance is a documented feature of mycetoma. In most studies, the reported male/female ratio is 3–4: 1 [23,24]. In this study, the disease prevalence was slightly higher in females, contradicting all the previous reports. The prevalence reported here may be more accurate as it is a community-based study, and most of the previous studies were hospitalbased. Females are less likely to seek medical treatment and often present late, suggesting previous studies may have been biased by female health-seeking behaviour [4,19].

In this study, individuals in the age group 16–45 years were most affected. This concurs with the literature, and the results are not surprising given that this is the most active group in society and is often involved in farming and animal grazing practices [25–27]. A fifth of the affected individuals were students who help with farming and animal care during vacations. Also, students have to walk long distances to and from schools in the rural communities and hence are more exposed to the environment.

The medical literature documented a high incidence of mycetoma among farmers and labourers. It was postulated that the direct and continuous contact with the environment and soil where the causative organisms reside and minor trauma and thorn pricks are important disease predisposing factors [9]. In the present study, arable farmers had a higher prevalence of mycetoma compared to shepherds. Furthermore, individuals with a history of local trauma and thorn pricks had a higher mycetoma prevalence, which is in line with the reported studies

[21,28,29].
Several studies suggested a possible role of animal dung in causing mycetoma as some organisms were isolated from the dung, which may act as a reservoir for them [21,30–32], allowing the direct transmission to the human. Most of the individuals living in rural areas are in direct contact with animals such as cattle, donkeys, dogs, sheep and chickens. We found no strong evidence supporting a role for animals or their dungs in the development of mycetoma, which needs further in-depth study [19,31].

In this study, the disease duration ranged between one month and 40 years, with a mean duration of 4.5 years before presentation. A study conducted in West Bengal showed that the disease period could vary according to causative organisms such as Nocardia, Streptomyces, Actinomadura species, *and Madurella*. *grisea*. The least duration was three months before presentation for all organisms and three years *for Nocardia* and *Actinomadura species*, while *Madurella grisea* organisms reached up to nine years [24]. However, due to the painless nature of the disease, duration is subject to memory bias.

Mycetoma was reported to affect different body parts, but the foot and hand are affected the most [33]. In this study, the obtained data align with this: the lower extremities (67%) and upper extremities (22.6%) were most affected. This result is expected since men mostly work barefooted farming, allowing exposure to injury and inoculation with mycetoma-causative organisms. In this study, only 2.3% of male cases presented with trunk mycetoma, which could be attributed to the nature of rural residents' occupation that makes them prone to injuries in upper or lower extremities. In contrast, trunk mycetoma was recorded in 19% in a study conducted in Mexico for mycetoma patients recorded in mycological centres from 1958 to 2012 and 10% from a single centre study in patients recorded in the period between 1980 to 2013 as they consistently carry woods for domestic activities on their backs [34,35].

An interesting observation was noted in this study, that females have more hand mycetoma. This can be attributed to the fact that females are commonly responsible for cooking and getting wood from forests to be used as a fuel source and are therefore prone to minor hand injuries. Also, they are involved in different indoor activities such as cleaning the floor and removing animal dung from within the houses where the organisms possibly reside.

The triad of subcutaneous swelling, multiple sinuses and discharge that contain grains is pathognomonic of mycetoma [1,36,37]. In our study, 80.8% of the patients presented with swelling, 38.4% had sinuses, and 32% of these sinuses produced purulent and sero-purulent discharge with mostly black grains. Black grains are usually produced in the fungal form of the disease eumycetoma, and in Sudan, eumycetoma accounts for 60–70% of mycetoma cases and the same pattern is observed for patients in Eastern Sennar locality [15]. Only 3% had white grain discharge, which could be attributed to actinomycetoma, the bacterial type of mycetoma. Actinomycetoma is not widespread in Sudan but constitutes the majority of mycetoma cases in Central and South America [20,34].

Currently, there is no evidence-based preventive or control programme or notification system for mycetoma as most of its epidemiological characteristics are not well known. Wearing shoes is considered a preventative measure for mycetoma since mycetoma is most commonly seen in the foot and is believed to be contracted via injuries by sharp objects [28].

In this study, we found that wearing shoes did not affect the prevalence of mycetoma in the region, as people who wore shoes had almost the same prevalence as people that did not wear them. However, in mycetoma endemic regions, people frequently work in the fields and walk barefooted as the available shoes can be an obstacle to executing these activities and sometimes are regarded as a hindrance. Due to the hot weather in Sudan, people tend to wear light open shoes, which offer little foot protection.

Even though individuals living in endemic areas share the same environment and are exposed to similar risk factors, only a proportion develop mycetoma. This supports the hypothesis that genetic factors play a role in mycetoma. In our study, 35.7% of mycetoma patients had a family history of mycetoma. It is important to highlight that consanguineous marriage are common in rural areas where mycetoma is endemic, which may explain this observation. In previous studies, family history was strongly associated with disease recurrence; individuals with a positive family history might be more genetically susceptible to contracting the disease initially and getting recurrent disease [38].

In conclusion, this clinical epidemiological community-based study is the first of its kind to be reported. The disease prevalence reported here may be more accurate as it was generated from a large study population. It revealed an equal sex ratio which contradicts most of the previous reports. Other findings are in line with those reported previously. Further epidemiological studies are needed to determine mycetoma prevalence in Sudan and bridge the gaps in our understanding of the epidemiology of mycetoma, which is vital to design evidence-based control and prevention programmes. Furthermore, these surveys are also helpful in early case detection and treatment, health education, disease awareness, and advocacy, reducing the disease burden and improving the disease prognosis. However, implementing surveys in rural areas in Sudan could be difficult, particularly during the rainy seasons, and proper team training, good facilities and collaboration between different stakeholders are all required.

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Formal analysis: Rowa Hassan.

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Appendix 4 – Paper 2 Role of socioeconomic factors in developing mycetoma: results from a household survey in Sennar State, Sudan

PLOS NEGLECTED TROPICAL DISEASES

RESEARCH ARTICLE

Role of socioeconomic factors in developing mycetoma: Results from a household survey in Sennar State, Sudan

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Abstract

Background

Mycetoma is a chronic, progressively destructive disease of subcutaneous tissues and bones caused by certain species of bacteria or fungi. We conducted a cross-sectional community-based study alongside mapping of mycetoma in five administrative units with high mycetoma endemicity in the Eastern Sennar Locality, Sennar State, Sudan.

Methods

A household survey was administered which included questions about the household members, household characteristics, economic activity and history of mycetoma. A clinical examination was conducted on all members of the household. If mycetoma was suspected, an individual questionnaire was completed collecting demographic, clinical and epidemiological data as well as information on the use of health care and associated costs. Geographical coordinates and photos of the lesions were taken, and the affected persons were referred to the medical centre for confirmation of the diagnosis and treatment. We compared the char- acteristics of households with confirmed cases of mycetoma with those without confirmed cases, and individuals with confirmed mycetoma with those in whom mycetoma was not confirmed.

Results

In total 7,798 households in 60 villages were surveyed; 515 suspected cases were identified and 359 cases of mycetoma were confirmed. Approximately 15% of households with mycetoma had more than one household member affected by this disease. Households with mycetoma were worse off with respect to water supply, toilet facilities, electricity and electri- cal appliances compared to the survey households. Only 23% of study participants with mycetoma had sought professional help. Of these, 77% of patients travelled an average of six hours to visit a medical facility. More than half of patients had to pay towards their

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treatment. The estimated average cost of treatment was 26,957 Sudanese pounds per year (566 US dollars, exchange rate 2018).

Conclusions

Results of this survey suggest that agricultural practices and reduced access to sanitation and clean water can be risk factors in developing mycetoma. Poor access to health care and substantial financial costs were barriers to seeking treatment for mycetoma.

Author summary

Mycetoma is a neglected tropical disease caused by certain species of bacteria or fungi. The pathogens enter the body through a break in the skin and infect underlying soft tis- sues, leading to tissue destruction, loss of function, amputations and disability. Early diag- nosis of mycetoma requires a clear understanding of disease prevalence, distribution and the contributing risk factors. In this study, we describe the results from a cross-sectional community-based study conducted in five administrative units with high mycetoma endemicity in the Eastern Sennar Locality, Sennar State, Sudan. 7,798 households in 60 villages were randomly selected across the entire locality. A household survey was admin- istered and household members underwent clinical examination. In case of suspected mycetoma, geographical coordinates and photos of the lesions were taken, further demo- graphic and clinical data were collected as was information on the use of health care and associated costs, and the individual was referred to the medical centre for confirmation of the diagnosis and treatment. This study aimed to identify socioeconomic risk factors con- tributing to mycetoma. We compared the characteristics of the households with con- firmed

Introduction

Mycetoma is a neglected tropical disease caused by certain species of bacteria and fungi. It is endemic in many tropical and subtropical countries such as Sudan, Somalia, Senegal, India, Yemen, Mexico, Venezuela, Columbia and Argentina [1,2]. The pathogens enter the body through a break in the skin and infect underlying soft tissues, muscles and bones leading to tis- sue destruction, deformity, loss of function, amputation, disability and occasionally death. The disease is mainly painless in the early stages, therefore many patients present late to health care facilities with advanced infection. The foot and hand are the most frequently affected body parts, accounting for 82% of cases [3]. Socioeconomic factors are thought to play an important role in developing mycetoma. Mycetoma occurs more frequently in young working-age adult men (20–40 years) who practice agriculture barefooted [4]. The pathogens causing mycetoma are present in soil and water [5,6]. Cattle dung has also been suggested as a source of myce- toma pathogens [7]. The infection may result from inoculation via small injuries and thorn pricks [4,6]. In Sudan, animal enclosures made of thorny branches are frequently used to pro- vide a safe place for livestock [8]. Areas harbouring *Acacia mellifera (*currently *Senegalia* *mellifera)* have been shown to overlap with the spatial distribution of mycetoma [9]. Popula- tions living in endemic areas are advised to wear protective shoes, clear thorny bushes and keep animals in special enclosures [5,8].

Although the epidemiology of mycetoma is not fully understood, the disease can be effec- tively managed if diagnosed early. The diagno- sis of mycetoma is based on clinical presentation, imaging studies and identification of the causative organisms in relevant clinical samples taken from affected tissues using fine-needle aspiration, or surgical biopsy. Microscopy and cytologi- cal, histopathological, immunohisto- chemical and molecular techniques based on the polymer- ase chain reaction (PCR) are applied to these samples [10]. Imaging including X-ray, ultrasound, MRI and CT scan examinations may be required to characterise the spread and extent of disease. These costly diagnos- tic modalities are only available in specialised medical centres located in big cities, which are not easily accessible to rural populations for a variety of reasons including the lack of infrastructure (e.g. roads that are unpassable during the rainy sea- son) and cost of transport. Per- sonal costs associated with the diagnosis and treatment of myce- toma are largely unknown. Religious beliefs and the use of traditional medicine by people with mycetoma are additional barriers to timely access to medical care [11].

Early diagnosis of mycetoma requires a clear understanding of disease prevalence, distribu- tion and the contributing risk factors. A spa- tial geographical distribution study that mapped all cases of mycetoma presented to the Mycetoma Research Centre in Khartoum from the East- ern Sennar Locality, Sennar State, Sudan between 1991 and 2001 showed that most cases were located in the southern part of the locality along the Blue Nile river valley and its tributaries [12]. A modelling study of the spatial distribution of mycetoma among patients attending the Mycetoma Research Centre (University of Khartoum) between 1991 and 2018 suggested that aridity, proximity to water, low soil calcium and sodium levels, and the distribution of various species of thorny trees are strong predictors of the occurrence of mycetoma [13].

Here we report the results from the cross-sectional population survey conducted alongside spatial mapping across all five highly endemic administrative units of Eastern Sennar Locality, Sennar State, Sudan in 2019 [14]. The study estimated the overall prevalence of myce- toma to be 0.87% with cases clustered within the central and north-eastern part of the locality. The prevalence was highest in the 31–54 years old, and illiteracy, being married and having a his- tory of skin trauma and thorn pricks were associated with mycetoma [14].

This study aimed to identify the socioeconomic factors contributing to mycetoma. Our objectives were: i) to compare characteristics of households with cases of mycetoma with those without cases, and ii) to compare individuals with confirmed mycetoma with those in whom mycetoma was not confirmed.

Methods

Ethics statement

Ethics approval for this study was obtained from the Mycetoma Research Centre, Khartoum, Sudan Institutional Review Board (Approval no. SUH 11/12/2018) and from the Brighton and Sussex Medical School Research Governance and Ethics Committee (ER/BSMS435/1). Written informed consent was obtained from each adult patient and parents or guardians of the popu- lation under 18 years old. Con- firmed mycetoma cases were referred for management at Wad Onsa Regional Mycetoma Centre or the Mycetoma Research Centre (MRC).

Study characteristics

A detailed description of the survey is published elsewhere [14]. Briefly, a cross-sectional com- munity-based survey was conducted in June-July 2019 in the Eastern Sennar locality, Sennar

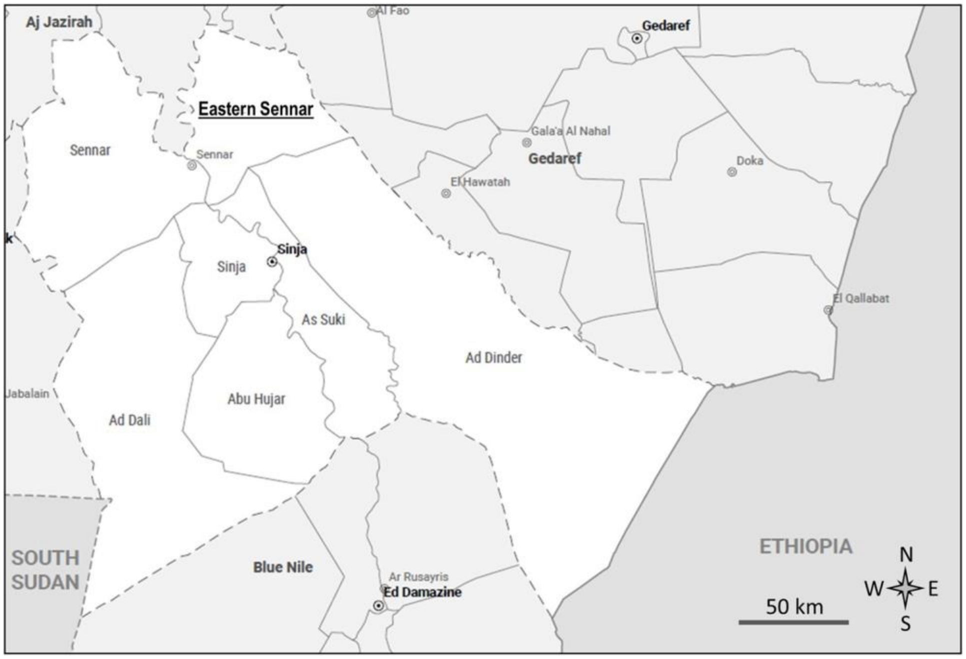

Fig 1. Administrative map of Sennar State, Sudan [15].

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State, Sudan (Fig 1). The research area covered five administrative units with high mycetoma endemicity: Doba, El-Reif El-Shargi, Wad al Abbas, Wad Onsa and Wad Taktok. Sixty villages were randomly selected across the entire locality to participate in the survey. The sample size was calculated based on an estimated prevalence of 5 cases of mycetoma per 10,000 population, an average population size of the villages of 935 people (average household size of 7), the popu- lation of Eastern Sennar locality of 353,196 people, design effect of 2.5, the precision of 3 per 10,000 population and a community participation rate of 80%. To obtain a 95% confidence interval with a half-width of 3 per 10,000 population around an estimated prevalence of 5 per 10,000 requires 21,332 participants (53,330 upon inflating by the design effect of 2.5). Inflating this further for the anticipated 80% participation rate gives a total sample size of 66,663. Apply- ing the finite population correction factor, nN/(n+N-1) (where N is population size 353,196 and n is the sample size 66,663) this becomes 56,079 which is rounded up to 56,100 i.e. 60 vil- lages of 935 people.

Survey population

The survey population included mainly subsistence farmers and their family members. All household members for whom informed consent was obtained were included in the survey. Written informed consent was obtained from the head of the household and verbal consent was obtained from each individual before the examination. There were no restrictions con- cerning age, sex, or health status.

Survey process

A survey coordinator was appointed in each administrative unit who was responsible for the overall leadership, management, liaison and compliance of the project. A field logistics coordi- nator was responsible for moving the survey team (which included a coordinator, a doctor, and a medical assistant) between the villages, transporting suspected cases to Wad Onsa

Mycetoma Centre for the confirmation of diagnosis, and providing study supplies. The coordi- nators were responsible for locating the targeted villages, making pre-survey arrangements, sensitizing regional leaders, explaining the objectives of the survey, community sensitization and mobilization. The data collectors were health assistants and doctors in the area who were responsible for case detection, data collection, and referral to the Wad Onsa Regional Myce- toma Centre. They were trained on field procedures, use of tablets, data entry and identifica- tion of mycetoma lesions. The coordinators visited the targeted villages before the actual day of the survey to accomplish the following tasks: to inform local leaders and community repre- sentatives about the objectives of the survey and to get permission, and to select a suitable day for the survey in consultation with the community members. A community member from each village was selected to assist the survey.

Data collection

On the day of the survey, the team members visited the targeted household and provided a brief introduction to the head of the household and the household members about the purpose of the study. When one of the household members was missing during the survey administra- tion the household was visited again up to three times. Written consent was taken from the head of the household. For illiterate individuals, a fingerprint was obtained. The data collectors started with the household survey including questions about the household members, house- hold characteristics and economic activities (see the section below for more details). They also asked questions about the history of skin trauma and family history of mycetoma. After indi- vidual verbal consent was given, a clinical examination was conducted on all members of the household. For cases of suspected mycetoma (those with swelling of any part of the body or sinus formation with or without grain discharge), an individual questionnaire was completed asking about the disease symptoms, contacts with the environment and actions taken toward managing the disease. Geographical coordinates, as well as photos of the lesions, were col- lected. The individuals who were suspected to have mycetoma were transported to the regional Wad Onsa Mycetoma Cente by the study team for the confirmation of the diagnosis and treat- ment. They were provided a barcoded form which was scanned to link the questionnaire data with the results of subsequent investigations. The diagnosis was based on a combination of clinical and ultrasound examinations. Patients with grains, capsules, and the accompanying inflammatory granuloma on ultrasound scans in addition to clinical presentation (swelling and sinus formation with or without grain discharge) were classified as individuals with "con- firmed mycetoma". Those who presented with swelling or sinus formation but had negative ultrasound examinations were classified as individuals with non-confirmed mycetoma. Those in whom mycetoma diagnosis was not confirmed were referred for treatment to the nearest medical facility.

Questionnaires

The household questionnaire included the personal details of the head of the household, the number of household members; the number of rooms in the household; types of materials used for the floor, walls and roof; type of cooking fuel used; toilet facilities; waste management; availability of electricity, drinking water and household appliances; vehicle ownership; land ownership, agricultural activities including growing crops, animal farming including types of animals, and animal shed location. Each household was assigned a unique identification num- ber that could be linked to the individual questionnaires completed for all household members with suspected mycetoma.

The individual questionnaires collected information on age, place of birth; animals owned by the household; cultivated plants; species of trees in the vicinity of the household; the season of acquiring the disease, its duration and symptoms; the presence of other mycetoma cases in the household, contacts with health care professionals and associated costs; medication; hospi- talisations; contact with traditional healers; money borrowed; the number of days unable to work because of the disease; and the number of days when help with household chores was required. The questionnaires were administered in Arabic.

All questionnaires were completed using tablet computers, checked for completeness and validity, and then data were transferred to the main server. Each questionnaire had a unique automatically generated identification number which was linked to the barcode on the referral form to the Wad Onsa Mycetoma Center.

Data analysis

Data analysis was conducted in SPSS 25 (SPSS, Chicago, IL, USA) and Stata 17 (StataCorp, 2021). The questionnaire data were anonymised, checked for misspellings, and coded if required. The data from the individual questionnaire were merged with the data from the household questionnaire using the household identification number to enable comparisons of households with confirmed cases of mycetoma with households without confirmed cases. At the point of data analysis, the mycetoma diagnosis was not confirmed for 23 survey partici- pants (21 of whom were traveling outside Sudan and two others who died). Therefore, the fol- lowing analytical approaches were used:

1. The primary comparisons were conducted between the households with cases of mycetoma with those without cases, and individuals with confirmed mycetoma with those in whom mycetoma was not confirmed.
2. Additional analyses were conducted using imputed datasets to compare individuals with confirmed and non-confirmed mycetoma. Multiple imputations of missing diagnoses were conducted for the dataset including 23 participants who were lost to follow-up. Missing diagnoses were assumed to be missing at random. Five datasets were imputed for further analysis. Covariates included in the imputations were: the presence of swelling, sinuses and/or discharge, history of skin trauma, family history of mycetoma, and practices such as arable farming and animal grazing.

The statistical significance of differences between the samples was tested using mixed- effects models to adjust for the clustering effect. The household-level data were analysed using the "administrative unit" variable as a fixed effect and "village" as a random effect. The individ- ual-level data were analysed using variables "village" and "household" as first- and second- level random effects. The models were compared using the likelihood-ratio test in Stata 17.

Multivariable analysis was used for the multiple-choice questions.

Opportunity costs were calculated using the minimum monthly wage of 425 Sudanese pounds (SDG) [16] and 246 working days (2018). Costs were converted to US dollars (USD) using the exchange rate of 1 USD/47.65 SDG (December 31, 2018) [17].

Results

Socioeconomic characteristics of survey households

The data on household characteristics were collected from 7,798 households in 60 villages. A summary of the household characteristics is provided in Table 1. The number of people in a

Table 1. Socioeconomic characteristics of survey households (n = 7,798).

(*Continued*)

	Characteristics	n (%) unless indicated
1	**Number of surveyed households,** n = 7,798	
	Doba	1,397 (17.9)
	El-Reif El-Shargi	1,183 (15.2)
	Wad al-Abbas	463 (5.9)
	Wad Onsa	1,809 (23.2)
	Wad Taktok	2,946 (37.8)
2	**Number of people in household**, n = 7,798 mean (SD); median; min-max	5.6 (2.7); 5; 1–35
	1–3	1,784 (22.9)
	4–6	3,332 (42.7)
	7–10	2,375 (30.5)
	11 and more	307 (3.9)
3	**Number of rooms in household,** n = 7,797 mean (SD); median; min-max	1.9 (1.1); 2; 0–13
	0–2	6,159 (79.0)
	3–4	1,430 (18.3)
	5 and more	208 (2.7)
4	**Wall materials,** n = n = 7,798	
	brick	2,664 (34.2)
	concrete	212 (2.7)
	mud/dung	3,306 (42.4)
	stone	57 (0.7)
	wood	885 (11.3)
	no walls	2,122 (27.2)
5	**Floor materials,** n = 7,798	
	brick	30 (0.4)
	ceramic	174 (2.2)
	earth/sand	7,537 (96.6)
	other (e.g. plastic cover)	455 (5.8)
6	**Roof materials,** n = 7,798	
	cardboard	12 (0.2)
	concrete	2,045 (26.2)
	plastic	108 (1.4)
	traditional	6,331 (81.2)
	wood	467 (6.0)
	zinc	743 (9.5)
7	**Cooking fuel,** n = 7,798	
	animal dung	169 (2.2)
	coal	3,144 (40.3)
	gas	3,234 (41.5)
	electricity	34 (0.4)
	wood/plants	6,093 (78.1)
	Balanites aegyptiaca[*]	159 (2.6)
	Capparis decidua[*]	157 (2.6)
	Eichhornia azurea	74 (1.2)
	Faidherbia albida[*]	7 (0.1)
	Gossypium barbadense	1,419 (23.3)

Table 1. (Continued)

	Characteristics	n (%) unless indicated
	Henlianthus annuus (sunflower)	419 (6.9)
	Indigofera oblongifolia	410 (6.7)
	Lens culinaris (lentil)	87 (1.4)
	*Prosopis chilensis**	242 (4.0)
	Sorghum bicolor (sorghum)	2,981 (48.9)
	*Vachellia nilotica**	3,872 (63.5)
	*Vachellia nubica**	684 (11.2)
	*Vachellia seyal var. fistula**	1,161 (19.1)
	*Vachellia seyal var. seyal**	1,134 (18.6)
	*Ziziphus spina-christi**	704 (11.6)
8	**Water supply,** n = 7,798	
	piped	4,862 (62.3)
	tank	1,803 (23.1)
	well	1,905 (24.4)
	other (e.g. canal, river)	198 (2.5)
9	**Water purification,** n = 600	
	adding bentonite clay	396 (66.0)
	settling	192 (32.0)
	straining	11 (1.8)
	other (boiling, adding chemicals)	10 (1.7)
10	**Toilet facility,** n = 7,798	
	flush toilet	55 (0.7)
	pit latrine	3,328 (42.7)
	ventilated pit latrine	267 (3.4)
	no facility	4,141 (53.1)
11	**Waste disposal,** n = 7,798	
	burning	2,247 (28.8)
	burying	26 (0.3)
	throwing in a designated place	6,898 (88.5)
12	**Electricity,** n = 7,798	5,394 (69.2)
13	**Mobile phone,** n = 7,798	5,934 (76.1)
14	**Radio,** n = 7,798	1,711 (21.9)
15	**Refrigerator,** n = 7,798	2,162 (27.7)
16	**Television,** n = 7,798	3,419 (43.8)
17	**Transport,** n = 7,798	
	animal-drawn cart	1,965 (25.2)
	car or truck	844 (10.8)
	raksha	106 (1.4)
	No transport	5.218 (66.9)
18	**Agricultural land ownership,** n = 7,798	4,561 (58.5)
	Doba, n = 1,397	1,056 (75.6)
	El-Reif El-Shargi, n = 1,183	550 (46.5)
	Wad al-Abbas, n = 463	233 (50.3)
	Wad Onsa, n = 1,809	1,089 (60.2)
	Wad Taktok, n = 2,946	1,633 (55.4)
19	**Animal ownership**, n = 7,798	3,766 (48.4)

(*Continued*)

Table 1. (Continued)

	Characteristics	n (%) unless indicated
	Doba, n = 1,397	809 (57.9)
	El-Reif El-Shargi, n = 1,183	596 (50.4)
	Wad al-Abbas, n = 463	195 (42.1)
	Wad Onsa, n = 1,809	1,001 (55.3)
	Wad Taktok, n = 2,946	1,165 (39.5)
20	**Farm animals,** n = 3,776	
	camels	31 (0.8)
	chicken	835 (22.1)
	cows	985 (26.1)
	donkeys	870 (23.0)
	ducks	7 (0.2)
	goats	2,838 (75.2)
	horses	7 (0.2)
	pigeons	88 (2.3)
	rabbits	4 (0.1)
21	**Other animals,** n = 239	
	cats	20 (8.4)
	dogs	219 (91.6)
22	**Animal shed ownership,** n = 7,798	3,193 (40.9)
23	**Animal shed location,** n = 3,193	
	shed inside house	2,853 (89.4)
	shed outside house	828 (25.9)
	near house	471 (14.8)
	away from house	357 (11.2)

*thorny plant species. The percentages were rounded to one decimal place. Questions 4–11, 17, 20–22 and 23 were multiple-choice questions; the sum of percentages may exceed 100%.

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household varied from 1 to 35 with an average number of 5.6. The number of rooms in the household varied from 0 to 13. Most of the dwellings (79.0%) had 1–2 rooms.

Dwellings

The walls of the dwellings were made of various materials including mud and animal dung (42.4%), brick (34.2%) and wood (11.3%). A small proportion of dwellings had concrete (2.7%) or stone walls (0.7%). Approximately 27% of dwellings had no built walls (semi-perma- nent structures). Nearly all (96.6%) of dwellings had earth or sand floors in at least a part of the house. Other materials such as bricks, ceramic tiles and plastic covers were occasionally used as flooring materials. The majority of roofs (81.2%) were of traditional style made of sticks, thatch and mud. Other roof materials included concrete (26.2%), zinc (9.5%), plastic (1.4%) and cardboard (0.2%).

Cooking fuel

Wood/plants were used as cooking fuel in 78.1% of households; 41.5% of households used gas, 40.3% coal, 2.2% animal dung and 0.4% electricity. More than half of households (58%) relied on more than one source of cooking fuel. Wood was the sole source of cooking fuel in 40% of households.

Water supply

Piped water (from a central pipe in the village) was available in 62.3% of households, well water in 24.4%, tank water in 23.1% of households and 2.5% of households used river water. Approximately 36% of households used water from multiple sources. Only 7.7% of households purified water for drinking. The most common purification methods were adding bentonite clay to absorb impurities [18] and settling. Straining, boiling and adding chemical agents (chlorine and alum) were rarely used (<1%).

Sanitation

Approximately half of the surveyed households (53.1%) had no toilet facilities; 42.7% had pit latrines, 3.4% ventilated pit latrines, and 0.7% had flush toilets.

The main approach to waste disposal was throwing in a designated place (88.5%), followed by burning (28.8%) and burying (0.3%). Approximately 13% of households combined different ways of waste disposal.

Electricity and household appliances

Electricity was available in 69.2% of households, television in 43.8%, radio in 21.9%; refrigera- tor in 27.7% and mobile phone in 76.1% of households.

Transport

Vehicles were owned by 33.1% of households. These were animal-drawn carts (25.2%), cars/ trucks (10.8%) and "rakshas"—three-wheeled motorised rickshaw-type vehicles (1.4%).

Agricultural activities

Overall, approximately 58.5% of households owned agricultural land, varying from 46.5% in El-Reif El-Shargi to 75.6% in Doba. Farm animals were raised by 48.4% of households, from 39.5% in Wad Taktok to 57.9% in Doba. The commonest farm animals owned were goats, cows, donkeys, chickens, pigeons, camels, horses, rabbits and ducks. Approximately 41% of households had animal sheds. The majority of sheds were inside houses (89.4%). Outside sheds were located near the houses (14.8%) or further away from the houses within the bound- aries of the village (11.2%) (Table 1).

Comparison of households with cases of mycetoma with households without cases

Data for 7,206 households were available for comparative analysis (identification numbers allowing to link household-level data to individual-level data were missing for 592 house- holds). Out of 7,206 households, 399 households with suspected mycetoma were identified, and for 288 households the mycetoma diagnosis was confirmed. Out of these, 234 households had one mycetoma case per household; 38 households had two cases; 10 households had three cases; four households had four cases, and two households had six cases. Table 2 shows a com- parison between the households with confirmed cases of mycetoma and households without confirmed cases. The proportion of households with mycetoma varied among the survey areas from 2.3% in Wad Taktok to 11.9% in Wad al-Abbas. There were no significant differences between the households with confirmed cases of mycetoma and households without con- firmed cases with respect to the number of people in the household, or the number of rooms for living. The proportion of households with piped and well water was lower in the mycetoma group (Table 2). Households with mycetoma were more likely to use tank water. Toilet facilities were unavailable in 66.3% of households with cases of mycetoma compared to 55.0% in households without cases. There were some differences in waste disposal between the two groups with burning and burying used more often in households with cases of mycetoma. The latter households were less likely to have electricity and electrical appliances (radio, refrigerator and television) compared to the households without mycetoma cases, although these differ- ences were not statistically significant. Ownership of agricultural land, farm animals and ani- mal sheds did not differ significantly between the two groups (Table 2).

Health care costs of patients with mycetoma

Table 3 summarises the use of health care services and associated costs, money borrowing and daily activities by people with confirmed mycetoma over the past year. The majority of survey participants with mycetoma (76.9%) did not seek professional help with respect to their dis- ease. Out of 83 patients who contacted health care professionals, 75 had an appointment with a doctor, 7 with a medical assistant, and one patient was seen by a nurse. The average cost of contacts with health care professionals was 7,271 SDG (153 USD) per person per year. Approx- imately 77.1% of these patients (64/83) had to travel outside their village to see a health care specialist. On average, they spent six hours traveling to a medical facility. The majority of these patients (82.8%, 53/64) had to pay for their travel. A third of these patients were accompanied by another person. Approximately 5.8% of patients with mycetoma had been hospitalised in the previous year, and more than half of them had additional expenses such as food or over- night stay for the accompanying person. The average out-of-pocket expenses associated with hospitalisation were 8,182 SDG (172 USD) per person.

Only 3.9% of people with mycetoma received medication with an average cost of 9,369 SDG (197 USD) per year. Traditional healers were attended by 10.6% of patients, with an aver- age cost of 1,048 SDG (22 USD) per year. Approximately 7.8% of families had to borrow money from relatives or the community to meet their needs. The average amount of borrow- ings was 4,814 SDG (101 USD) per year. The estimated average cost of treatment was 26,975 SDG (567 USD) per year.

Approximately 15% of survey participants with mycetoma reported a reduction in daily activities due to their disease. They were completely unable to work on average for 73 days a year. The opportunity cost of illness estimated using the minimum monthly wage of 425 SDG

[16] was 1,513 SDG (32 USD) per year. Approximately 10.3% of patients required help with everyday chores on average 65 days per year with an opportunity cost of 1,348 SDG (28 USD). Approximately 5.3% of patients were helped by children. (Table 3)

Comparison of individuals with confirmed mycetoma with individuals in whom mycetoma was not confirmed

Out of 515 survey participants with suspected mycetoma, 359 had their diagnosis confirmed. Mycetoma was excluded in 133 participants (other diagnoses in this group included foreign body granuloma, fibroma and lipoma). Twenty-three participants did not attend for further investigation to confirm the presence or absence of mycetoma. The proportion of people with confirmed mycetoma varied between the administrative units from 2.8% in El-Reif El-Shargi to 37.6% in Wad Taktok. Table 4 summarises the characteristics of participants with con- firmed and non-confirmed mycetoma. The average age of people with confirmed mycetoma was 30.0 (median 27) years, compared to 27.8 (median 25) years in those without mycetoma. The proportion of people with a family history of mycetoma was higher in the confirmed group (35.7%) than in the non-confirmed group (29.5%). The two groups were similar with respect to the number of household members (on average 5 people) and the number of rooms

Table 2. Comparison of households with confirmed cases of mycetoma and households without confirmed cases.

Characteristics	Households with mycetoma cases (n = 288)	Households without mycetoma cases (n = 6,918)	*p*-value
Number of surveyed households, n (%)			N/A
Doba	55 (4.4)	1,200 (95.6)	
El-Reif El-Shargi	31 (3.5)	850 (96.5)	
Wad al Abbas	55 (11.9)	408 (88.1)	
Wad Onsa	82 (4.7)	1,660 (95.3)	
Wad Taktok	65 (2.3)	2,800 (97.7)	
Number of people in household mean (SD); median; min-max	5.4 (2.4); 5; 1–14	5.5 (2.7); 5; 1–35	0.18
Number of rooms in household mean (SD); median; min-max	1.8 (1.0); 2; 1–6	1.9 (1.1); 2; 0–13	0.12
Water supply, n (%)			0.02
piped	159 (55.2)	4,236 (61.2)	
tank	102 (35.4)	1,685 (24.4)	
well	44 (15.3)	1,766 (25.5)	
other (e.g. river, canal)	2 (0.7)	212 (3.1)	
Toilet facility, n (%)			<0.01
flush toilet	1 (0.3)	47 (0.7)	
pit latrine	85 (29.5)	2,820 (40.8)	
ventilated pit latrine	11 (3.8)	236 (3.4)	
no facility	191 (66.3)	3,808 (55.0)	
Waste disposal, n (%)			0.03
burning	82 (28.5)	1,910 (27.6)	
burying	2 (0.7)	19 (0.3)	
throwing in a designated place	240 (83.3)	6,145 (88.8)	
Cooking fuel, n (%)			0.18
animal dung	8 (2.8)	160 (2.3)	
coal	96 (33.3)	2,788 (40.3)	
gas	118 (41.0)	2,784 (40.2)	
wood/plants	223 (77.4)	5,567 (80.5)	
other (e.g. electricity)	2 (0.7)	16 (0.2)	
Electricity, n (%)	184 (63.9)	4,788 (69.2)	0.19
Mobile phone, n (%)	224 (77.8)	5,220 (75.5)	0.17
Radio, n (%)	49 (17.0)	1,441 (20.8)	0.12
Refrigerator, n (%)	65 (22.6)	1,871 (27.0)	0.16
Television, n (%)	107 (37.2)	2,968 (42.9)	0.18
Transport, n (%)			
animal-drawn cart	90 (31.3)	1,759 (25.4)	
car or truck	32 (11.1)	730 (10.6)	
raksha	4 (1.4)	97 (1.4)	
no transport	178 (61.8)	4,634 (67.0)	
Agricultural land ownership, n (%)	164 (56.9)	3,999 (57.8)	0.16
Farm animal ownership, n (%)	143 (49.7)	3,304 (47.8)	0.19
Farm animals, n (%)			
camels	0 (0.0)	31 (0.4)	
chicken	27 (9.4)	725 (10.5)	
cows	43 (14.9)	833 (12.0)	
donkeys	34 (11.8)	816 (11.8)	
goats	107 (37.2)	2,520 (36.4)	

(*Continued*)

Table 2. (Continued)

Characteristics	Households with mycetoma cases (n = 288)	Households without mycetoma cases (n = 6,918)	*p*-value
pigeons	2 (0.7)	75 (1.1)	
other (ducks, horses, rabbits)	0 (0.0)	17 (0.2)	
Animal shed ownership, n (%)	121 (42.0)	3.193 (41.0)	0.19
Animal shed location, n (%)			0.18
inside house	98 (34.0)	2,236 (32.3)	
outside house	42 (14.6)	693 (10.0)	
near house	27 (9.4)	411 (5.9)	
away from house	15 (5.2)	275 (3.7)	

The percentages were rounded to one decimal place. For the multiple-choice questions, the sum of percentages may exceed 100%. *p*-values were derived using mixed- effects models with an administrative unit as a fixed effect and a village as a random effect. Multiple-choice questions were analysed using multivariable analysis.

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(on average 2 rooms). There were no statistically significant differences between the groups with regard to water supply, toilet facilities, waste disposal, utilities and appliances, vehicles and farm ownership.

More than half of the respondents in both groups were arable farmers. The most frequently cultivated crops were sorghum, wheat, cotton and millet. Other crops included sunflowers, sesame and various fruit and vegetables (e.g. tomatoes, cucumbers and watermelons). The pro- portion of people involved in agricultural activities was significantly higher in those with mycetoma (64.6%) compared to those who did not have mycetoma (54.9%). People with

Table 3. Use of healthcare services and associated costs, money borrowing and daily activities over the past year by people with confirmed mycetoma (n = 359).

Activity	Number of users n (%)	Paid for services n (%)	Cost, SDG mean (min-max),
Contacts with healthcare specialists	83 (23.1)	60 (16.7)	7,271 (5–100,000)
doctor	75 (20.9		
medical assistant	7 (1.9)		
nurse	1 (0.3)		
no contact	276 (76.9)	N/A	N/A
Travel to healthcare specialists	64 (17.8)	53 (14.8)	1,086 (2–10,000)
Hospital admissions	21 (5.8)	18 (5.0)	8,182 (250–29,000)
Medication	14 (3.9)	14 (3.9)	9,369 (60–50,000)
Contacts with traditional healers	38 (10.6)	25 (7.0)	1,048 (10–5,000)
Borrowed money	28 (7.8)	N/A	4,814 (250–30,000)
Daily activities			
number of people with reduced activities	53 (14.8)	N/A	N/A
number of people required help	37 (10.3)	N/A	N/A
number of people helped by children	19 (5.3)	N/A	N/A
number of days completely unable to work, mean (min-max)	73 (1–365)	N/A	1,513 (21–5,100)
number of days required help, mean (min-max)	65 (1–365)	N/A	1,348 (21–5,100)

Costs are rounded to the nearest Sudanese pound (SDG)

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mycetoma were more likely to practice agriculture regularly (23.4% versus 17.3%), although this difference was not statistically significant.

Over a third of respondents were involved in raising animals, goats, cows, donkeys, chick- ens, sheep and pigeons. There were no statistically significant differences between the two groups regarding the types of animals raised, although the overall livestock ownership was higher in the confirmed mycetoma group (42.3% vs 36.6%). Animal sheds were owned by 43.2% of households with mycetoma cases and by 46.2% of households without mycetoma cases. No statistically significant differences were found with respect to the shed ownership and location (inside/outside or near/away from homes). The majority of animal sheds were located inside the houses (34.8% in the confirmed group and 35.9% in the group without mycetoma)

The proportion of land with trees was higher in the confirmed mycetoma group (46.5% vs 33.3%). Thorny trees comprised 90% of species growing on the land. The proportion of thorny tree species in the vicinity of the household was also higher in the confirmed mycetoma group (42.9% vs 28.2%) (Table 4).

Comparison of imputed datasets for the groups with confirmed and non- confirmed mycetoma

Multiple imputations were conducted for 23 survey participants who were unavailable for the confirmation of the diagnosis. The resulted dataset included 373 confirmed diagnoses and 142 not confirmed diagnoses. S1 Appendix summarises the characteristics of the populations with and without a diagnosis of mycetoma. Comparisons of imputed data revealed similar results to the analysis of non-imputed datasets. People with mycetoma were more likely to practice agri- culture, have mycetoma in their family, grow farm animals and use animal-drawn vehicles, although these differences did not reach statistical significance (S1 Appendix).

Discussion

Role of economic factors in developing mycetoma

The burden of mycetoma is heavily concentrated in low- and middle-income countries. Pov- erty is often seen as a root cause of neglected tropical diseases because of its association with deprived living and working conditions, and limited access to health care services. This survey was conducted in rural, remote and economically-deprived areas in Sudan. The surveyed households were lacking essential utilities such as electricity (30% of households), piped water (38%) and toilet facilities (53%). Less than half of households owned a TV (44%) and less than a third (28%) had a refrigerator. Animal-drawn carts were the dominant type of vehicle (67%). The average number of people in a household was 5.6 and the average number of rooms was 2. Drinking water was purified in less than 10% of households. Despite widespread overall pov- erty, the households with confirmed cases of mycetoma were worse off with respect to the sup- ply of water and electricity, toilet facilities, and ownership of electrical appliances (Table 2)

In agreement with previous studies, this survey found that the prevalence of mycetoma was higher in the populations involved in agricultural activities (Table 4). Sennar state is located in central Sudan with an area of 40,680 square kilometers. Almost half of this territory is a low rainfall savannah with a total area of rainfed agriculture of approximately 23,000 square kilo- meters [19]. Both traditional rain-fed and modern irrigated farming are practiced in Sudan [20], but the majority of people affected by mycetoma come from rural, isolated communities involved in manual agriculture. They are more likely to contract injuries when working in the field without protective footwear. Our survey has demonstrated that 64.6% of people affected

Table 4. Comparisons of individuals with confirmed and non-confirmed mycetoma.

Characteristics	Confirmed mycetoma (n = 359)	Non-confirmed mycetoma (n = 133)	*p*-value
Number of individuals, n (%)			N/A
Doba	80 (22.3)	57 (42.9)	
El-Reif El-Shargi	10 (2.8)	0 (0.0)	
Wad al Abbas	30 (8.4)	1 (0.8)	
Wad Onsa	104 (29.0)	47 (35.3)	
Wad Taktok	135 (37.6)	28 (21.2)	
Age, years, mean (SD); median; min-max	30.0 (16.3); 27; 1–85	27.5 (17.1); 25; 5–100	0.68
Family history of mycetoma, n (%)	128 (35.7)	38 (28.6)	0.31
Number of people in household mean (SD); median; min-max	5.6 (2.4); 6; 1–14	5.4 (2.5); 5; 1–13	0.58
Number of rooms in household mean (SD); median; min-max	1.9 (1.0); 2; 1–6	1.9 (1.2); 2; 1–9	0.49
Water supply, n (%)			0.06
piped	193 (53.8)	76 (57.1)	
tank	127 (35.4)	47 (35.3)	
well	54 (15.0)	24 (18.0)	
other (e.g. river, canal)	37 (10.3)	5 (3.8)	
Toilet facility, n (%)			0.14
flush toilet	1 (0.3)	0 (0.0)	
pit latrine	107 (29.8)	32 (24.1)	
ventilated pit latrine	13 (3.6)	10 (7.5)	
no facility	234 (65.2)	91 (68.4)	
Waste disposal, n (%)			0.22
burning/burying	104 (29.0)	38 (24.4)	
throwing in a designated place	295 (82.2)	123 (92.5)	
Electricity, n (%)	208 (57.9)	68 (51.1)	0.42
Mobile phone, n (%)	277 (77.2)	106 (79.7)	0.51
Radio, n (%)	63 (17.5)	39 (29.3)	0.04
Refrigerator, n (%)	73 (20.3)	32 (24.1)	0.30
Television, n (%)	125 (34.8)	49 (36.8)	0.34
Transport, n (%)			0.42
animal-drawn cart	116 (32.3)	32 (24.1)	
car or truck	43 (12.0)	18 (13.5)	
raksha	5 (1.4)	3 (2.3)	
no vehicles	218 (60.7)	91 (68.4)	
Land ownership, n (%)	240 (66.9)	80 (60.2)	0.60
Practicing agriculture, n (%)	233 (64.9)	72 (54.1)	0.03
Regularly practicing agriculture, n (%)	84 (23.4)	24 (18.0)	0.86
Type of agriculture, n (%)			0.69
manual	224 (62.4)	69 (51.9)	
mechanical	5 (1.4)	1 (0.8)	
Years of practicing agriculture, mean (SD); median; min-max	14.1 (14.1); 8.0; 0–65	12.3 (12.9); 5; 1–60	0.75
Crops, n (%)			0.62
cotton	53 (14.8)	14 (10.5)	
millet	27 (7.5)	1 (0.8)	

(*Continued*)

Table 4. (Continued)

Characteristics	Confirmed mycetoma (n = 359)	Non-confirmed mycetoma (n = 133)	*p*-value
sorghum	165 (46.0)	58 (43.6)	
wheat	59 (16.4)	14 (10.5)	
other (e.g. sunflower, sesame and vegetables)	92 (25.6)	18 (13.5)	
Practice animal grazing, n (%)	106 (29.5)	46 (34.6)	0.30
Animal ownership, n (%)	182 (50.7)	56 (42.1)	0.13
Farm animals, n (%)			0.89
chicken	36 (10.0)	10 (7.5)	
cows	58 (16.2)	15 (11.3)	
donkeys	42 (11.7)	15 (11.3)	
goats	138 (38.4)	40 (30.1)	
pigeons	2 (0.6)	1 (0.8)	
sheep	34 (9.5)	8 (6.0)	
Trees on the land, n (%)	167 (46.5)	44 (33.1)	0.12
Animal shed ownership, n (%)	155 (43.2)	62 (46.6)	0.98
Shed location, n (%)			0.74
inside house	125 (34.8)	49 (36.8)	
outside house	53 (14.8)	21 (15.8)	
near house	34 (9.5)	13 (9.8)	
away from house	19 (5.3)	8 (6.0)	

The percentages were rounded to one decimal place. For the multiple-choice questions, the sum of percentages may exceed 100%. *p*-values were derived using mixed-effects models using variables "village" and "household" as first- and second-level random effects, respectively. For the multiple-choice questions, multivariable analysis was used.

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by mycetoma practice subsistence farming growing sorghum, millet, wheat, maize, barley and various vegetables, and raising cattle, camels, goats and sheep. Animal farming has previously been identifed as a contributing factor in developing mycetoma [4,7]. In Sudan, animals are often kept inside homes or in outside enclosures surrounded by thorny tree branches. The floors of the animal enclosure are covered with animal dung and thorns, which can cause skin injuries and inoculation with mycetoma pathogens [8]. It has been suggested that animal dung can promote the growth of environmental microorganisms that cause mycetoma [7]. Results of this survey suggest that families with mycetoma are more likely to own farm animals and animal sheds, although no statistically significant differences were found with respect to the animal ownership, type of farmed animals or animal shed location between populations with and without confirmed mycetoma cases (Table 4).

Role of sanitation in developing mycetoma

The results of our survey suggest that poor sanitation and limited access to clean water are fac- tors contributing to mycetoma. Lack of toilet facilities in economically deprived communities increases the risk of contracting waterborne infections such as cholera, dysentery, hepatitis A, typhoid and polio, as well as wound infections. According to the UNICEF report on water, sanitation and hygiene in Sudan [21], approximately 34.6% of households in Sudan practice open defecation. Our study has demonstrated that over half of households in the survey area (53.1%) lacked toilet facilities (Table 1). This proportion was higher (66.3%) in the households with confirmed mycetoma (Table 2.)

The shortage of clean drinking water in rural Sudan contributes to poor domestic hygiene.

According to published data, 68% of households in Sudan have access to improved water sources such as piped water, protected wells and boreholes [21]. Coverage for piped water in the survey area was less than the national coverage (62.3% of households), and only 55.2% of households with cases of mycetoma had access to piped water (Table 2). Person-to-person and water-borne modes of pathogen transmission typically associated with poor sanitation have not been reported for mycetoma. However, poor sanitation and lack of clean water may facili- tate the acquisition of mycetoma in individuals predisposed by skin trauma, which could explain multiple cases of mycetoma in some households, especially those which cannot afford footwear. A family history of mycetoma was reported in 13% of patients who underwent surgi- cal treatment for mycetoma at the Mycetoma Research Centre, Khartoum, Sudan in the period 1991–2015 [22], and 15% of children seen at the Centre during 1991–2009 [23]. Results of our survey showed that 35.7% of people with confirmed mycetoma reported a family history of the disease (Table 4), and 18.8% of households with confirmed mycetoma had more than one affected household member. This could reflect the shared environment that families are exposed to but could also be explained by genetic factors harboured by relatives within the same household [22,24].

Role of environmental factors

Mycetoma is endemic in many tropical and subtropical countries where the climate is charac- terised by low annual rainfall, a dry winter season, a relatively short but heavily rainy summer season, and high year-round temperatures. It is thought that these conditions may contribute to the survival of microorganisms that cause mycetoma [25]. Analyses of soil samples collected from highly endemic areas in Khartoum State, Sudan confirmed the presence of *Madurella mycetomatis* DNA fragments identical to those from biopsies of patients with mycetoma [6]. *Madurella mycetomatis* eumycetoma is the most common cause of mycetoma in Sudan, accounting for around 70% of cases [6]. The prevalence of these fungi in soil samples suggests that soil may be the prime reservoir from which the infection originates. Our survey shows that the majority of mycetoma infections (83%) were acquired during the rainy seasons (sum- mer and autumn), which can be due to the high prevalence of mycetoma-causing pathogens in wet soil.

The abundance of thorny trees in endemic areas could contribute to skin injuries through which pathogens can penetrate [4,9]. Our study revealed that 70% of farms in endemic areas were surrounded by trees, of which 90% of species had thorns. These include a group of *Vachellia* species as well as other thorny plants such *as Balanites aegyptiaca*, *Capparis decidua*, *Prosopis chilensis* and *Ziziphus spina-christi*. Branches from thorny trees are used by the farm- ers to build animal enclosures to secure livestock [8]. In this survey, we found that trees were used as the main source of cooking fuel in more than 70% of households. Trees were also used as a building material, for example, in traditional roofs found in over 80% of households (Table 1). Activities such as working in the field without protective footwear, collecting fuel wood, repairing roofs and animal enclosures pose a significant risk of skin injuries allowing entry of pathogens that are present in the environment.

Access to health care

Lack of qualified medical help and substantial healthcare costs are important factors contribut- ing to the progression of mycetoma. Our survey showed that almost 80% of people with sus- pected mycetoma did not seek professional help. There are several reasons for this. Due to the lack of pain at the onset of mycetoma patients tend to present at later stages of the disease when surgical treatment (including amputation) may be required. The analysis of 6,792 patients with mycetoma managed at the Mycetoma Research Centre of the University of Khar- toum, Sudan showed that over half of them (57%) had previous surgery elsewhere with limited success [22]. The identification of the mycetoma-specific pathogen is a prerequisite for the suc- cessful treatment of the disease (for example, differentiating bacterial and fungal causes which require different antimicrobial treatments). The methods allowing identification of mycetoma pathogens as well as techniques required to localise the main focus and extent of disease are not widely available in Sudan. Our study showed that patients with suspected mycetoma have to travel long distances to the nearest health care facility. Many patients need to be accompa- nied by another person. Over half of patients had to pay towards their treatment with cost being a further barrier. The average annual cost of treatment including consultations, hospita- lisations, travel, medication, and help from traditional healers amounted to 26,957 SDG (566 USD) per year (Table 3). Such expenses are unaffordable for many households. For comparison, the minimum monthly wage in Sudan in 2018 was 425 SDG (9 USD) [16]. According to our survey, approximately a third of people who sought medical help with respect to suspected mycetoma had to borrow on average 4,249 SDG (90 USD) per year to meet their needs and the needs of their families.

Comparison with published studies

The majority of epidemiological data on mycetoma come from case reports and single-cen- tre studies. Large population studies are costly and labour-intensive, and therefore rare. A large historic survey of 2,150 cases (1956–1985) was carried out in Mexico to determine the incidence and epidemiological characteristics of the population affected by mycetoma [26]. This survey was updated to 3,933 cases in 2012 [27]. The collected data included age, sex, occupation, geographical area, affected body area and aetiological agent. The affected popu- lation were predominantly farmers and their family members, 76.1% male and 16–50 years old [26,27].

A retrospective epidemiological study of 264 cases (1981–2000) was carried out in West Bengal, India [28]. The population characteristics were similar to those reported in the previ- ous studies and included agricultural workers, predominantly male (68.6%) with mycetoma onset between 16 and 25 years and a history of pricks and skin trauma. Actinomycetoma was the most common type of mycetoma in this population(74.6%) [28].

A retrospective review of biopsy reports for 279 mycetoma cases (1950–2019) was con- ducted to estimate mycetoma prevalence in Uganda [29]. A mapping study of 337 mycetoma cases (1993–2016) was conducted in Senegal [30]. However, no demographic or socioeco- nomic data were reported in these studies [29,30].

In Sudan, a study of the spatial geographical distribution of mycetoma was conducted using data from 594 patients with confirmed mycetoma (1991–2020) [12]. Demographic characteris- tics were collected alongside geographic information including geological, soil, temperature and land cover details. The main group affected by mycetoma included 20–40 years old males (77.9%), aged <40 years at presentation, with eumycetoma being the most common type of mycetoma (82%) [12].

The current study (see also the accompanying paper [14]) presents, to our knowledge, the most comprehensive characterisation of the population affected by mycetoma. In addition to clinical epidemiology data, we provided characteristics of households, economic activities and use of health care services by people with mycetoma. The results from our study suggest that socioeconomic factors in combination with the natural occurrence of mycetoma pathogens contribute to the high mycetoma prevalence in Sudan.

Conclusions

Results of this survey suggest that agricultural practices and reduced access to sanitation and clean water can be risk factors in developing mycetoma. Poor access to health care and sub- stantial financial costs were barriers to seeking treatment for mycetoma.

Limitations

1. The diagnosis of mycetoma is based on clinical presentation, typical radiological findings and microbiological identification of the causative organisms (e.g. using microscopy and cytological, histopathological, immunohistochemical and molecular techniques) [10]. In the current study, the diagnosis was based on a combination of clinical and ultrasound examinations since mycetoma grains and the accompanying inflammatory granulomata have a characteristic ultrasonic appearance [31]. Given that microbiological identification of the mycetoma-causative pathogen was not possible due to the lack of available facilities locally, there was a possibility of not confirming true cases of mycetoma in individuals who presented with swelling or sinus formation but had negative ultrasound examinations.
2. At the time of data analysis, 23 out of 515 cases with suspected mycetoma were not confirmed due to the loss to follow-up. The primary analysis compared cases with a confirmed diagnosis of mycetoma to those with another diagnosis combined with those lost to follow-up. This means that there is a possibility of positive mycetoma cases among those lost to follow-up. To address this uncertainty, multiple imputations were conducted for participants with a missing diagnosis. Analysis using the imputed data (S1 Appendix) revealed similar results to the analysis of the non-imputed datasets (Table 4).
3. To identify factors associated with mycetoma we compared individuals with confirmed cases of mycetoma with those in whom mycetoma had been not confirmed and an alternative diagnosis was made. This group was not necessarily representative of the general study population and bias may have been introduced to the analysis. However, this group is a useful comparator as individual data for the general population was not collected in this survey.
4. Due to the multiple comparisons of survey variables (Tables 2 and 4), some statistically significant differences may be found by chance.

Supporting information

S1 Appendix. Comparison of imputed datasets for the populations with confirmed and non-confirmed mycetoma.
(DOCX)

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Appendix 5 – Paper 3 Individual Risk Factors of Mycetoma Occurrence in Eastern Sennar Locality, Sennar State, Sudan: A Case-Control Study

Tropical Medicine and Infectious Disease

Article

Individual Risk Factors of Mycetoma Occurrence in Eastern Sennar Locality, Sennar State, Sudan: A Case-Control Study

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Abstract: Mycetoma is a serious chronic subcutaneous granulomatous inflammatory disease that is endemic in tropical and subtropical regions, where it impacts profoundly on patients, families, and communities. Individual-level risk factors for the disease are poorly understood. To address this, a case-control study was conducted based on data collected from 60 villages in Eastern Sennar Locality, Sennar State, Sudan. Based on the presence of swelling in any part of the body, or sinus formation with or without grain discharge evident from the lesion by ultrasound examination, we diagnosed 359 cases of mycetoma. For each case, we included three healthy sex-matched persons, with no evidence of mycetoma, from the same village as the control group (n = 1077). The odds for mycetoma were almost three times higher in individuals in the age group 16–30 years (Adjusted Odds Ratio (AOR) = 2.804, 95% CI = 1.424–5.523) compared to those in age group ≤ 15 years. Other factors contributing to the odds of mycetoma were history of local trauma (AOR = 1.892, 95% CI = 1.425–2.513), being unmarried (AOR = 3.179, 95% CI = 2.339–4.20) and owning livestock (AOR = 3.941, 95% CI = 2.874–5.405).
In conclusion, certain factors found to be associated with mycetoma in this study could inform a high index of suspicion for mycetoma diagnosis, which would improve early case detection. Other factors found to be associated could inform the development of an interventional program for mycetoma control in Sudan, including education on healthy farming practices and the risks of puncture wounds for individuals residing in endemic areas. However, this work was conducted in one endemic state, while mycetoma cases occur in all states of Sudan. Replicating this study over a wider area would give a fuller picture of the situation, providing the control program with more comprehensive information on the risk factors for the disease.

Keywords: case-control study; mycetoma; risk factors; determinants; Sennar; Sudan

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Introduction

Mycetoma is a devastating, neglected tropical disease that occurs in tropical and subtropical regions [1]. It is characterized by painless subcutaneous masses with multiple sinuses draining seropurulent discharge and grain, most frequently affecting the feet and hands, though other parts of the body may be involved [2,3]. The disease progresses to involve the skin, deep tissues, and bone, sometimes resulting in massive tissue de- struction leading to significant deformities and disability [4]. The disease is associated with massive morbidity, stigma and reduced economic productivity [4,5]. Mycetoma is reported worldwide and Sudan is considered the most endemic country and, yet, there is no community-based study to feed national control programs with comprehensive infor- mation. Mycetoma is commonly reported from Mexico, Venezuela, Mauritania, Senegal, Chad, Ethiopia, Somalia, Yemen, and India [6]. Most of the mycetoma cases in Sudan are attributed to fungal organisms, and *Madurella mycetomatis* is the most common causative agent [7]. While actinomycetoma cases in Sudan are commonly caused by *Streptomyces somaliensis*, *Actinomadura madurae* and *Actinomadura pelletierii* [8].

The geographical distribution of mycetoma depends on a range of environmental factors, such as rainfall, humidity, and temperature [9]. A study conducted in Sudan indicated mycetoma is more likely to occur in arid areas proximal to water sources, soil with low calcium and sodium concentrations, and a variety of thorny tree species [10].

There is controversy on the route of infection in mycetoma; however, subcutaneous traumatic inoculation of the causative organism is the most favored hypothesis, based on current evidence [4].

The disease is most frequently reported in young adults aged 20–40 years, but people of all ages are at risk [9]. Males are reported to be at higher risk, but a community-based study in White Nile, Sudan, showed almost equal risk for both sexes [10,11]. Mycetoma commonly affects those who work in close contact with the environment, such as farmers and shepherds [12]. Affected communities are generally of low socioeconomic status and underdeveloped infrastructure to provide clean water and sanitation [13].

There are currently no dedicated control programs for mycetoma, and the only avail- able tools to reduce the disease burden are early case finding and appropriate management. These are hampered by challenges in the diagnosis and treatment of the disease and knowl- edge gaps surrounding its incidence, prevalence, and determinants of susceptibility [11–13]. Diagnosis requires numerous invasive and time-consuming investigations led by an ex- perienced physician, and available treatment options are limited and expensive, require a long time to effect cure, and have a high recurrence rate [14–17]. The promotion of early case detection is costly and relies on appropriate public health messaging and community education. Existing knowledge gaps surrounding the route of infection, factors favoring transmission, and determinants of susceptibility to the disease impede the design and implementation of effective prevention strategies [4,18–20].

We sought to address this issue using a case-control study to determine risk factors for mycetoma infection in a highly endemic area of Sudan.

Methods

This population-based case-control study was conducted in Eastern Sennar Locality, Sennar State, central-eastern Sudan, and included 41,176 individuals as total population covered in the study. Eastern Sennar Locality is highly endemic according to surveillance databases from the Mycetoma Research Centre (MRC), a World Health Organization (WHO) Collaborating Centre on Mycetoma, and the reference center for mycetoma management and research in Sudan.

The rationale for the sample size was based on the assumption that the prevalence of mycetoma is five cases per 10,000 population, with the average population size of 935 people in the village, and the population of Eastern Sennar locality of 353,196 people. The calculations suggested that sampling 60 villages would give 80% power of detecting mycetoma at 5% significance level. Cases were identified through an exhaustive survey of 60/292 randomly selected villages in the five administrative units of the locality, described in more detail elsewhere [21]. The survey was conducted from June 2019–July 2019, and every household in these sixty villages was visited. Cases were identified through clinical examination of all individuals by a medical doctor. When an individual was not at home for the examination, the household was revisited. All individuals with swelling of any part of the body or sinus formation, with or without grain discharge, were considered suspected cases of mycetoma. All suspected cases were referred to Wad Onsa Regional Mycetoma Centre, where an experienced radiologist performed lesion ultrasound examination to ascertain mycetoma diagnosis. A confirmed mycetoma case was defined as an individual with swelling or sinuses in any body part, with a pocket of fluid containing echogenic grains on ultrasound examination [22].

For each patient, three healthy controls were selected by simple random sampling. We considered that a 3:1 ratio of controls to cases would provide sufficient power to the statistical analyses. Controls were matched on community and gender (i.e., 3 controls of the same gender and from the same village were selected for each case identified), and selected from households where no suspected case was detected.

After village leaders and the study population were informed about the survey ob- jectives and process, all households in the study villages were visited. The questionnaire was written in English and was validated by a team, including a medical doctor and a statistician at the MRC. Responses were captured through electronic data capture forms through open-source software called Open Data Kit (ODK), which collects, manages, and uses data in resource-constrained environments, running via Android devices. It allows for offline data collection with mobile devices in remote areas.

Household-level data included the type of material of the floor, roof, external walls of the dwelling, and hygiene and sanitation amenities. Individual-level factors included age, sex, marital status, educational level, occupation, swelling, history of trauma and wearing shoes/slippers at home and work. Further details, including clinical features, lesion onset, duration, site, and mycetoma family history, were collected from suspected cases.

Data were sent directly to a server at the MRC and imported to the Statistical Package for Social Sciences, SPSS 25 (SPSS, Chicago, IL, USA) for analysis. Descriptive analysis was performed, and bivariate analysis (chi-square test) was used to identify any statis- tically significant associations between explanatory variables and the outcome variable (confirmed mycetoma).

Univariate and multivariate analyses were undertaken to assess the strength of associa- tion of individual and household-level variables with disease status (i.e., being a mycetoma case or control). A stepwise forward and backward selection procedure was used to select inclusion variables in a conditional logistic regression model. A p-value of 0.05 or less was used to enter variables into the model and 0.1 or above for removal from the model. The strength of association of each retained variable with mycetoma was expressed using adjusted odds ratios with their 95% confidence interval (CI). The STROBE case-control reporting guidelines were used in this study [23].

Results

1.1. Demographic Characteristics of Cases and Controls

A total of 1436 individuals (359 mycetoma patients and 1077 controls) were included in the case control analysis. The mean age of confirmed mycetoma patients was 27.0 (SD = 16.3) years, ranging from 1–85 years. The mean age of controls was 37.2 (SD = 16.9) years, ranging from 1–105.

The patient group comprised 174 males (48.5%) and 185 females (51.5%), and the control group 537 (49.9%) males and 540 (51.1%) females. Sixty-seven (18.7%) cases and 236 (21.9%) of controls worked as shepherds or farmers. One hundred and thirty-nine cases (38.7%) and 525 controls (48.7%) were illiterate.

1.2. Clinical Characteristics of Cases

In total, 290 cases (80.8%) presented with visible swelling, and 245 (68.2%) had lesions in the lower extremities. Sinuses were present in 138 (38.4%), and, of those, 122 (33.9%) patients had discharge. One hundred and twenty-nine cases (35.9%) had a history of local trauma at the mycetoma site.

Age, history of trauma, marital status, education, raising animals within the household and livestock ownership were strongly associated with the odds of mycetoma (p-value < 0.05). Compared to individuals aged 0–15, the odds of mycetoma were more than six times higher in individuals in the age group 16-30 years (OR = 6.467, 95% CI = 3.421–12.224, p < 0.001), and around two times higher for those aged 31–45 or 46–60 years. The likelihood of mycetoma in individuals with a history of trauma was 71% higher than in those without (OR = 1.710, 95% CI = 1.323–2.209, $p < 0.001$). The odds of mycetoma in unmarried individ- uals were over four times that in married individuals (OR = 4.117, 95% CI = 3.151–5.381, $p < 0.001$), while illiterate individuals had lower odds (OR = 0.664, 95% CI = 0.521–0.848, $p = 0.001$). Compared to individuals in other occupations, housewives had increased odds of mycetoma (OR = 4.945, 95% CI = 2.747–8.902, $p < 0.001$), as did those who were desk em- ployees (odds ratio, 1.251, 95% CI = 1.279–3.961, $p = 0.005$). Individuals living in households with animals raised within the dwelling had lower odds (OR = 0.557, 95% CI = 0.435–0.713, $p < 0.001$). Ownership of animals increased the odds of mycetoma by more than two times (OR = 2.15, 95% CI = 1.687–2.742, $p < 0.001$) (Table 1).

Table 1. Demographic characteristics of confirmed cases and controls, showing unadjusted odds ratios (OR) for mycetoma risk factors (n = 1436).

Characteristic	Cases No. (%)	Controls No. (%)	Crude OR (95% CI)	*p*-Value
Individual factors				
Age group				
0–15 Years	70 (19.5%)	70 (6.5%)	1.0	
16–30 Years	136 (37.9%)	391 (36.3%)	6.467(3.421–12.224)	<0.001
31–45 years	101 (28.1%)	344 (31.9%)	2.249 (1.262–4.008)	0.006
46–60 Years	37 (10.3%)	175 (16.2%)	1.899(1.055–3.416)	0.032
>60 Years	15 (4.2%)	97 (9.0%)	1.367(0.714–2.617)	0.345
Sex				
Female	185 (51.5%)	540 (50.1%)	1.0	
Male	174 (48.5%)	537 (49.9%)	1.069(1.414–2.290)	0.583
Trauma history				
Yes	129 (35.9%)	266 (24.7%)	1.710 (1.323–2.209)	<0.001
No	230 (64.1%)	811 (75.3%)	1.0	
Marital status				
Married	207 (57.7%)	905 (84.0%)	1.0	
Unmarried	152 (42.3%)	172 (16.0%)	3.864(2.963–5.038)	<0.001
Education level				
Literate	220 (61.3%)	552 (51.3%)	1.0	
Illiterate	139(38.7%)	525 (48.7)	0.664 (0.521–0.848)	0.001
Wear shoes/Slippers				
Both work and home	261 (72.7%)	828 (76.9%)	1.0	
At work or at home only	32 (9.8%)	84 (7.8%)	0.788 (0.574–1.083)	0.142
Not at all	66 (18.4%)	165 (15.3%)	0.952 (0.579–1.566)	0.847
Occupation				
Farmers or shepherds	67 (18.7%)	236 (21. 9%)	1.0	
Students	67 (18.7%)	60 (5.6%)	1.257 (0.728–2.170)	0.411
Housewives	114 (31.5%)	447 (41.5%)	4.945 (2.747–8.902)	<0.001
Merchants	15 (4.2%)	76 (7.1%)	1.129 (0.674–1.893)	0.644
Unemployed and underage of work	61 (17.3%)	120 (11.1%)	0.874 (0.422–1.811)	0.717
Desk employee	5 (1.4%)	29 (2.7%)	1.251 (1.279–3.961)	0.005
Freelancer	21 (5.8%)	93 (8.6%)	2.491 (0.969–6.403)	0.058
Other jobs	9 (2.4%)	16 (1.5%)	0.764 (0.264–2.205)	0.618
Household factors				
Source of fuel for cooking				
Wood and Animal dung	243 (67.7%)	433(40.2%)	0.600 (0.429–0.839)	0.003
Gas and Coal	77 (21.4%)	130 (12.1%)	1.0	
Any source of fuel available	35 (9.7%)	500 (46.4%)	0.403 (0.286–0.569)	<0.001
No food cooked in the house	4 (1.1%)	14 (1.3%)	0.476 (0.151–1.498)	0.205
Main material of dwelling floor				
Brick/cement/ceramic	20(5.6%)	50 (4.6%)	2.160 (1.372–3.400)	0.001
Earth/soil and/or sand	326 (90.8%)	1007 (93.5%)	1.0	
Combination	13 (3.6%)	20(1.9%)	1.271 (0.554–2.913)	0.571
Main material of dwelling roof				

Table 1. *Cont.*

Characteristic	Cases No. (%)	Controls No. (%)	Crude OR (95% CI)	*p*-Value
Main material of dwelling exterior walls				
Wood	40 (11.1%)	104 (9.7%)	0.824 (0.525–1.294)	0.401
Mud and/or animal dung	114 (31.8%)	371 (34.4%)	0.847 (0.626–1.147)	0.284
Red bricks and/or concrete	97 (27.0%)	324 (30.1%)	0.786 (0.572–1.079)	0.137
No walls	108 (30.1%)	278 (25.8%)	1.0	
Animal raised within the dwelling				
Yes	127 (35.4%)	534 (49.6%)	0.557 (0.435–0.713)	<0.001
No	232 (64.6%)	543 (50.4%)	1.0	
Livestock ownership				
Yes	183 (51.0%)	351 (32.6%)	2.151 (1.687–2.742)	<0.001
No	176 (49.0%)	726 (60.4%)	1.0	

OR = Odds Ratio, CI = Confidence Interval.

1.3. Multivariate Analysis

After adjusting for other significant variables, being unmarried had higher odds of mycetoma (AOR = 3.179, 95% CI = 2.339–4.20, p < 0.001). The odds of the disease were roughly double for patients with a history of local trauma compared to those with- out (AOR = 1.892, 95% CI = 1.425–2.513, p < 0.001). Those aged 16–30 had higher odds (AOR = 2.804, 95% CI = 1.424–5.523, p = 0.003) compared to those aged ≤15 years. Il- literate individuals had lower odds of mycetoma (AOR = 0.685, 95% CI = 0.521–0.900, p = 0.007). Individuals who owned animals had higher odds of mycetoma (AOR = 3.914, 95% CI = 2.874–5.405, p < 0.001), but those keeping animals within their own dwelling had lower odds of disease (AOR = 0.310, 95% CI = (0.303, 95% CI = 0.220–0.416, p < 0.001)
(Table 2).

Table 2. Individual risk factors for mycetoma at Eastern Sennar locality, Sennar State (n = 1436).

Individual Characteristics	Cases No. (%)	Controls (%)	AOR (95% CI)	*p*-Value
Age group				
0–15 years	70 (19.5%)	70 (6.5%)	1.0	
16–30 years	136 (37.9%)	391 (36.3%)	2.804 (1.424–5.523)	0.003
31–45 years	101 (28.1%)	344 (31.9%)	1.564 (0.852–2.871)	0.149
46–60 years	37 (10.3%)	175 (16.2%)	1.469 (0.791–2.726)	0.223
>60 years	15 (4.2%)	97 (9.0%)	1.218 (0.617–2.405)	0.570
Trauma history				
Yes	129 (35.9%)	266 (24.7%)	1.892 (1.425–2.513)	<0.001
No	230 (64.1%)	811 (75.3%)	1.0	
Marital status				
Married	207 (57.7%)	914 (84.9%)	1.0	
Unmarried	152 (42.3%)	163 (15.1%)	3.179 (2.339–4.20)	<0.001
Education level				
Literate	220 (61.3%)	559 (51.9%)	1.0	
Illiterate	139 (38.7%)	518 (48.1)	0.685 (0.521–0.900)	0.007
Animal raised within the dwelling				
Yes	127 (35.4%)	534 (49.6%)	0.303 (0.220–0.416)	<0.001
No	232 (64.6%)	543 (50.4%)	1.0	
Livestock ownership				
Yes	183 (51.0%)	351 (32.6%)	3.941 (2.874–5.405)	<0.001
[illegible]	[illegible]	[illegible]	[illegible]	

AOR = Adjusted Odds Ratio, CI = Confidence Interval.

Discussion

Although mycetoma is a serious disease, inflicting disability and stigma on patients across many parts of the world, there remain essential questions about its epidemiology, particularly the risk factors for the disease. In the current study, patients with confirmed mycetoma were compared to community- and sex-matched controls to identify determinants of risk in the study population. To the best of our knowledge, this is the first population-based case-control study of sociodemographic risk factors for mycetoma, pro- viding evidence likely to apply to other settings and support global control efforts.

One of the risk factors we identified was a history of trauma, which roughly doubled the odds of mycetoma. This finding represents the most substantial epidemiological evidence for the theory that skin trauma may facilitate the inoculation of mycetoma- causative organisms into the subcutaneous tissue; although this does not rule out other possible routes of transmission [24]. This hypothesis is supported by the fact that mycetoma- causative organisms typically reside in the soil and evidence that certain *Acacia* trees, whose thorns may facilitate inoculation, are associated with environmental suitability for the disease [10]. However, previous analyses of mycetoma cases have shown a history of trauma in only a few patients, hypothesized to reflect that the trauma may be minor and pass unnoticed by the patient [11]. Moreover, and due to the nature of work for individuals living in endemic areas, causative organisms can be contracted from the environment through previous injuries. The case-control design we employed, including many patients and community-matched controls, allowed us to demonstrate a significant difference in the history of trauma between these two groups.

Many reports in the literature show that young adults and children are the most affected populations [2,6,25,26]. The data obtained in this study showed that the most common age group affected was the category aged 16–30 years. The higher odds in younger adult groups is likely to reflect the fact that these individuals are more likely to be actively engaged in activities, such as agricultural work and animal grazing, which exposes them to mycetoma-causative agents in endemic areas.

As expected, livestock ownership was a strong risk factor for mycetoma. There are several possible mechanisms by which people who raise animals may be at increased risk of mycetoma. People living in rural areas tend to make animal enclosures from the wood of thorny trees and may be at risk of thorn pricks during the construction and maintenance of these structures [11]. As well as being in close contact with the environment, they are likely to be at higher risk of ticks, which are highly prevalent in domestic animals in the Eastern Sennar Locality and hypothesized to play a role in the transmission of mycetoma causative agents to humans [27]. The evidence for this route of transmission is not conclusive, however. While the DNA of mycetoma-causative organisms has been isolated from ticks, this does not prove they are involved in transmission.

We found that the odds of unmarried individuals contracting mycetoma was four times that of their married counterparts. This is likely to reflect the social stigma of mycetoma, which may mean affected individuals are less likely to get married, or more likely to get divorced if they contract the disease after marriage. In rural communities, early marriage is often considered mandatory and social pressure is put on young adults to get married by their early teen years. If they fail to get married early, this pressure can cause psychological distress in addition to the stress and depression occurring as a result of such a stigmatizing disease [28].

Interestingly, there was no significant difference between literate and illiterate pop- ulations and there were increased odds for mycetoma in desk employees, contradicting previous reports in the literature that mycetoma is commonly prevalent among poor communities with low education levels [4]. This may be explained by the fact that in mycetoma-endemic areas, most individuals share the same social and economic activities and behavior irrespective of their educational level due to economic constraints. Another factor may be that schools in rural areas are limited to certain areas, and students often have to walk for long distances, putting them at higher risk of contact with mycetoma-causative organisms. We also observed that housewives and desk employees had increased odds of mycetoma. Regarding housewives, they are usually responsible for the cooking, and, hence, they walk long distances to collect wood to use as fuel. Furthermore, housewives work in farming, especially in the harvesting season, which may mean that they have a similar level of exposure.

There are some limitations to this study. The field-based case definition we used lacks specificity compared to the gold standard techniques of microbiological or PCR confirmation of the causative organisms, which were not feasible within our study setting. Nevertheless, our study is, to our knowledge, the first community-based study to apply clinical examination of all individuals, followed by ultrasound examination to ascertain mycetoma cases. Within the limited timeframe of our study, we were also unable to assess end-line treatment outcomes. Another limitation was that this work was conducted in one endemic state, while mycetoma cases occur in all states of Sudan. The factors we identified may not be applicable to all endemic settings. Finally, some suspect cases were lost to follow-up and were not included in the comparative analysis. However, we have no reason to believe that the absence of these individuals was due to their disease status or other systematic factors, so considered this data to be randomly missing.

Conclusions

In this population-based case-control study on mycetoma, the first of its kind at this scale, the high number of participants enabled precise estimates for the strength of association of various risk factors with disease.

The results of this study could be applied to inform future control of mycetoma. Efforts to raise awareness among clinicians of mycetoma risk factors, particularly those of age, history of trauma, and ownership of animals, could promote earlier diagnosis and treatment of mycetoma in patients presenting swellings or wounds in endemic regions of Sudan. These factors could also inform the design of health awareness campaigns in communities at risk, educate the population about activities that may put them at risk of the disease and encourage them to present early to health facilities if they experience early signs. In addition, education about hygienic practices after encountering local trauma, including cleaning and disinfecting the injured area to avoid contracting mycetoma, is advocated. Finally, this study adds further evidence for the substantial social impact of the disease and the stigma associated with it, which should not be overlooked in assessments of its global burden.

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Informed Consent Statement: Written informed consent was obtained from each adult patient, and parents or guardians of the population under the age of 18 years.

Data Availability Statement: The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request on the following email: roaal- basha2016@outlook.com.

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Appendix 6: Paper 4 - Modelling the spatial distribution of mycetoma in Sudan

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ORIGINAL ARTICLE

Modelling the spatial distribution of mycetoma in Sudan

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Background: Mycetoma is a neglected tropical disease that is reported worldwide and Sudan has the highest reported number of mycetoma infections across the globe. The incidence, prevalence and burden of mycetoma globally are not precisely known and its risk factors remain largely unelucidated.

Methods: This study aimed to identify the environmental predictors of fungal and bacterial mycetoma in Sudan and to identify areas of the country where these niche predictors are met. Demographic and clinical data from confirmed mycetoma patients seen at the Mycetoma Research Centre from 1991 to 2018 were included in this study. Regression and machine learning techniques were used to model the relationships between mycetoma occurrence in Sudan and environmental predictors.

Results: The strongest predictors of mycetoma occurrence were aridity, proximity to water, low soil calcium and sodium concentrations and the distribution of various species of thorny trees. The models predicted the occurrence of eumycetoma and actinomycetoma in the central and southeastern states of Sudan and along the Nile river valley and its tributaries.

Conclusion: Our results showed that the risk of mycetoma in Sudan varies geographically and is linked to identifiable environmental risk factors. Suitability maps are intended to guide health authorities, academic institutes and organisations involved in planning national scale surveys for early case detection and management, leading to better patient treatment, prevention and control of mycetoma.

Keywords: ensemble models, environmental modelling, machine learning, mycetoma, Sudan

Introduction

Mycetoma is a neglected tropical disease (NTD) that, in common with other NTDs, has numerous health and socioeconomic impacts on patients and communities.[1–3] It is a chronic infectious disease that leads to destructive granulomatous inflammation, eventually causing deformity to the affected body part limb, impeding mobility.[4,5] Mycetoma is reported worldwide, but it is endemic in tropical and subtropical regions.[6,7] Mycetomaendemic areas are characterised by moderate aridity, low humidity and a short rainy season.[8–10] Sudan is considered one of the endemic regions for mycetoma among a list of other countries.[11,12] In a recent review, mycetoma was noted to be present in 102 countries around the world and Sudan had the highest burden of cases.[13] More than 70 microorganisms have been incriminated in causing mycetoma and, according to the causative organism, it is classified into eumycetoma, caused by fungi, and actinomycetoma caused by bacteria.[14,15] Eumycetoma is frequently reported in Africa and India, whereas actinomycetoma is more predominant in Asia, and in Central and South America.[16,17] In Sudan, *Madurella mycetomatis* is the leading causative agent of eumycetoma, while *Streptomyces somaliensis*, *Actinomadura madurae* and *Actinomadura pelletierii* are the most frequently reported actinomycetoma causative organisms.[18–21]

Mycetoma is a painless disease in the initial stages, of gradual onset and progress. Most affected patients are of low

socioeconomic status and have poor levels of health education.[22] Furthermore, in mycetoma-endemic regions, the medical and health facilities are meager so most patients report late to medical care with advanced disease.[19,20] For this reason, most of the reported patients are hospitalised rather than diagnosed at an earlier, more treatable stage of the disease.[21,23] Late presentation of mycetoma patients leads to a reduced cure rate and a higher chance of recurrence, which in turn increases the rate of morbidity and disability.[24,25] Despite the long history of mycetoma in Sudan and efforts to fight it, the incidence, prevalence and geographical distribution remain unclear.[9] Mycetoma patients in Sudan are often poor and illiterate and contracting mycetoma adds to their socioeconomic burden.

Most of the epidemiological characteristics of mycetoma disease are uncertain. The route of infection, the incubation period and the factors that determine susceptibility and resistance to disease are largely unknown.[22,26] Likewise, its incidence, prevalence and burden have not been properly estimated at global or national levels. This lack of data is one reason for the absence of control and prevention programmes targeting the

disease.22,27

Although the infection pathway is not completely known, bacteria and fungi causing mycetoma are thought to penetrate through skin lesions and wounds.[28] It is hypothesised that thorns from trees and shrubs of the *Acacia* genus are a major cause of wounds, through which mycetoma infectious agents can infect humans. In most mycetoma-endemic countries, thorny trees and shrubs such as *Acacia* species and other species from the pea family (Family: Fabaceae) are present. Many *Acacia* species bear sharp thorns that protect their leaves from herbivorous animals and, which on the ground, pose a threat to individuals walking barefoot or in open shoes such as sandals.[29] Other potential environmental risk factors that have been identified include hygiene practices and proximity to animals.[22] Many individuals living in rural areas have poor access to water of adequate quality and sanitation facilities, which impedes good hygiene and could be one of the factors allowing the organism to establish infection, in the context of direct and sustained contact with soil, animals and their dung.[30] Many mycetoma-causative organisms have been found in dung-enriched soil, hence dung could also provide a natural habitat for mycetoma-causative agents.[26]

The distribution of mycetoma has previously been modelled using environmental niche modelling techniques, which suggested central Sudan is suitable for mycetoma occurrence due to an evident overlap of *Acacia* distribution and mycetoma cases, but only 44 mycetoma confirmed cases were used and they were distributed between 12ºS and 19ºN.[9] In this work, we aim to provide an update to these models by constructing models using a large occurrence dataset, categorised by type of mycetoma (actinomycetoma vs eumycetoma), using a wider set of environmental predictors and with a range of modelling algorithms. The presen study was conducted at the Mycetoma Research Centre (MRC), University of Khartoum, the only WHO collaborating centre on mycetoma globally.[31] The MRC was established in 1991 to tackle the burden of mycetoma in Sudan with well-trained dedicated staff for the diagnosis and management of mycetoma patients attending from all the states of Sudan. To date, more than 9500 patients have been seen and managed at the centre.

Materials and Methods

Data source

The data included in this study were extracted from the patient database at the MRC. The study included only patients from Sudan who were seen at the centre during 1991–2018. The variables of interest included the patients' demographic characteristics, details of their clinical presentation and diagnosis, place of birth, their current home address if different and their address at the time of onset of symptoms. For most people, the place of birth and current residence were within the same vicinity but if the person had moved outside of their birth area then the address where they were living at the onset of symptoms was used for the analysis. These locations were remotely georeferenced using ArcGIS 10.5 (Environmental Systems Research Institute [ESRI] Inc., Redlands CA, USA) to obtain geographical coordinates defining the mycetoma occurrence locations. The diagnosis of mycetoma and its classification into eumycetoma and actinomycetoma were based on a careful clinical interview and examination, grain culture, cytological examination of aspirates from mycetoma lesions and histopathological examination of surgical biopsy samples. Histopathological methods have been used for mycetoma diagnosis since the 1950s.[31] Molecular diagnosis using conventional PCR with pan-fungal/bacterial primers and species-specific primers was introduced in 2017.

Environmental predictors and variable selection

Candidate predictors

Because some determinants of mycetoma occurrence remain unelucidated, to avoid exclusion of relevant predictors, we started with a wide range of candidate predictors and used correlation analysis followed by principal components analysis (PCA), a technique used to reduce the dimensionality of large datasets.[32]

Continuous gridded datasets (rasters) of 52 environmental variables considered relevant to the mycetoma ecological niche were assembled. These raster datasets included precipitation and temperature, soil composition and pH, livestock distributions, proximity to water sources, elevation and related topographical variables, the predicted distributions of *Acacia* species and other thorny vegetation, measures of atmospheric moisture availability that determine potential vegetative growth and an index of vegetation coverage. Previous studies have indicated these variables to be associated with mycetoma occurrence in Sudan and

elsewhere.9,33–35

Climate variables such as minimum, maximum and mean temperature and precipitation levels were obtained from the WorldClim database (version 2.0), a repository of climatic indicators based on long-term data collection from weather stations.[36] In addition, from the Consortium for Spatial Information we obtained estimates of aridity (rainfall deficit) and potential evapo-transpiration (an indicator of atmospheric demand for moisture),[37] both modelled by using WorldClim datasets and a dataset of modelled elevation, based on data collected by the Shuttle Radar Topography Mission.[38]

We compiled soil composition and pH datasets from the International Soil Reference and Information Centre-World Soil Information project.[39] Raster datasets of predicted livestock

Table 1. Distribution by state of mycetoma patients from Sudan seen at the Mycetoma Research Centre during 1991–2018

State	Eumycetoma	Actinomycetoma	Total
Al Jazirah	2163	234	2397 (34.4%)
Khartoum	730	280	1010 (14.5%)
White Nile	753	101	854 (12.2%)
North Kordofan	479	341	820 (11.7%)
Sennar	562	69	631 (9%)
River Nile	128	59	187 (2.7%)
South Darfur	118	62	180 (2.6%)
North Darfur	97	73	170 (2.4%)
Al-Qadarif	108	42	150 (2.1%)
Kassala	95	40	135 (1.9%)
South Kordofan	80	46	126 (1.8%)
West Darfur	44	36	80 (1.1%)
Northern	45	29	74 (1.1%)
Blue Nile	38	10	48 (0.7%)
East Darfur	34	15	49 (0.7%)
West Kordofan	19	12	31 (0.4%)
Red Sea	13	13	26 (0.4%)
Central Darfur	7	8	15 (0.2%)
Total	5513	1470	6983

distributions (cattle, chickens, sheep and goats), modelled by collaborators from the International Livestock Research Institute, the Food and Agriculture Organization of the United Nations and the Université Libre de Bruxelles, were obtained from the Gridded Livestock of the World version 2 database.[40] We downloaded spatial data on waterbodies and waterways from the OpenStreetMap project through the *Geofabrik* platform and produced continuous surfaces of straight-line (Euclidean) distance in km to each.[41] We obtained the Enhanced Vegetation Index dataset, generated using satellite imagery data from the Moderate

Resolution Imaging Spectroradiometer satellite, from the Earth Explorer NASA site.[42] Full details of these data sources are shown in Supplementary File 1, Table 1.

Finally, we modelled the distributions of 11 species of thorny trees that are considered potential vehicles for the inoculation of mycetoma-causative organisms using occurrence records downloaded from the Global Biodiversity Information Facility[43] along with a suite of environmental variables. Full details of the modelling of these thorny plant species are given in Supplementary

File 2.

Each of these gridded datasets was resampled to 1 km^2 resolution using bilinear interpolation and clipped and aligned to the outline of Sudan. Raster processing was performed using the *raster* package in R v. 3.3.2 and the final map layouts were created with ArcGIS 10.5 software (ESRI Inc., Redlands, CA, USA).

Variable selection

Initially, we calculated correlation coefficients between all candidate predictors across Sudan and identified groups of predictors that were correlated with a Pearson's correlation coefficient >0.8. We then extracted values of each of the compiled raster datasets at each of the mycetoma-occurrence locations to produce a matrix of covariates (a table showing the x and y coordinates of the occurrences, and at each of these locations, the values of all candidate covariates). We then ran PCA separately for actinomycetoma and eumycetoma to identify the most relevant predictors of the distribution.

In this process, the original variables are reduced into a set of 'components' (or axes), which are linear combinations of variables and are uncorrelated with each other. The components are ordered by the fraction of the total dataset variance they contribute and the variance contribution of each component was summarised by a value termed an eigenvalue. From the minimum set of components that collectively contributed at least 80% of the total variation in occurrence locations, we identified the original variable with the highest influence (measured via the factor loading). We ensured that we did not select any variables that were found to be correlated with another included variable. This process resulted in a selection of uncorrelated variables that collectively contributed the greatest proportion of variance to the occurrence locations. We used the *prcomp* package in R to implement this analysis.

Ensemble modelling

We built separate ensemble distribution models for actinomycetoma and eumycetoma, using the occurrence records and the suite of selected covariates for each. The ensembles were constructed from four algorithms from the BIOMOD (Centre d'Ecologie Fonctionnelle et Evolutive of the CNRS, Montpellier, France) package: generalized boosted regression model, random forest (RF), generalized linear models and generalized additive models.[44] For each model, we used the default parameters set by the *biomod2* R package. These algorithms are all classified as presence-absence models, meaning that they require both occurrence and absence points to determine environmental suitability, absence points being locations confirmed by surveys to be clear of the outcome under investigation. To account for the lack of absence points within the dataset, we generated 'pseudoabsence' points, representing areas presumably unsuitable for mycetoma. The use of pseudoabsence points to represent areas presumed to be unsuitable for a species is a well-established approach in species distribution modelling.[45,46] We implemented pseudoabsence selection within the BIOMOD framework, using the 'surface range envelope' approach to define the area of assumed unsuitability. The envelope is estimated through a presence-only suitability model[47] that identifies the range of locations at which the values of the chosen environmental covariates are within a specified range (here between the 5th and 95th percentiles) of the covariate values at the occurrence locations.[44] A set of pseudoabsence samples (here a set of five samples), equal in size to the number of occurrence points, was randomly extracted from outside this envelope each time the model was run. Each algorithm was run 50 times with a random sample of 80% of the data points used for model training and the remaining 20% reserved for evaluation. The mean true skill statistic (TSS), the mean proportion correctly classified that is equivalent to kappa

and the mean area under the curve (AUC)

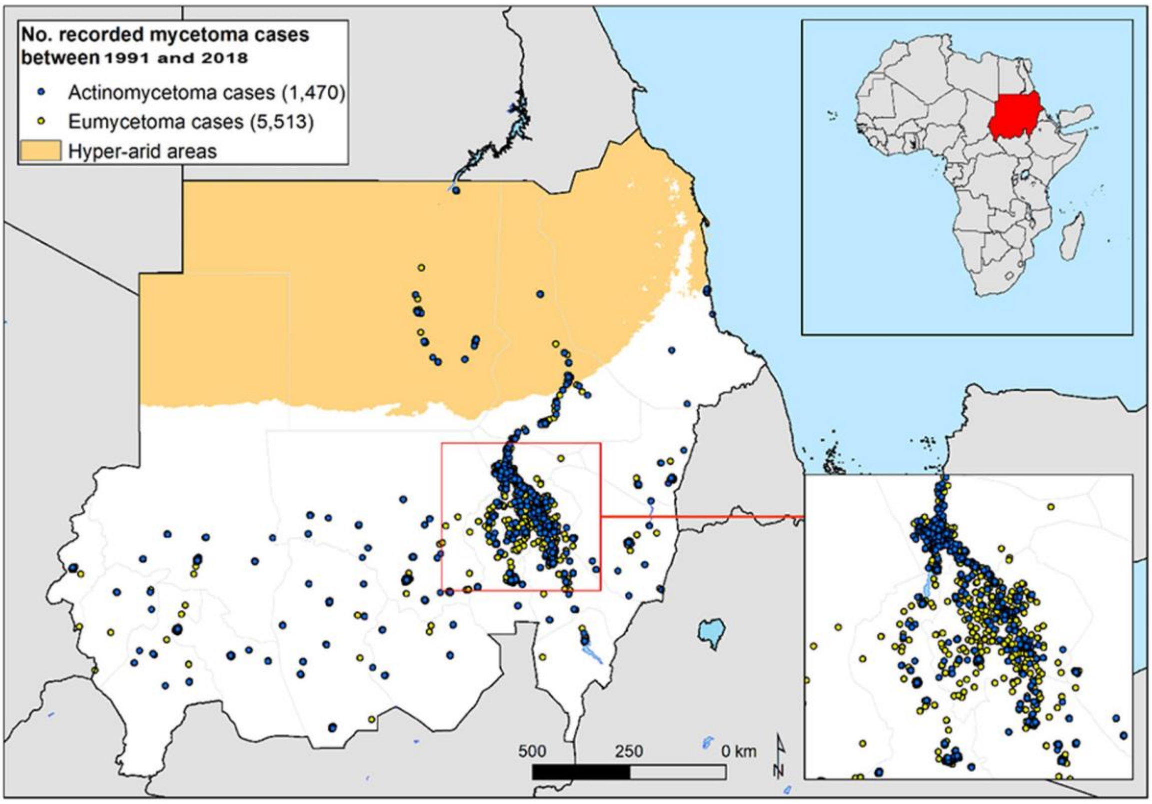


Figure 1. Records of mycetoma cases in Sudan (1991–2018); the upper right map shows where Sudan is situated in Africa; the blue dots represent Actinomycetoma, and the yellow dots represent Eumycetoma. Mycetoma cases are distributed along the River Nile with a focus on central Sudan.

Cases are distributed sporadically in the western part of Sudan.

of the receiver operating characteristic (ROC) were used to evaluate the performance of each algorithm.

Variables of final models

For eumycetoma, aridity index, soil calcium concentration, wetness index, mean diurnal temperature, distance to the nearest river, presence of cattle, goats and chickens, and predicted occurrence of *Acacia mellifera* and *Faidherbia albida* trees in the area, were included. For actinomycetoma, aridity index, distance to the nearest river, distance to the nearest water body (pond or lake), wetness index, soil sodium and iron concentrations, presence of cattle and sheep in the area, mean diurnal temperature and mean temperature in the coldest year quarter, were included.

All models with an AUC>0.8 were compiled into the final ensemble distribution model. Within the ensemble, the predicted suitability value was the mean of the included models, weighted by their relative AUC. Upper and lower limits of suitability were estimated by calculating confidence intervals around the ensemble mean suitability value for each cell within the grid. Variable contribution plots were produced to show the relative contribution of each variable to the model, along with marginal effect plots to show the response of both mycetoma types to changes in each modelled covariate.

Results

Dataset of mycetoma occurrence

The modelled data included 7812 unique points after the exclusion of patients (829) from outside Sudan and those lacking diagnostic or geographical information. The patients were seen at the MRC during 1991–2018 and they came from all the states of Sudan. The study included 5513 patients (79%) with confirmed eumycetoma and 1470 patients (21%) with actinomycetoma (Figure 1). Most of the mycetoma patients were from Al Jazirah State (34.4%) and Khartoum State (14.5%) (Table 1).

Environmental predictors of eumycetoma occurrence in Sudan

The PCA for eumycetoma occurrence in Sudan identified 10 variables that characterised the environmental conditions at the occurrence locations. These were aridity index, soil calcium concentration, wetness index, mean diurnal temperature, distance to the nearest river, presence of cattle, goats and chickens, as well as predicted occurrence of *A. mellifera* and *F. albida* trees in the area. Two variables—the predicted density of cattle and chickens—were excluded after the initial modelling step as each contributed less than 1% of the variability in the response variable (eumycetoma occurrence).

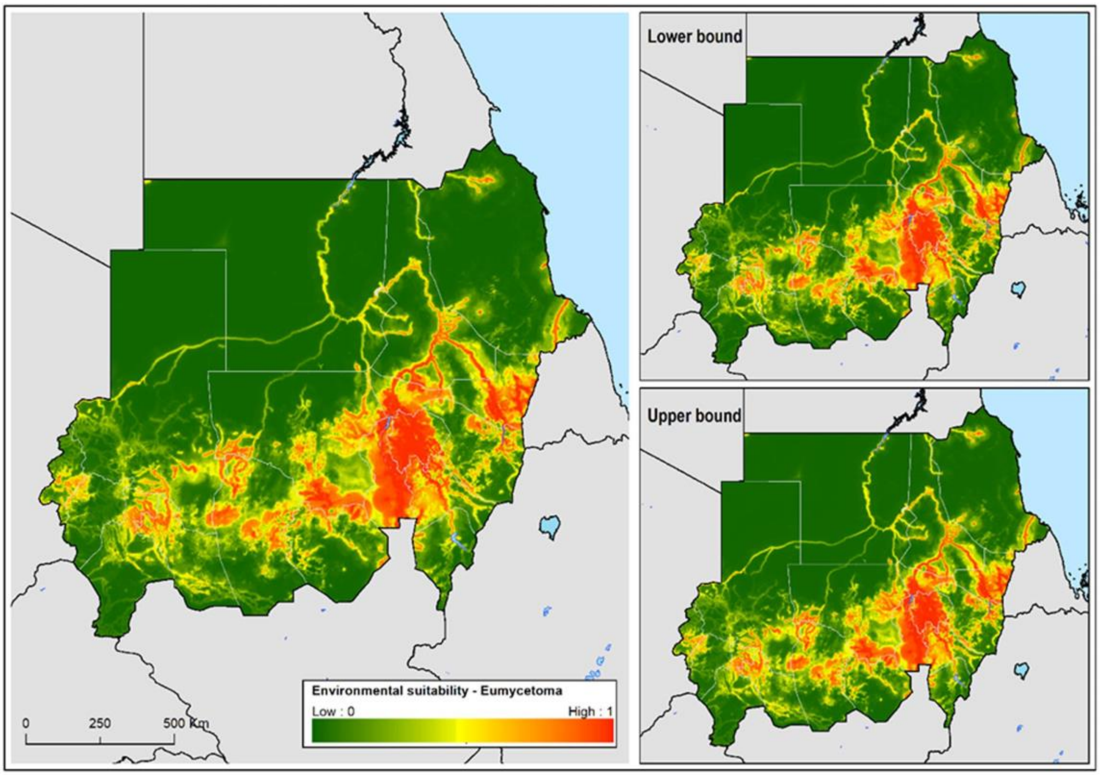


Figure 2. Predicted environmental suitability for eumycetoma in Sudan. Eumycetoma is predicted to occur along the River Nile and its tributaries with hotspots in the central and southeaster parts of Sudan.

Table 2. Validation metrics for ensemble models for eumycetoma and actinomycetoma suitability

		Weighted mean	Lower CI	Upper CI
Eumycetoma	TSS	0.909	0.909	0.911
	ROC	0.993	0.993	0.993
	kappa	0.898	0.897	0.898
Actinomycetoma	TSS	0.921	0.922	0.922
	ROC	0.995	0.995	0.995
	kappa	0.903	0.903	0.902

Abbreviations: ROC, receiver operating characteristic; TSS, true skill statistic.

All models for eumycetoma occurrence performed well with an ensemble ROC score of 0.993, sensitivity of 96.475% and specificity of 94.476%. The mean TSS was 0.909 and mean kappa score was 0.898 (Table 2).

Across the RF models, the distance to the nearest river was the strongest environmental predictor of suitability for eumycetoma occurrence and the suitability decreased with increased distance to the nearest river. The diversity of thorny trees, represented by the number of species predicted to be present, was the second most important predictor of suitability for eumycetoma occurrence, with a greater diversity of thorny trees associated with a higher probability of occurrence of the disease. We found the suitability for eumycetoma to be higher in areas with a lower probability of occurrence of *A. mellifera* and arid areas. Soil calcium concentration suitable for eumycetoma occurrence was from 3000 to 11 000 mg/kg, while mean diurnal temperature range and the probability of occurrence contributed to a lesser extent; goat density contributed the least (Supplementary File 1, Figure 1).

Environmental predictors of actinomycetoma occurrence in Sudan

The PCA for actinomycetoma occurrence also revealed 10 variables as potential environmental predictors. The variables were aridity index, distance to the nearest river, distance to the nearest water body (pond or lake), wetness index, soil sodium and iron concentrations, presence of cattle and sheep in the area, mean diurnal temperature and mean temperature in the coldest year quarter. After the primary analysis, four variables—distance to the nearest river, distance to the nearest water body (pond or lake), mean temperature in the coldest year quarter and variety in thorny trees—were included.

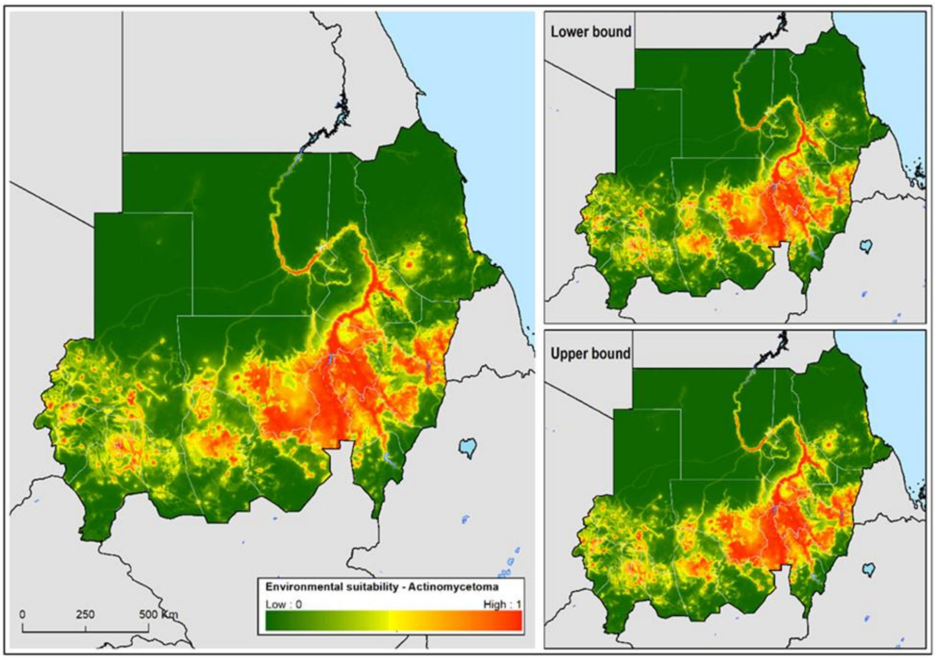


Figure 3. Predicted environmental suitability for actinomycetoma in Sudan. Actinomycetoma is predicted to occur along the River Nile and its tributaries with hotspots in the central and southeastern parts of Sudan with sporadic occurrence in the western part of Sudan.

All models for actinomycetoma occurrence performed well, with a ROC score of 0.995, sensitivity of 95.445% and specificity of 96.709%. The mean TSS was 0.921 and the mean kappa was

0.903 (Table 2).

Across the RF models, distance to the nearest water body was the major contributor to actinomycetoma occurrence, followed by the distance to the nearest river. Mean temperature during the coldest quarter over a range of 18–25ºC contributed to the predicted suitability for actinomycetoma occurrence by 13%. Arid areas with a low concentration of soil sodium contributed by almost 11%. The diversity of thorny trees in an area showed no difference between areas with no diversity of thorny trees and areas with a diverse group of thorny trees (Supplementary File 1, Figure 3).

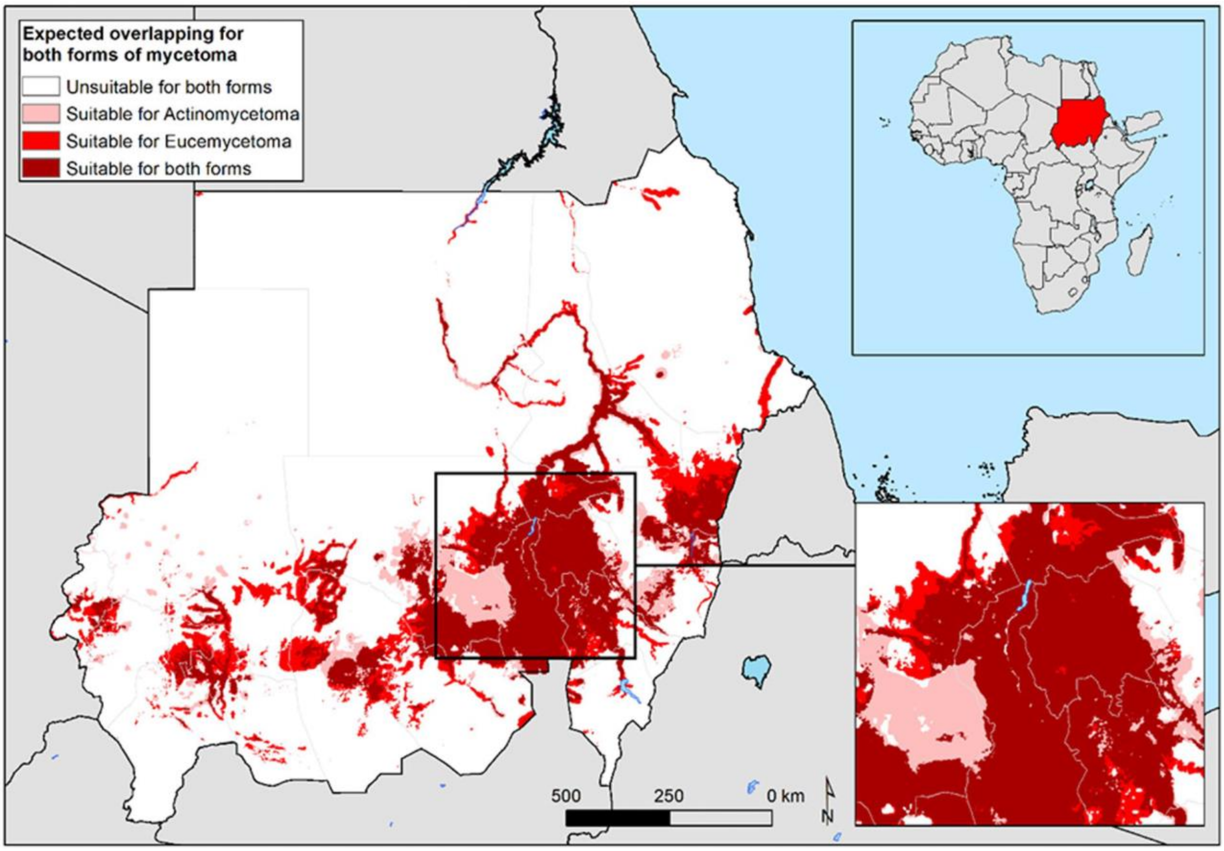
Predicted suitability for mycetoma occurrence in Sudan

Both eumycetoma and actinomycetoma were predicted to occur around the central and southeastern parts of Sudan and along the Nile river valley and its tributaries. The central states of Sudan, Al Jazirah, White Nile, Khartoum and Sennar State had the largest areas predicted as suitable for eumycetoma. The predicted distribution of actinomycetoma was slightly wider than that of eumycetoma, with more patchy suitability predicted in the western states of Sudan (Supplementary File 1, Figures 5 and 6) . The overlapping areas for actinomycetoma and eumycetoma risk are illustrated in Figure 4.

Discussion

We have applied ecological niche modelling techniques to identify environmental variables associated with the occurrence of mycetoma in Sudan. Models were run separately for eumycetoma and actinomycetoma and gave similar results in terms of the predicted distribution. Both types were predicted along the River Nile and its tributaries, mainly around the central and southeastern parts of Sudan covering Khartoum, Al Jazirah and White Nile States, the northern part of Sennar State and the eastern part of North Kordofan State, which are known to have a high burden of cases.[48] The models also predicted the occurrence of eumycetoma in the north and eastern parts of the country, where cases have not previously been recorded. This might indicate underreporting or misdiagnosis of cases from these areas. Such areas must be considered as priorities for future surveillance activities and to offer targeted training of medical personnel for early detection and better diagnosis of mycetoma.

This study showed the suitability for mycetoma occurrence was widespread in Khartoum State, and this can be attributed to the high influx of people from rural endemic areas to the state,



area for the occurrence of both types of mycetoma.

due to various socioeconomic difficulties, often secondary to having the disease. Also, Khartoum residents regularly visit their families in endemic regions and can contract the infection during such visits. Furthermore, much of the state beyond the city is undeveloped rural land that harbours the ecological and sociodemographic characteristics associated with mycetoma.

As previously mentioned, mycetoma is distributed globally in a belt form, and areas within the belt are dry with annual rainfall of 50–1000 mm and high temperatures.[49] Given the recognised influence of temperature on the survival of mycetoma-causative organisms,[50] it is not surprising that temperature-related variables had a large impact on the suitability for both types of mycetoma. Our results indicate that environments with mean temperatures in the coldest quarter of the year of 20–25°C are most suitable for actinomycetoma-causative organisms. Daily variation in temperature was a strong predictor of suitability for eumycetoma. It has been reported that fungal organisms that cause eumycetoma cannot survive in environments where the daily range in temperature is more than 15°C on average. This could be explained by the fact that the organisms do not live in regions that have extreme variations in temperature.[51] According to our results, mycetoma seems to be predicted in arid areas; as mentioned previously in the literature, mycetoma prevails in reasonably arid areas with a short rainy season of

4–6 mo.[50]

Analysis of the MRC data showed that most of the patients reside in locations close to water sources and the models predicted the same pattern. This observation is most likely explained by the fact that while mycetoma-causing species thrive in drier environments, people living in such environments settle near a water source to enable activities such as washing and growing crops. Thus distance to rivers correlates with mycetoma occurrence as a result of human behaviour, rather than environmental conditions near rivers supporting organism survival.

To our knowledge, the influence of the soil mineral components on the growth of mycetoma-causative organisms has not been studied thoroughly. Our findings suggested that soil calcium concentration had a moderate effect on suitability for eumycetoma and that sodium concentrations contribute to the disease occurrence suitability. In histopathological studies, calcium, among other elements, was found in abundance in the cell wall of *M. mycetomatis*. It is an important element in the formation of cement substance, which is a hard brown material containing melanin, heavy metal ions, proteins and lipids. Cement is a fungal protective mechanism, acting as a sink for harmful unpaired electrons and provides the cell walls with structural rigidity as well as storing water and ions to prevent desiccation.[52] This may indicate that calcium could be an element in the organism's virulence by contributing to the myelinisation and sclerosis of the grain.[51] In a recent cross-sectional study conducted in Mexico, cases were prevalent among three soil types that had a high level of calcium.[35] This observation warrants further laboratory studies to explore the possible role of these soil elements in the pathogens' survival.

We found that environments with a greater number of species of Acacia trees were more suitable for eumycetoma, supporting previous evidence that suggested a role for skin penetration by thorns in the transmission of the disease.[9] In our study, and in contrast to the study conducted by Samy et al. in 2014, not only was the distribution of Acacia trees tested, but also the role of diversity of these trees in mycetoma occurrence in both its types.[9] However, areas with either no thorny trees or areas with more than five species of thorny trees were both suitable for actinomycetoma occurrence. In our study, eumycetoma, but not actinomycetoma, occurrence seems to be predicted by the diversity of thorny trees. This could be explained by the observation that eumycetoma-causative organisms live on the thorns. This study showed no strong association between the individual species types and mycetoma occurrence. These observations on the association between disease occurrence and individual tree species, their botanical properties and other parameters, need further investigation. Such studies are important to gain more indepth knowledge about disease transmission and the life cycle of the causative microorganisms to develop ways to interrupt them.

In conclusion, the studied models have indicated that arid areas proximal to water sources, soil with low concentrations of calcium and sodium and with a variety of thorny tree species provide the most suitable environment for the occurrence of mycetoma. This work included environmental probable risk factors that have not been studied previously and which could be used to guide the implementation of preventive interventions and control strategies in the affected areas. Until there is robust evidence on disease transmission and the life cycle of the microorganisms involved, it may be advisable to limit the use of thorny trees in building animal enclosures and houses. However, such simple and effective interventions may not align with longstanding cultural practices and should be comprehensively discussed with all stakeholders and community members to ensure community acceptance. Protective measures such as the use of footwear should be encouraged in people who are in direct contact with these trees.

Our study also considered the suitable environmental indicators across the whole of Sudan with updated data from the MRC database records using a wide range of environmental risk factors. The results could help guide planning for national and international scale surveys for mycetoma identification and to design targeted training for medical staff on early detection and diagnosis. Moreover, they could aid in the design of national and global mycetoma advocacy and awareness programmes leading to early active case detection with early appropriate patient management in areas that are highly endemic for mycetoma.

Authors' contributions: RH, HS, JC, SB, EG, DA, MJN, KD and AHF conceived and designed the study; RH, HS, JC, SB, EG, DA, AHF, MJN and KD performed the study; RH, HS, JC, SB, EG, DA, MJN, KD and AHF analysed the data; RH and KD wrote the first draft of the manuscript. RH, HS, JC, SB, EG, DA, MJN, KD and AHF revised the manuscript. All authors read and approved the final manuscript.

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Competing interests: The authors declare that they have no conflicts of interest.

Ethical approval: This study was approved by the Mycetoma Research Centre, Khartoum, Sudan (IRB, No. SUH 11/12/2018) and from the BSMS Research Governance and Ethics Committee (ER/BSMS435/1). Written informed consent was obtained from each adult patient, and verbal assent was obtained from minors (aged <18 y) in addition to signed written informed consent from the parent/guardian.

Data availability: Data are available upon request.

Supplementary data: Supplementary data are available at *Transactions* online.

Supporting information:

S1 File: Covariates, marginal effect plots and predicted occurrences

S2 Text: Modelling the environmental suitability for the species of thorny vegetation present in Suda

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Appendix 7 - Supplemental materials for Paper 4 - Modelling the spatial distribution of mycetoma in Sudan

Supplementary information

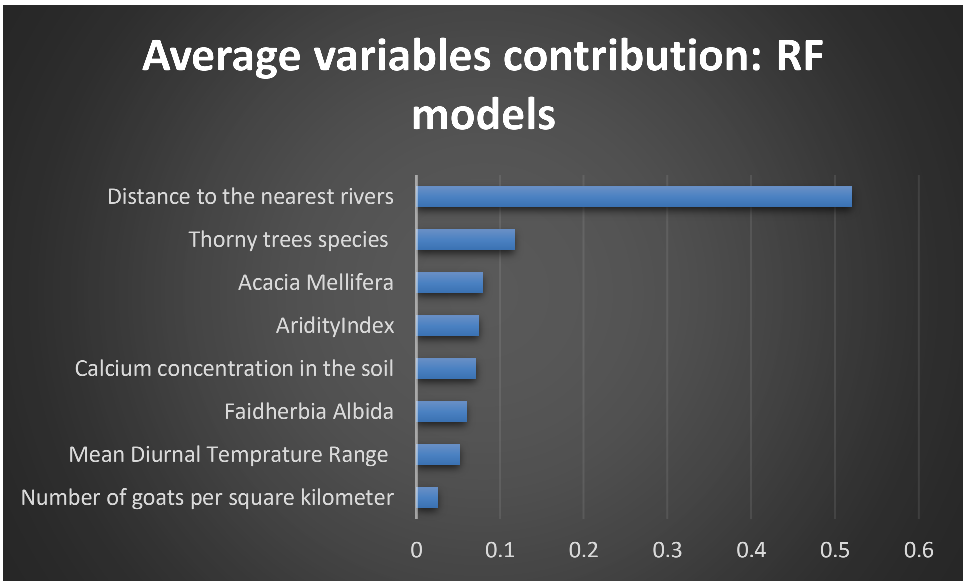
Table 1: The potential environmental predictors for eumycetoma (EUM) and actinomycetoma (ACM)). The gridded predictor variables were compiled at a 1 Km x 1 Km resolution within the boundaries of Sudan.

variables	ACM	EUM	Sources
bioclim_01: annual mean temperature			[140]
bioclim_02: mean diurnal range (mean of monthly (max temp - min temp))		x	
bioclim_03: isothermality (bio2/bio7) (* 100)			
bioclim_04: temperature seasonality (standard deviation *100)			
bioclim_05: max temperature of the warmest month			
bioclim_06: min temperature of the coldest month			
bioclim_07: annual temperature range (bio5-bio6)			
bioclim_08: mean temperature of wettest year quarter			
bioclim_09: mean temperature of driest year quarter			
bioclim_10: mean temperature of warmest year quarter			
bioclim_11: mean temperature of coldest year quarter	x		
bioclim_12: annual precipitation			
bioclim_13: precipitation of the wettest month			
bioclim_14: precipitation of the driest month			
bioclim_15: precipitation seasonality (coefficient of variation)			
bioclim_16: precipitation of wettest year quarter			
bioclim_17: precipitation of driest year quarter			
bioclim_18: precipitation of warmest year quarter			
bioclim_19: precipitation of coldest year quarter			
AF_WM: Acacia flava			[141]
AL_WM: Acacia Leta			
AM_WM: Acacia Mellifera		X	
AN_WM: Acacia Nilotica			
AS_WM: Acacia Senegal			
AT_WM: Acacia tortilis			
AY_WM: Acacia Seyal			
BA_WM: Balanites Aegyptiaca			
DC_WM: Dichrostachys Cinerea			
EC_WM: Euphorbia Candelabrum			
FA_WM: Faidherbia Albida		X	

Clay TopSoil			[142]
Water pH			
Sand TopSoil			
Silt Topsoil			
Flow Accumulation			
Wetness Index			
Aluminium concentration in the soil			
Calcium concentration in the soil		X	
Iron concentration in the soil			
Magnesium concentration in the soil			
Potassium concentration in the soil			
Sodium concentration in the soil	X		
Potential Evapo-Transpiration (PET)			
Aridity Index	X	X	[140]
Distance to nearest rivers		X	[143]
Distance to nearest water body	X		
Elevation			
Cattle density			[144]
Chicken density			
Goats density		X	
Sheep density			
Thorne variety		X	The presence of thorny trees was categorised as 0 to > 5 within areas

ACM: Actinomycetoma. EUM: Eumycetoma. X: predictors that were selected for the modelling

Figure 1: Variable contribution of final ensemble models for Eumycetoma in Sudan based on random forest (RF) and Generalized boosted regression model (GBM). Variable contribution is provided as percentage, and it shows the relative contribution of selected environmental predictors to the final ensemble model.



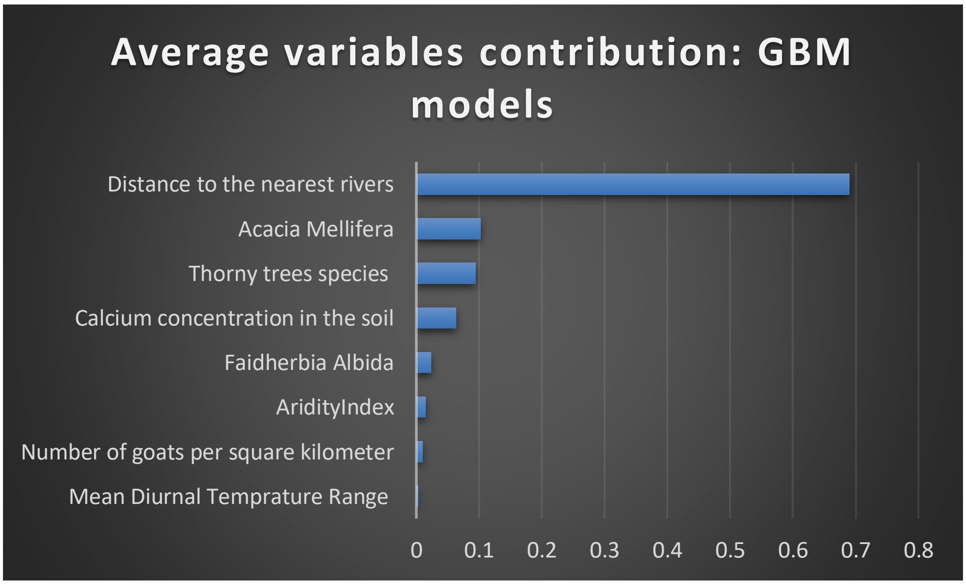


Figure 2: Marginal plots for all significantly contributing variables for the occurrence of eumycetoma,(distance to the nearest stream, thorn trees variety, extractable calcium , mean diurnal temperature range, aridity index, occurrence of white acacia , occurrence of acacia mellifera and number of goats in square kilometre) to Random Forest (RF), averaged over 50 ensembles. Blue lines represent the mean partial dependence over all 50 algorithms ensembles and grey envelopes the standard deviation from the mean. The y-axis is the transformed logit response and x-axis is the full range of covariates values.

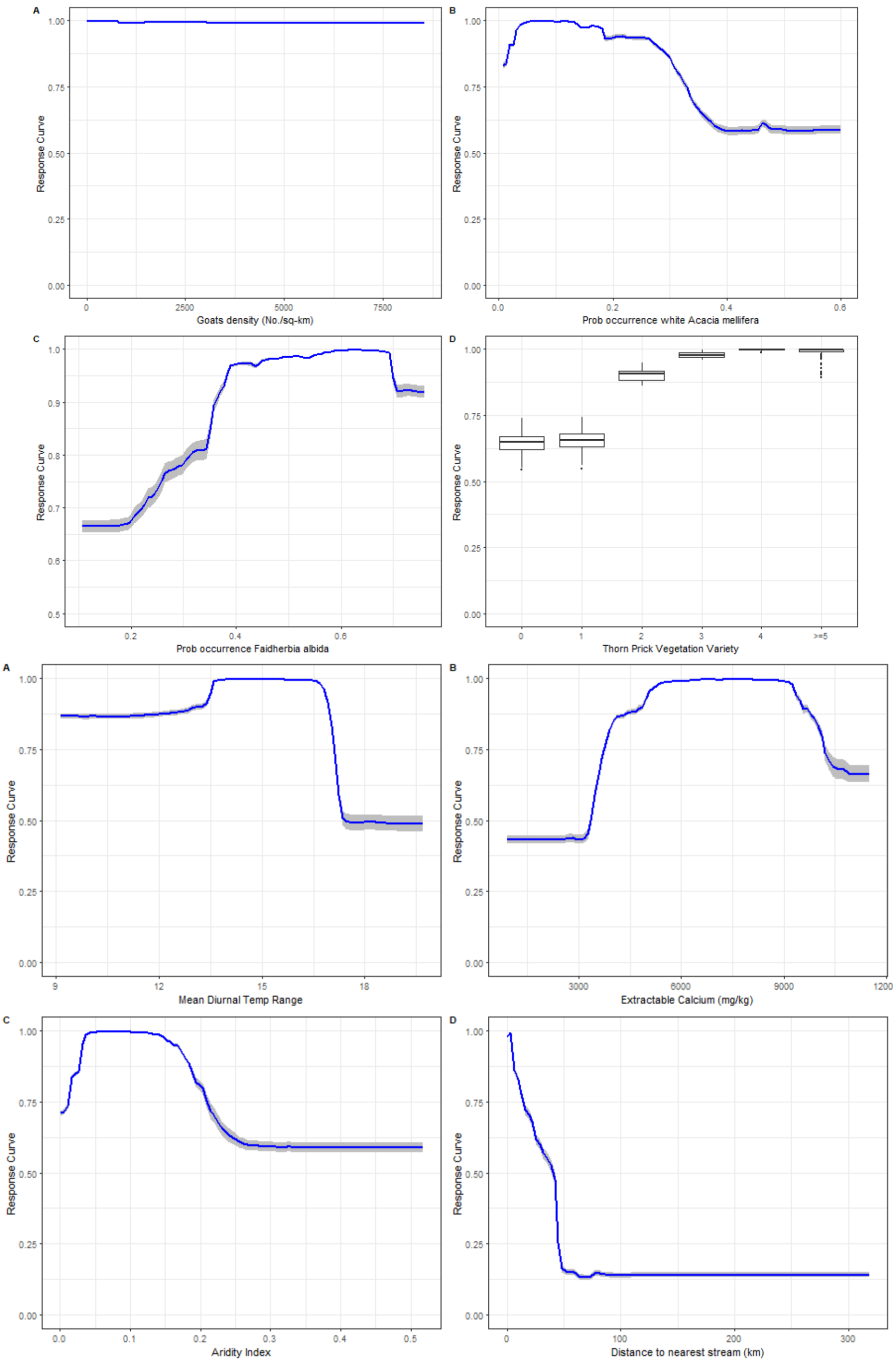
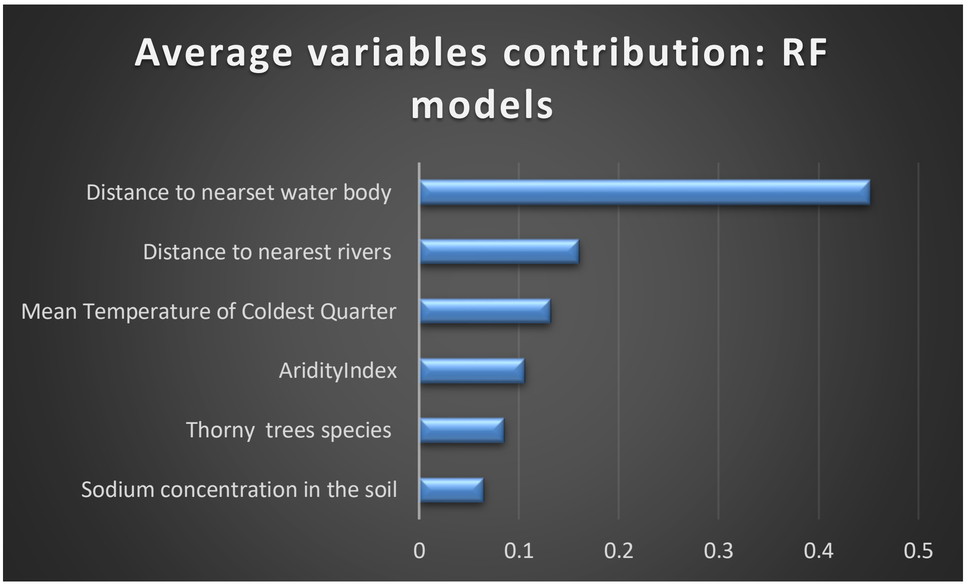


Figure 3: Variable contribution of final ensemble models for Actinomycetoma in Sudan based on random forest (RF) and Generalized boosted regression model (GBM). Variable contribution is provided as percentage, and it shows the relative contribution of selected environmental predictors to the final ensemble model.



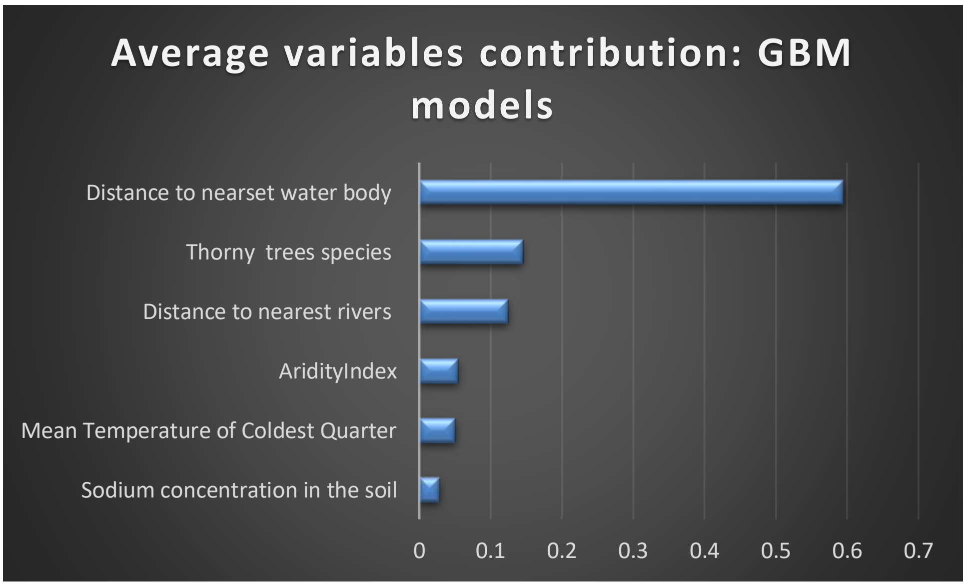


Figure 4: Marginal plots for all significantly contributing variables for the occurrence of actinomycetoma, (distance to the nearest stream, distance to nearest river, thorn trees variety, extractable calcium , Aridity index , mean diurnal temperature range) to the Random Forest (RF), averaged over 50 ensembles. Blue lines represent the mean partial dependence over all 50 algorithms ensembles and grey envelopes the standard deviation from the mean. The y-axis is the transformed logit response and x-axis is the full range of covariates values.

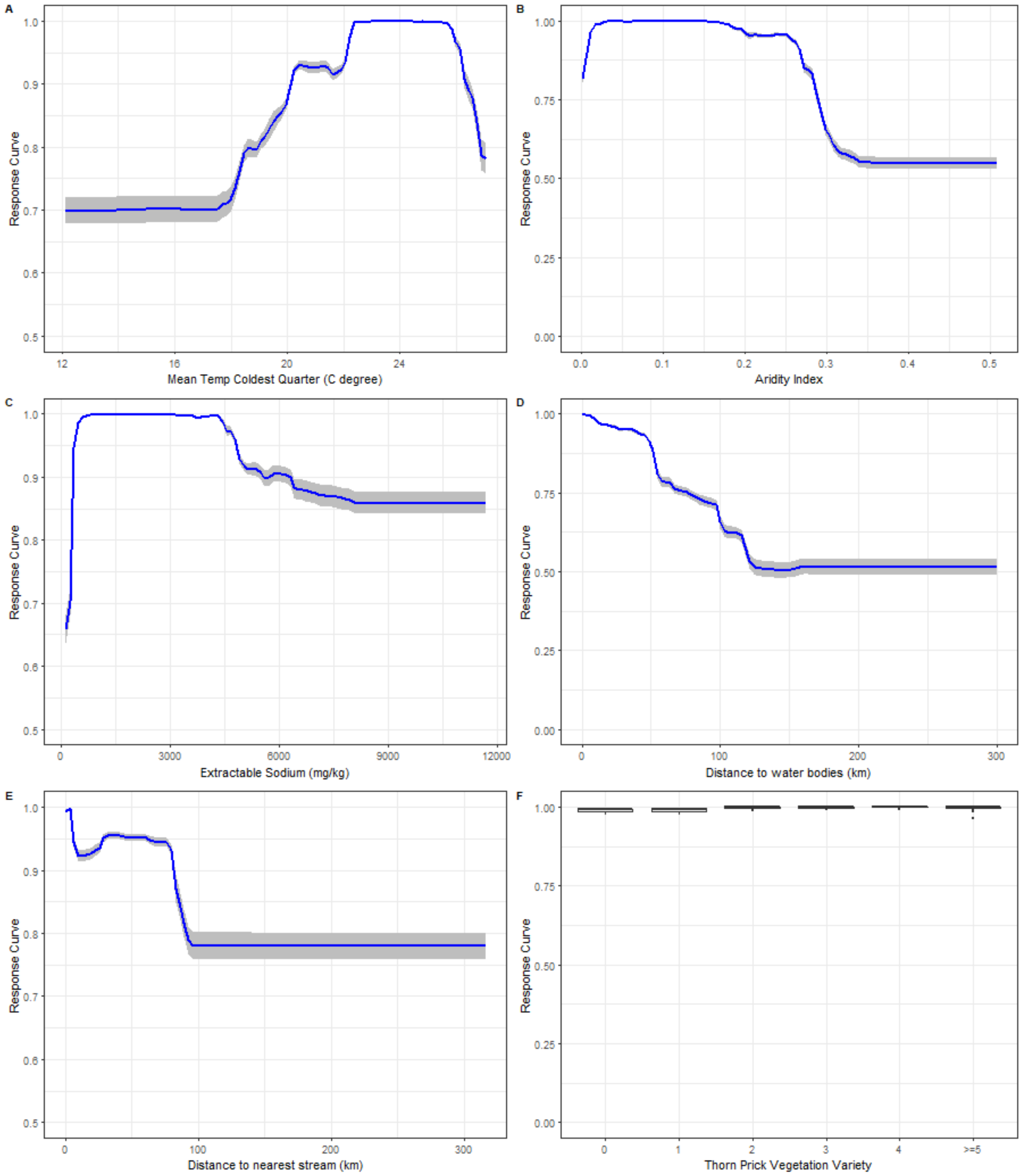


Figure 5: Predicted occurrence of eumycetoma in Sudan based on a cut-off (value: 0. 452) that optimizes sensitivity and specificity.

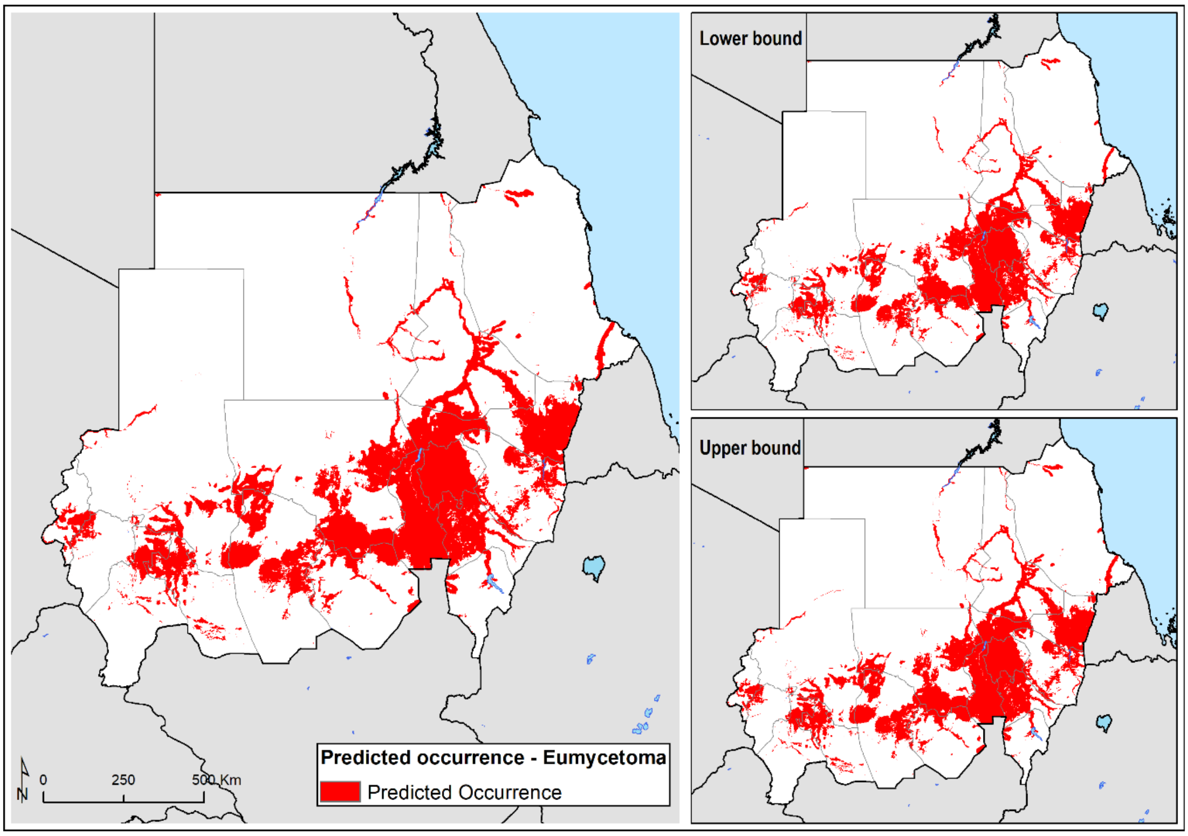
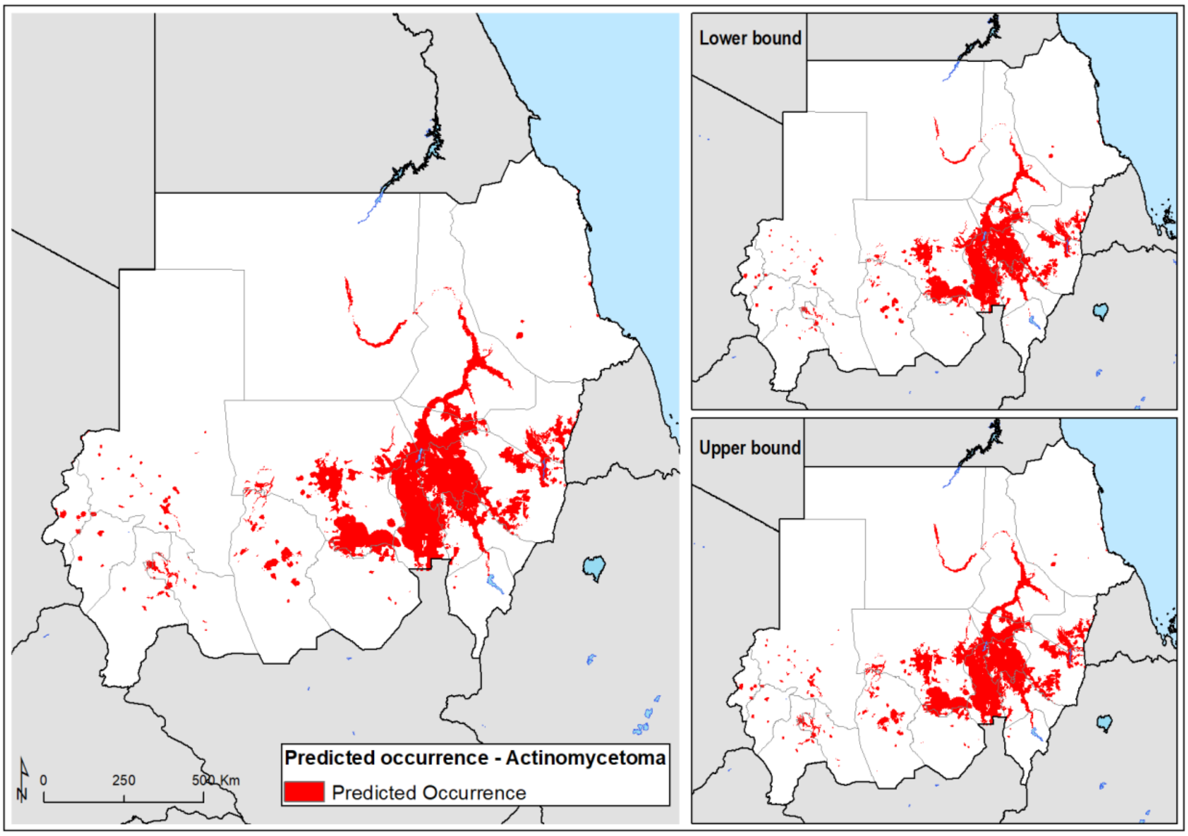


Figure 6: Predicted occurrence of actinomycetoma in Sudan based on a cut-off (value: 0. 578) that optimizes sensitivity and specificity.



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Supplementary file 2

Modelling the environmental suitability for the species of thorny vegetation present in Sudan

An ensemble of distribution models was generated for each species of thorny vegetation that are reportedly present in Sudan (see Table 1S) according to three sources: the Invasive Species Compendium (https://www.cabi.org/ISC), the ILDIS LegumeWeb (http://www.ildis.org/LegumeWeb/) and PROTAbase (https://www.prota4u.org/database/). These species are: *Acacia flava, A. laeta, A. mellifera, A. nilotica, A. senegal, A. seyal, A tortilis, Balanites aegyptiaca, Faidherbia albida, Dichrostachys cinerea, and Euphorbia candelabrum*. Geolocated (provided longitude and latitude) records of occurrences for each of the 11 species were downloaded from the GBIF database [145, 146]. In addition, a suite of 19 bioclimatic factors obtained from the WorldClim database were used to construct the ensemble models along with the georeferenced records of species occurrence [147]. The WorldClim (version 2.1) database provides a set of global climate and weather datasets obtained by interpolation of precipitation data for the period 1970–2000 collected in weather stations distributed across the world [148]. The datasets are provided as continuous raster surfaces at a minimum of 1 sq-km resolution.

We used seven algorithms available within the BIOMOD framework [149] to obtain those ensembles of predicted distributions: generalized linear models (GLM), generalized additive models (GAM), generalized boosted regression models (GBM), artificial neural networks (ANN), multiple adaptive regression splines (MARS), maximum entropy (MaxEnt) and random forest (RF). These models were run using the parameters set by default in the *biomod2* R package [149].

All these models are intended to discriminate the suitability of the environment for the presence of a particular organism, and for this they need to be trained with presence and absence records. When there are no available absences records, an alternative is to generate background points and/or pseudo-absences [150]. We generated sets of the same number of background points as presence data compiled for every species of thorny vegetation. Background points were randomly generated accounting for the underlying geographical bias on the occurrence data, as previously recommended [151]. For this, we created a sampling bias surface by counting the number of occurrence records within each grid cell (5km x 5km resolution) and then extrapolated these data across Africa using kernel density estimation using the R packages *kernlab*, *ks* and *sm* . Lastly, we generated the background points from random locations weighted by the sampling bias surface [152, 153]. In addition, pseudoabsences points, same number than occurrence, were randomly generated from countries where these species have not been reported (see Table 2S). Pseudoabsences points were given a weight of 0.5 due to the underlying uncertainty of these species not being reported in the three botanic libraries abovementioned.

Models were calibrated using an 80% random sample of the initial data and evaluated against the remaining 20% data using the area under the curve (AUC) of the receiver operation characteristic (ROC) and the true skill statistic (TSS) [154]. TSS compares the number of correct predictions, minus predictions attributable to random guessing [155], taking into account both sensitivity and specificity. Its value ranges from -1 to +1, where +1 indicates perfect score, 0 indicates random performance and values of 0.5 or higher are generally considered to be acceptable for well model performance [155, 156]. TSS value is not affected by the size of validation data set. Projections were performed 50 times per algorithm (350 in total), each time selecting a different 80% random sample while verifying model accuracy against the remaining 20%. The evaluation statistics (AUC and TSS) were used to select the models to be assembled on the basis of matching between predictions and observations. Here, models with AUC < 0.7 were disregarded when constructing the final assemble model.

The final assemble model was obtained by estimating a weighted mean of probabilities across the selected models per species of thorny plants and per grid cell [157]. The weight assign to each single model was given by the ROC value obtained through cross-validation with the 20% held-out subsample. The range of uncertainties obtained with the seven modelling techniques was also calculated by estimating the confidence intervals across the ensemble for each species and per grid cell (see Figures 1S-22S).

Sensitivity and specificity were calculated and a threshold value that maximizes it (optimal threshold value for each condition) was considered to generate binary maps that display areas where the presence of this species of thorny plants is more likely based on environmental suitability.

A final map of diversity of thorny plants across Africa was obtained by overlapping the maps of predicted occurrence for each species. Then, this map shows the number of species of thorny plants reportedly present in Sudan by 5km x 5km area (see Figure 23S).

Figure 1S. Environmental suitability for *Acacia flava* across Africa and prediction uncertainty (95% confidence interval).

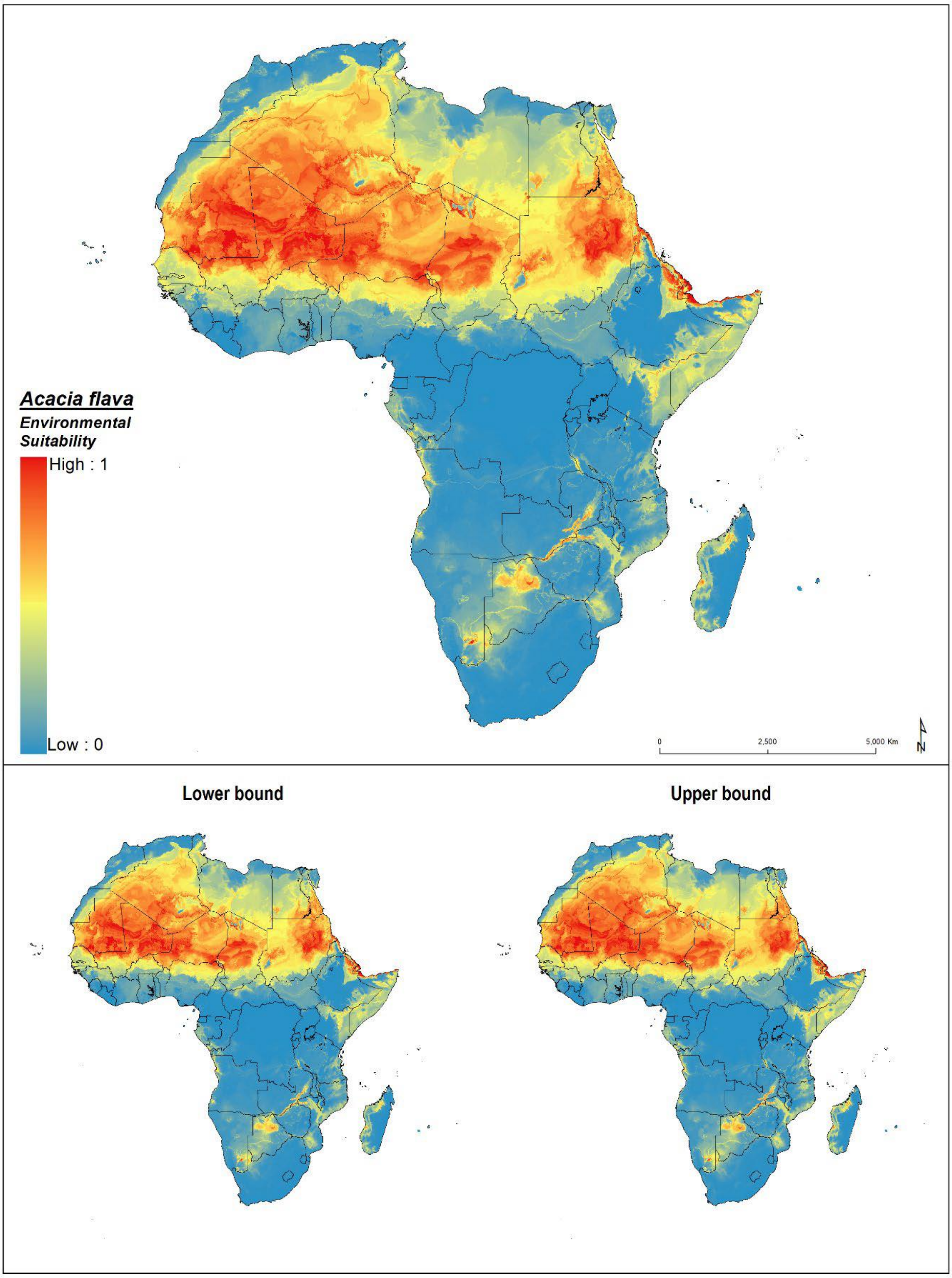

Figure 2S. Predicted occurrence for *Acacia flava* across Africa and uncertainty.

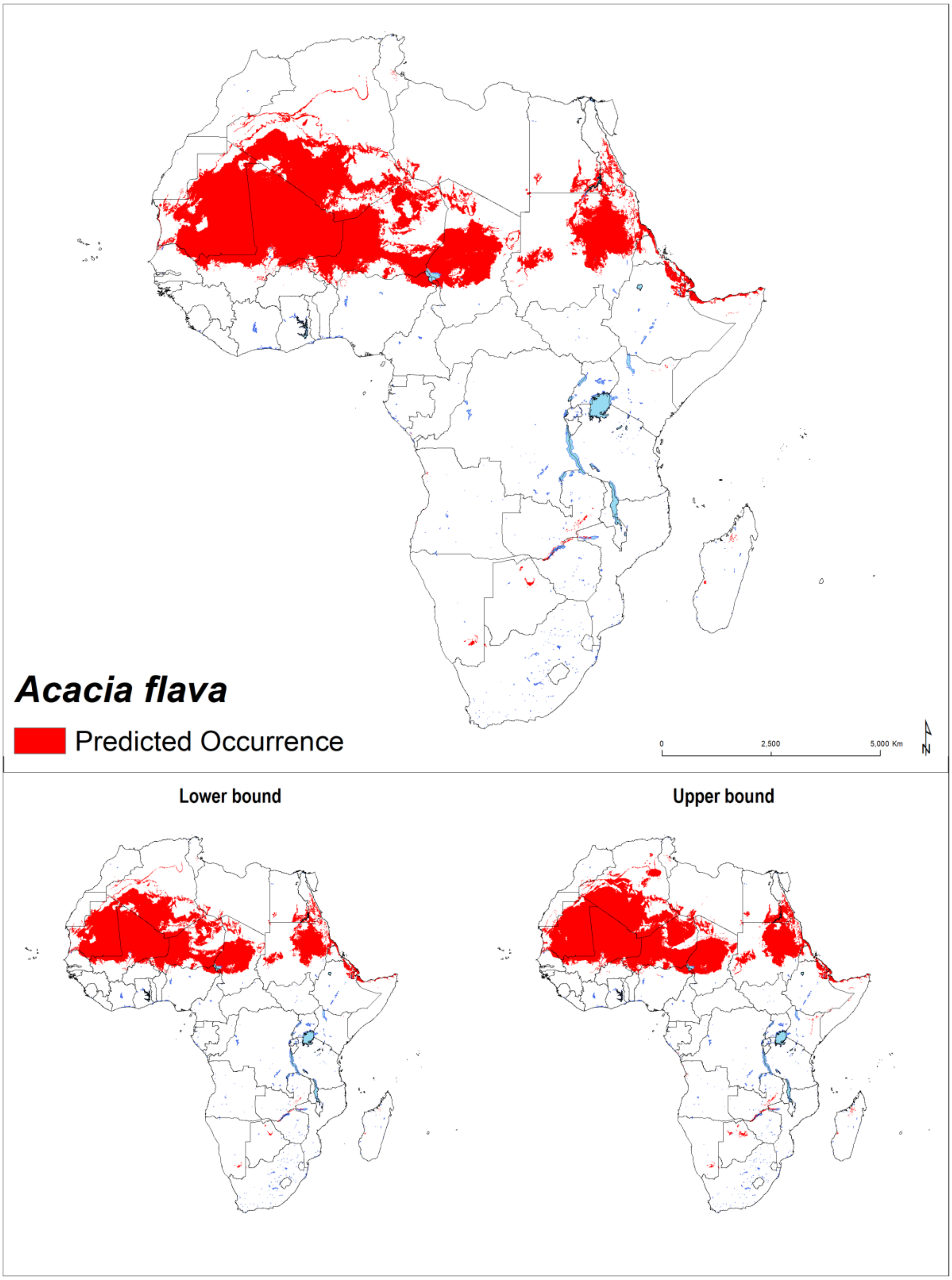

Figure 3S. Environmental suitability for *Acacia laeta* across Africa and prediction uncertainty (95% confidence interval).

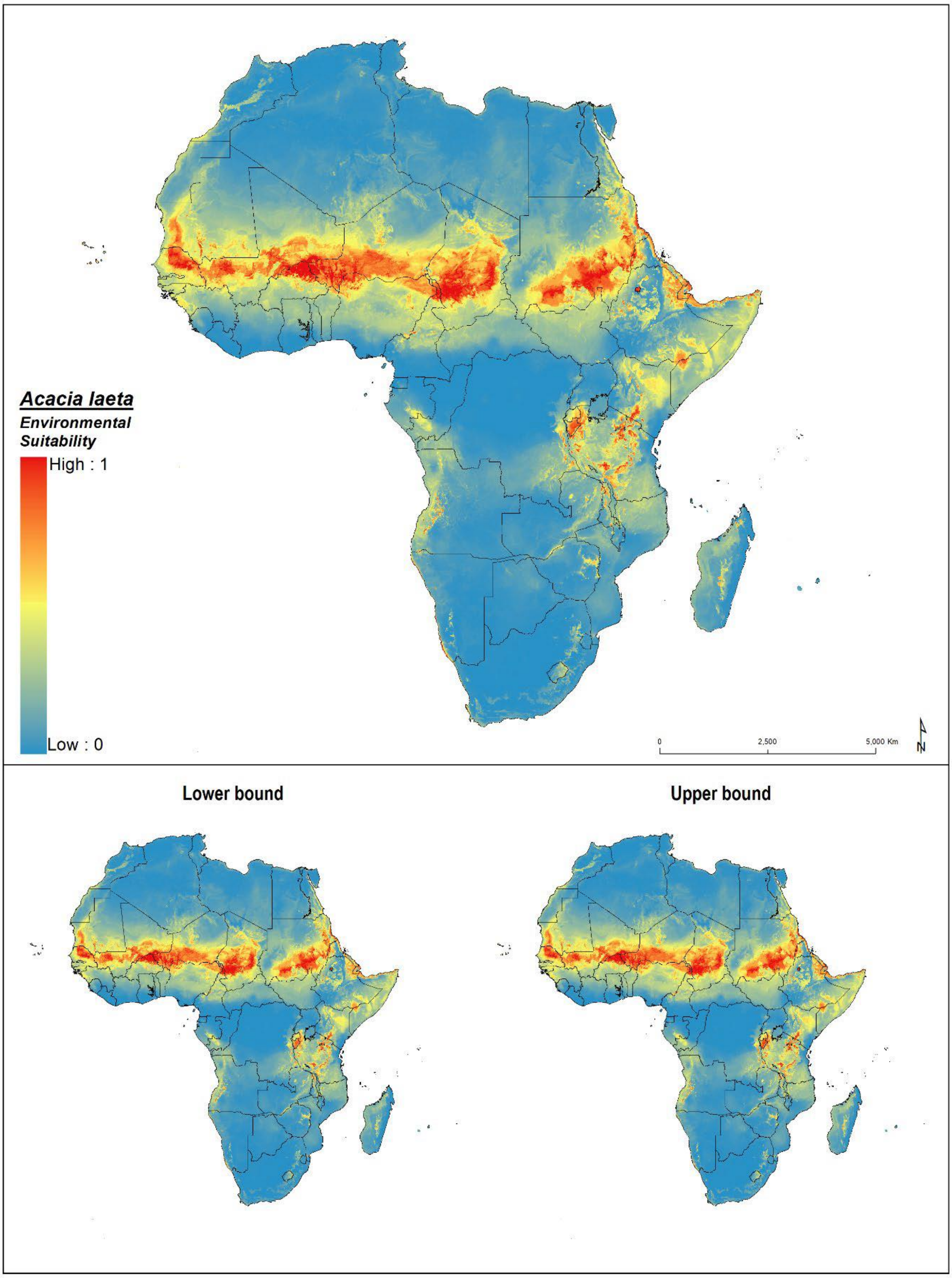

Figure 4S. Predicted occurrence for *Acacia laeta* across Africa and uncertainty.

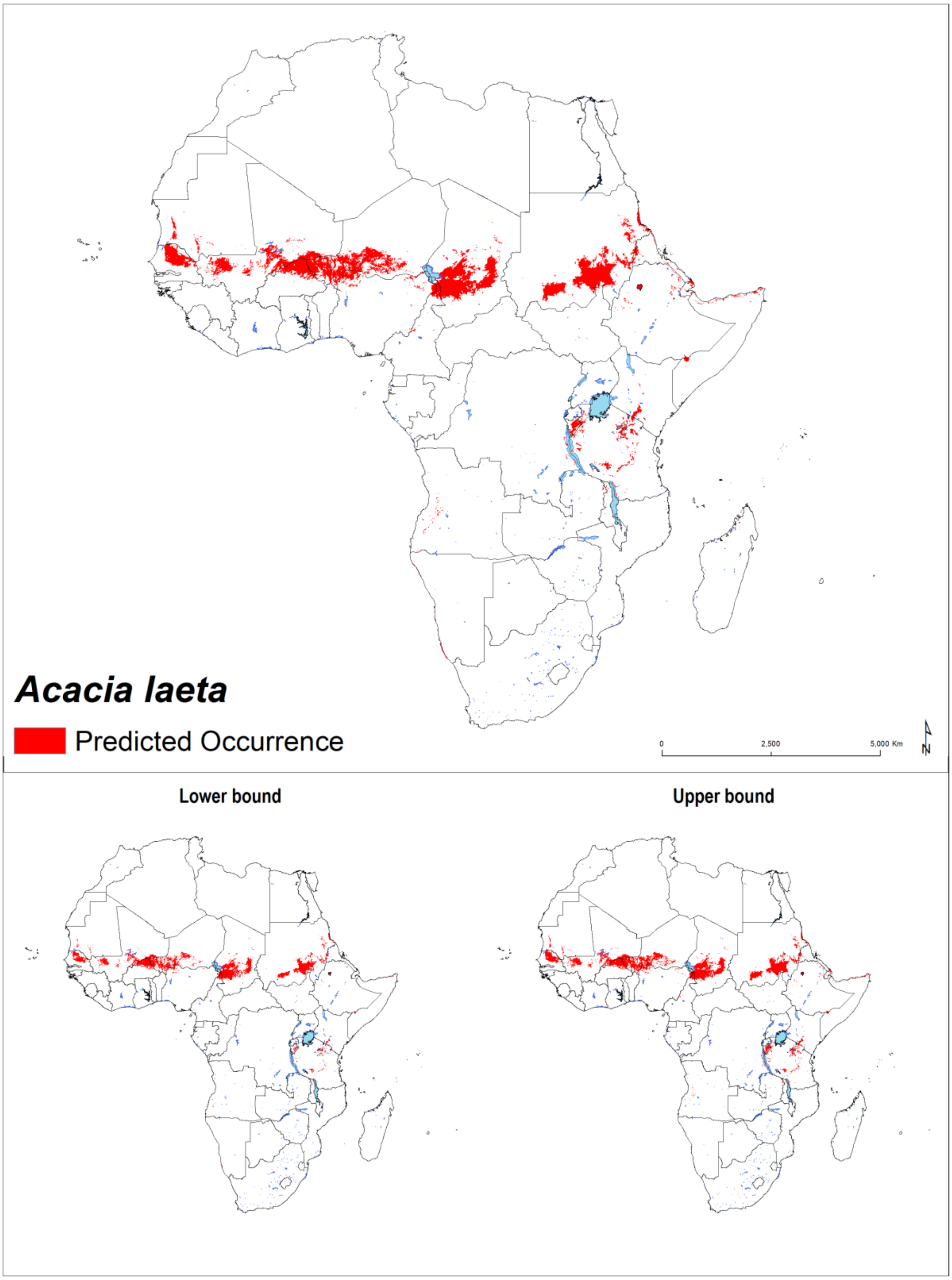

Figure 5S. Environmental suitability for *Acacia mellifera* across Africa and prediction uncertainty (95% confidence interval).

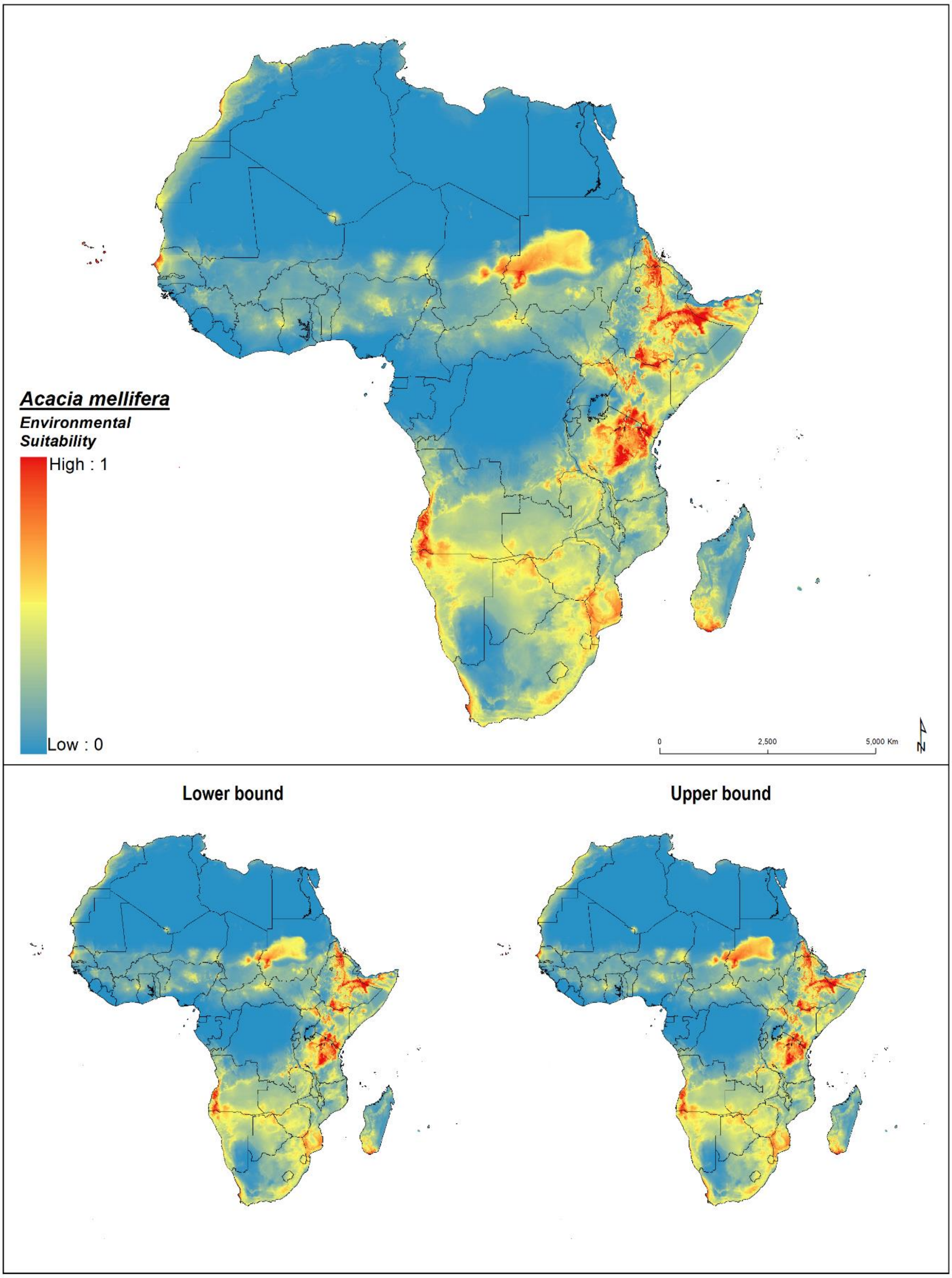

Figure 6S. Predicted occurrence for *Acacia mellifera* across Africa and uncertainty.

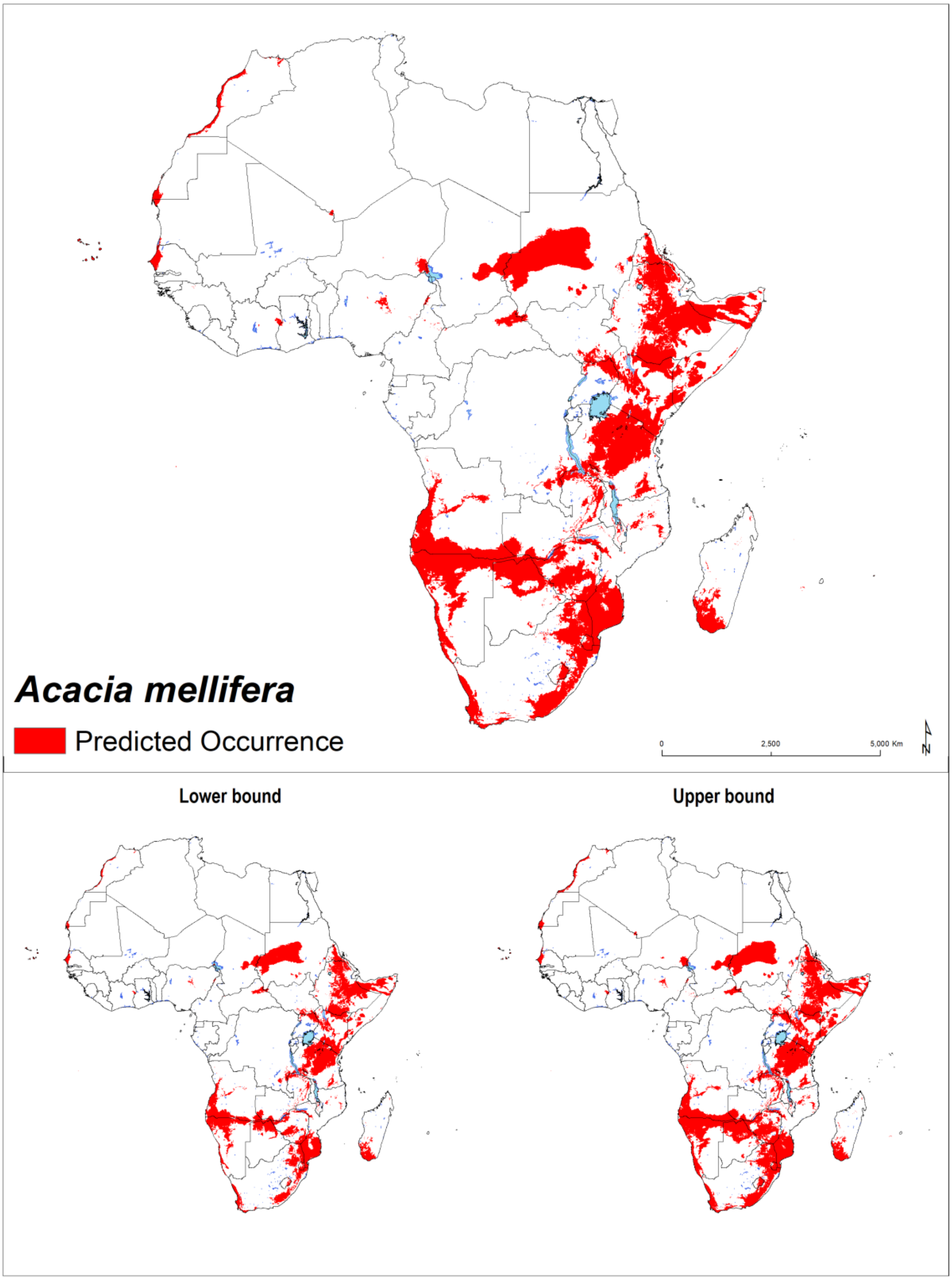

Figure 7S. Environmental suitability for *Acacia nilotica* across Africa and prediction uncertainty (95% confidence interval).

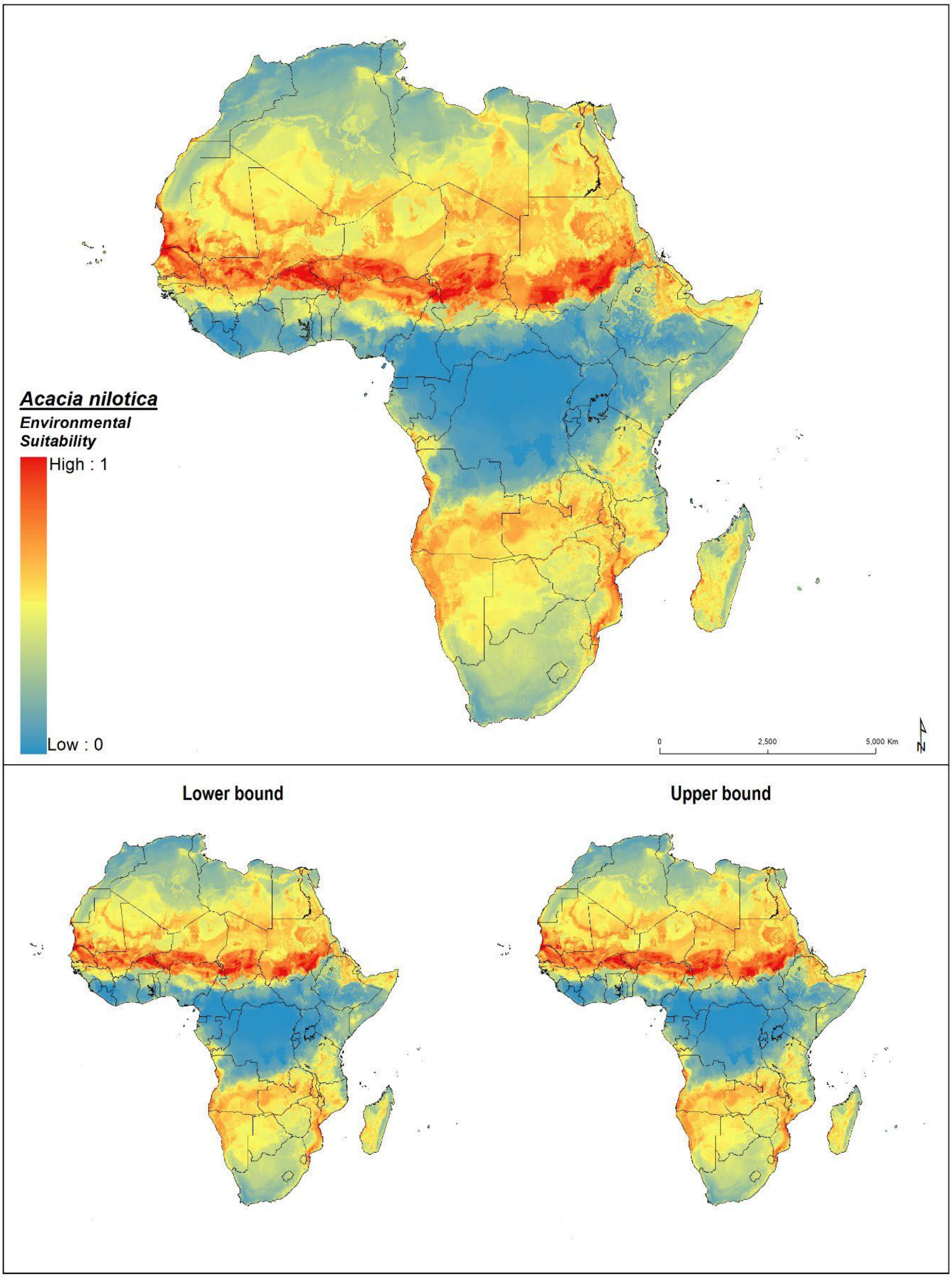

Figure 8S. Predicted occurrence for *Acacia nilotica* across Africa and uncertainty.

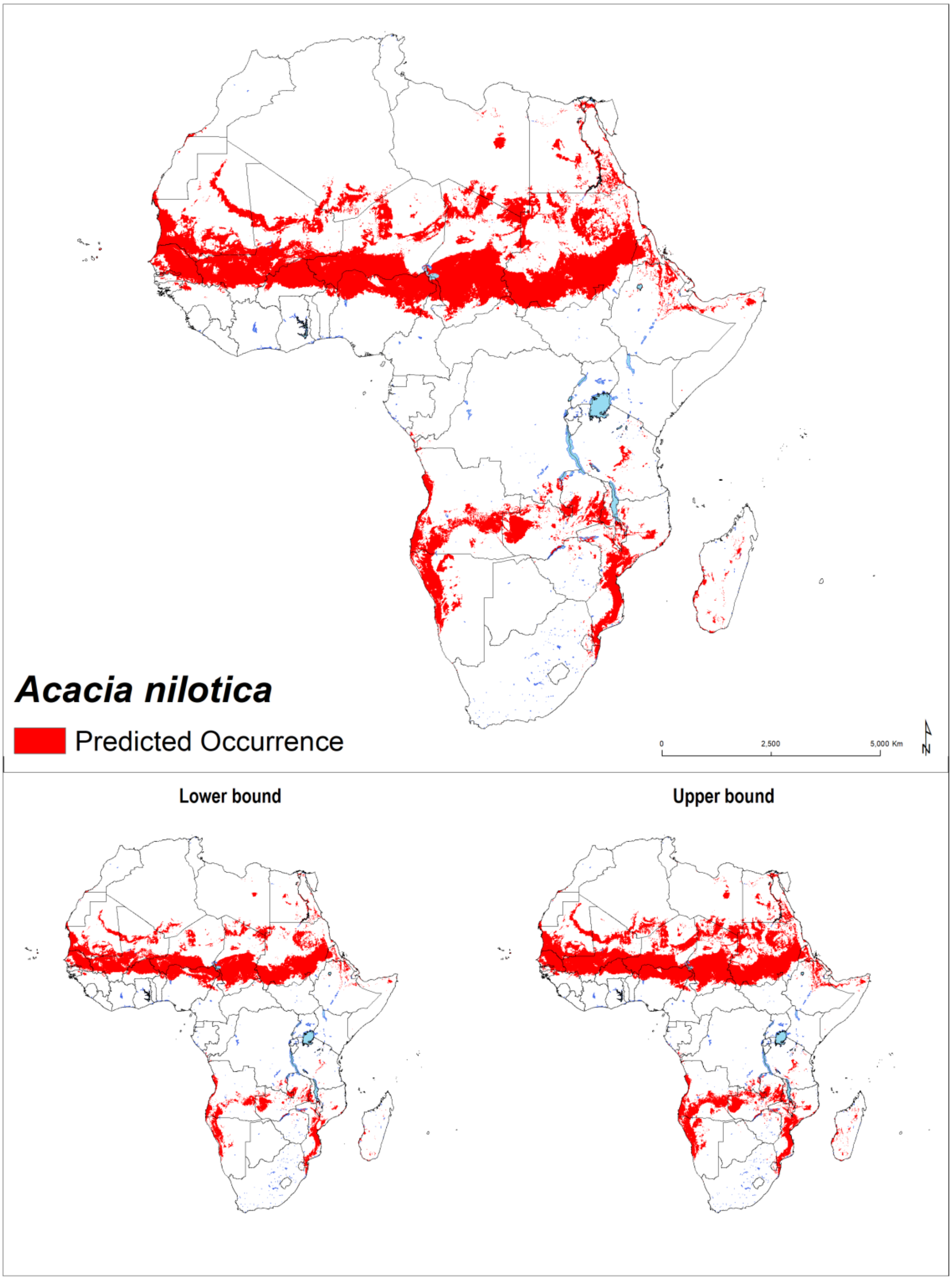

Figure 9S. Environmental suitability for *Acacia senegal* across Africa and prediction uncertainty (95% confidence interval).

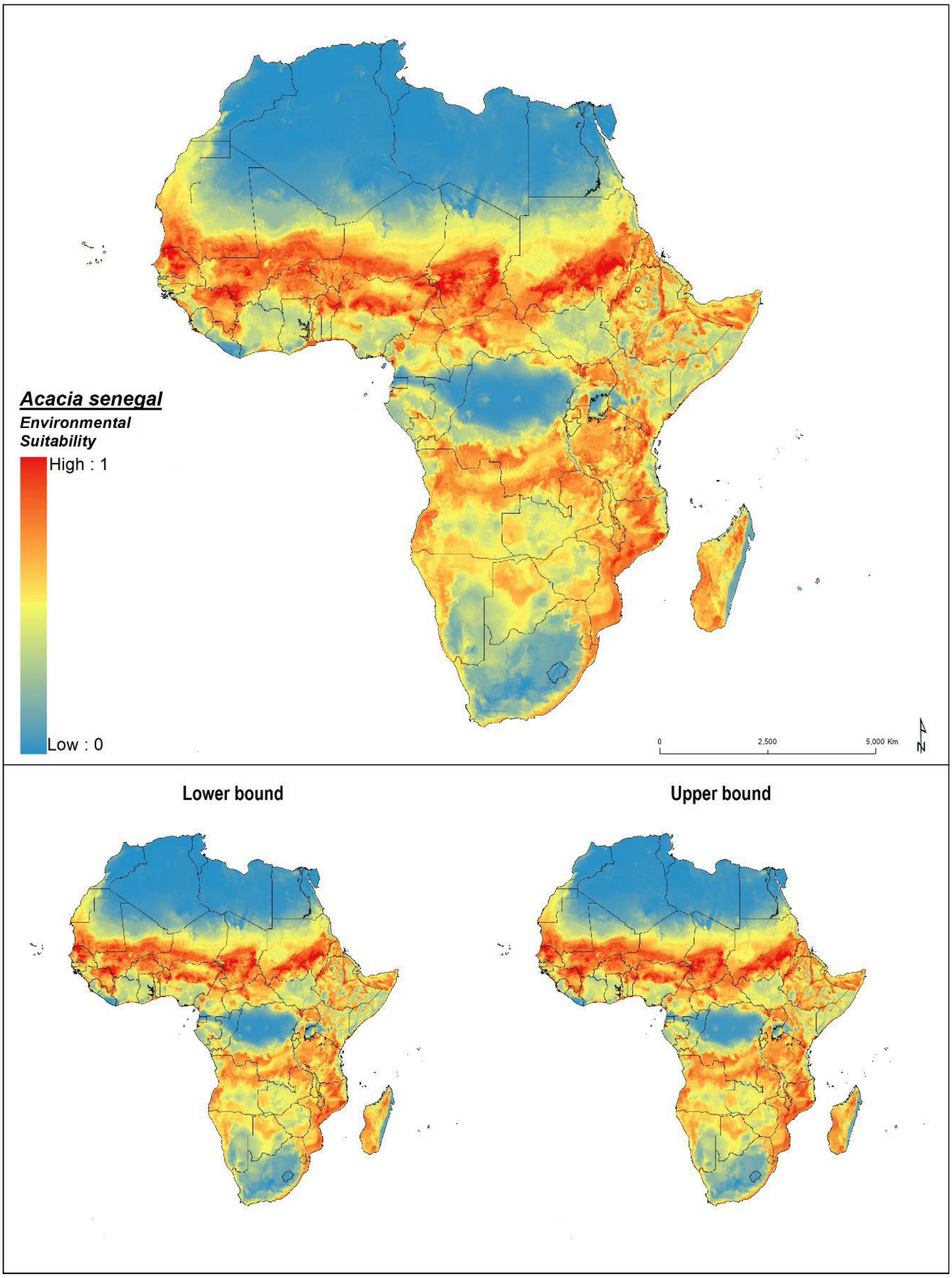

Figure 10S. Predicted occurrence for *Acacia senegal* across Africa and uncertainty.

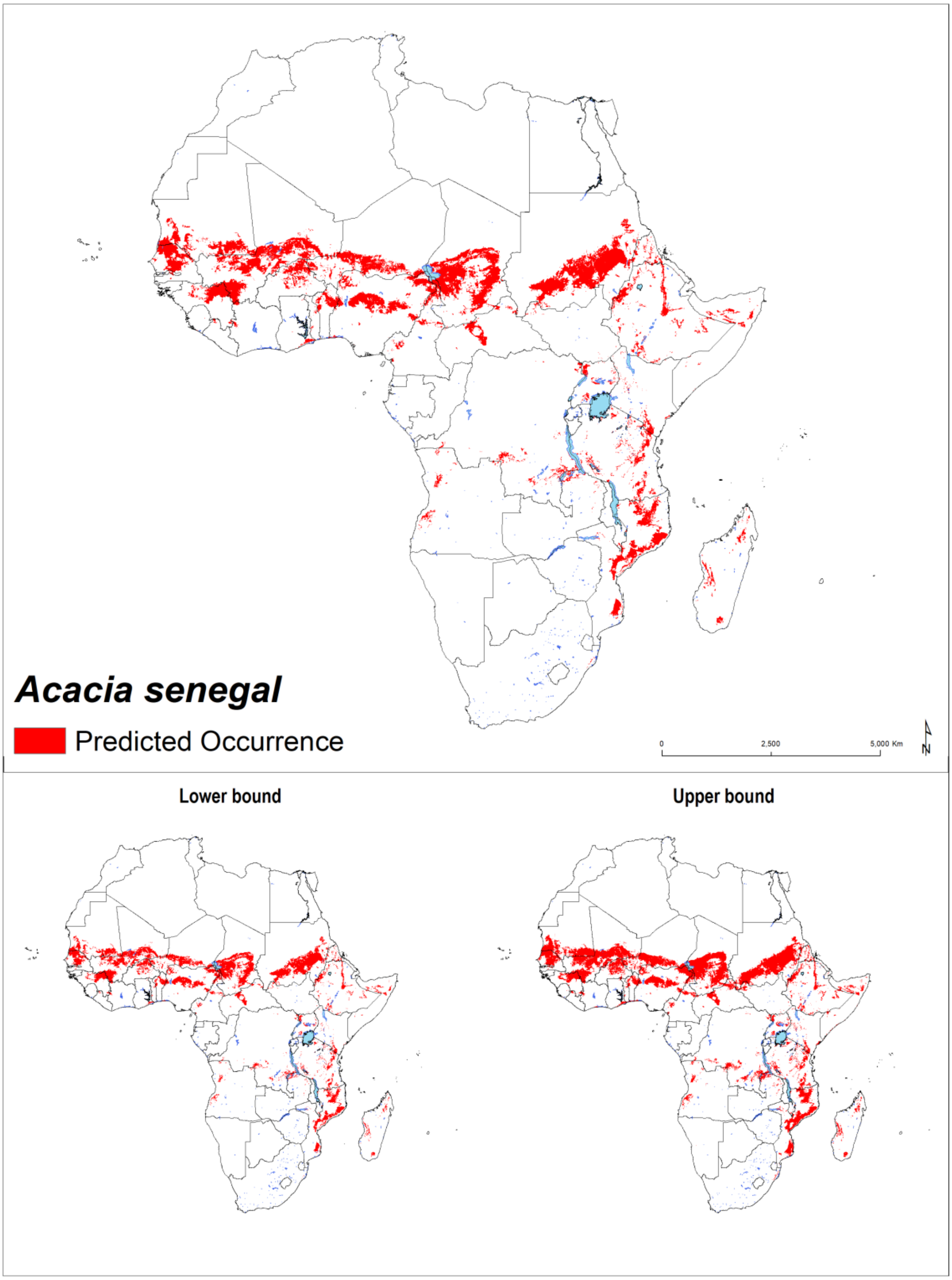

Figure 11S. Environmental suitability for *Acacia seyal* across Africa and prediction uncertainty (95% confidence interval).

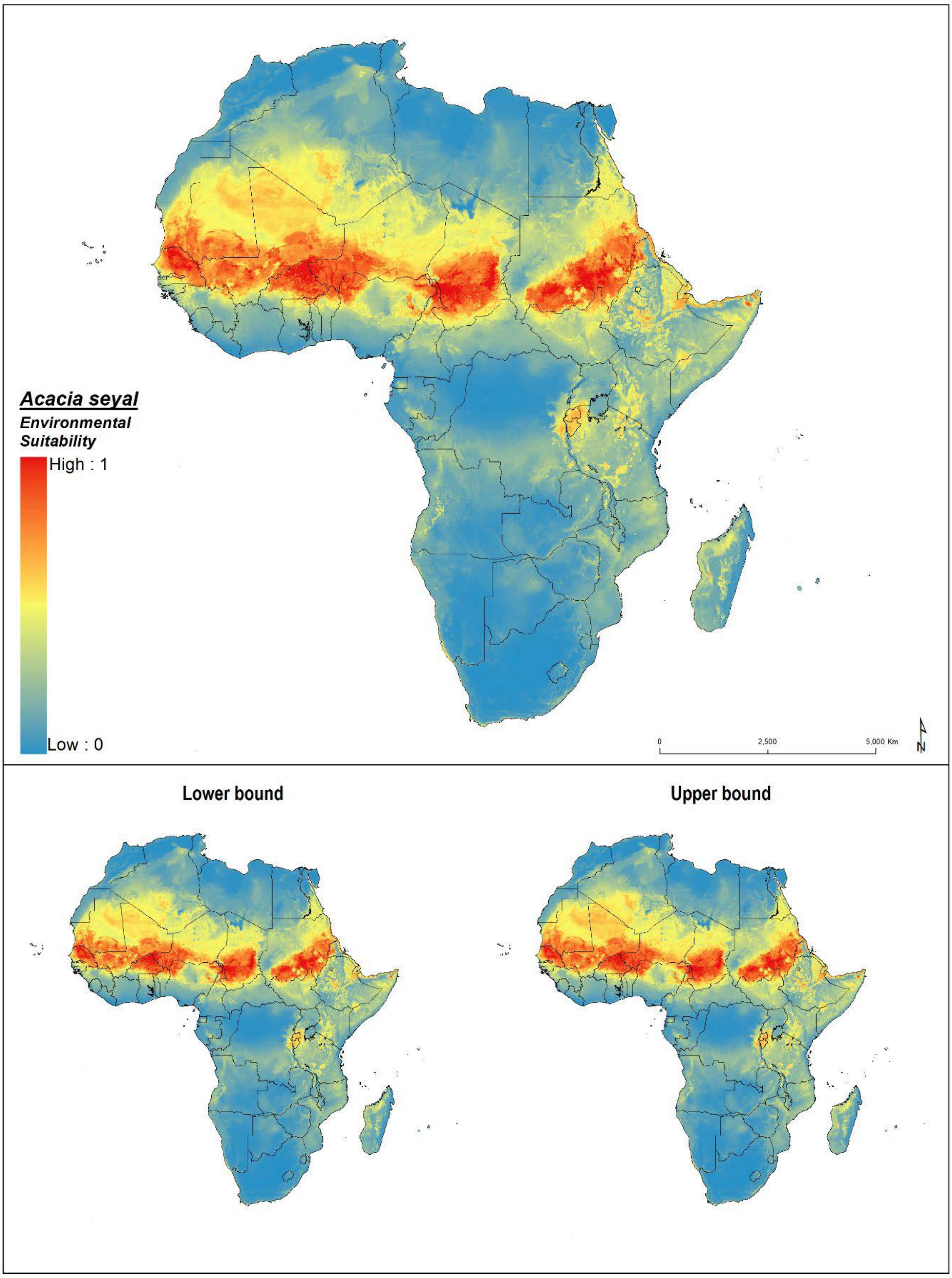

Figure 12S. Predicted occurrence for *Acacia seyal* across Africa and uncertainty.

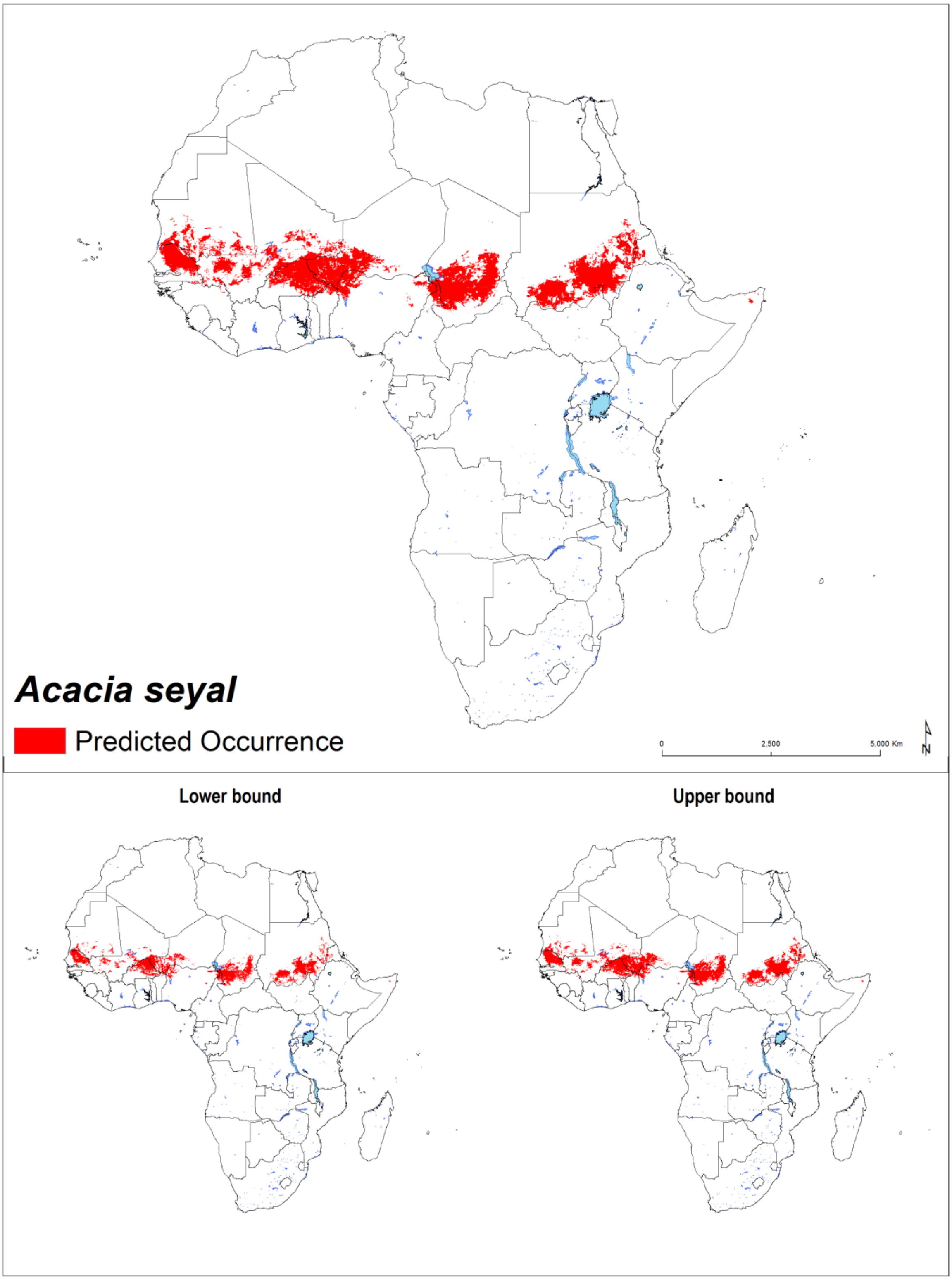

Figure 13S. Environmental suitability for *Acacia tortilis* across Africa and prediction uncertainty (95% confidence interval).

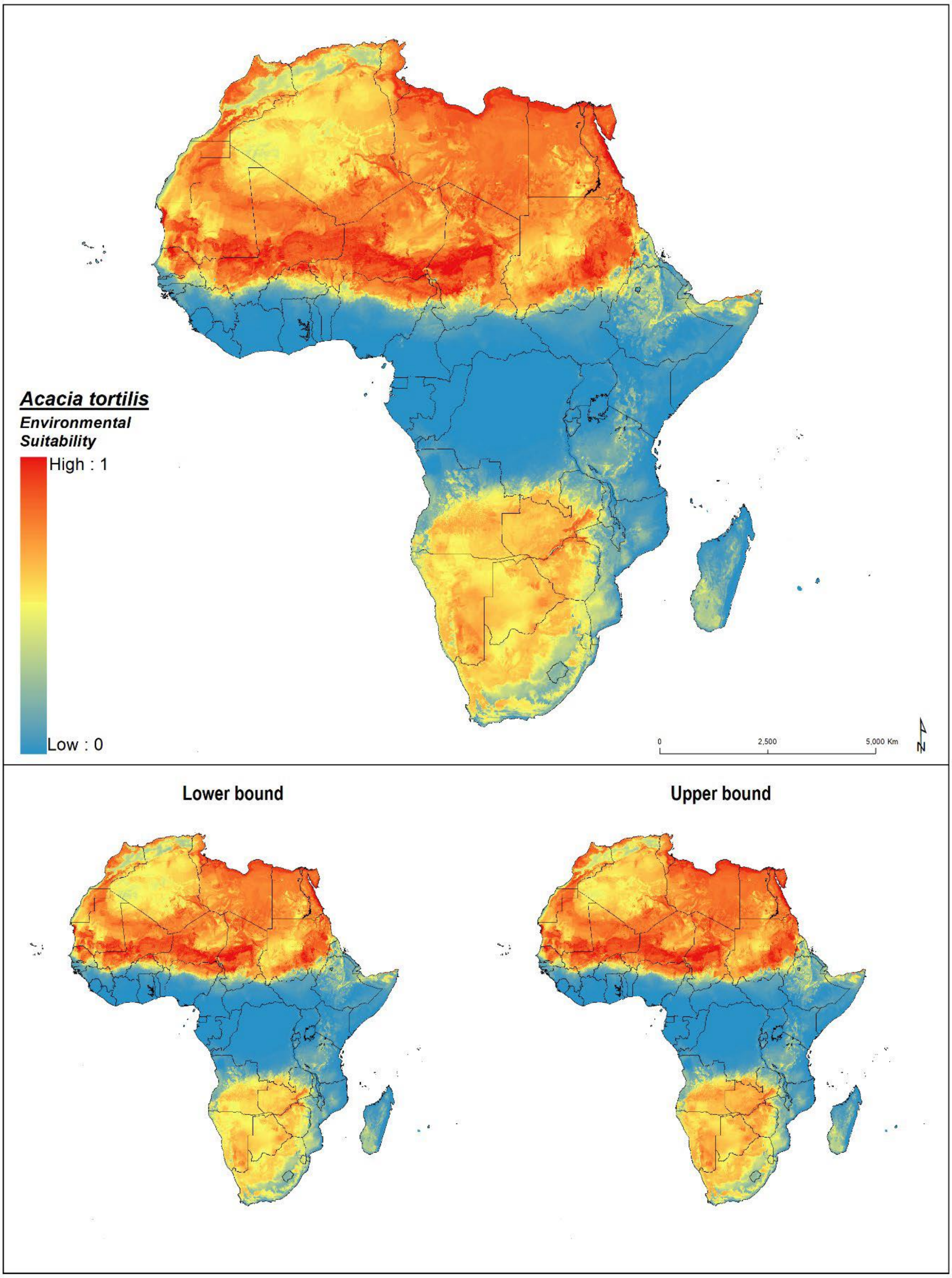

Figure 14S. Predicted occurrence for *Acacia tortilis* across Africa and uncertainty.

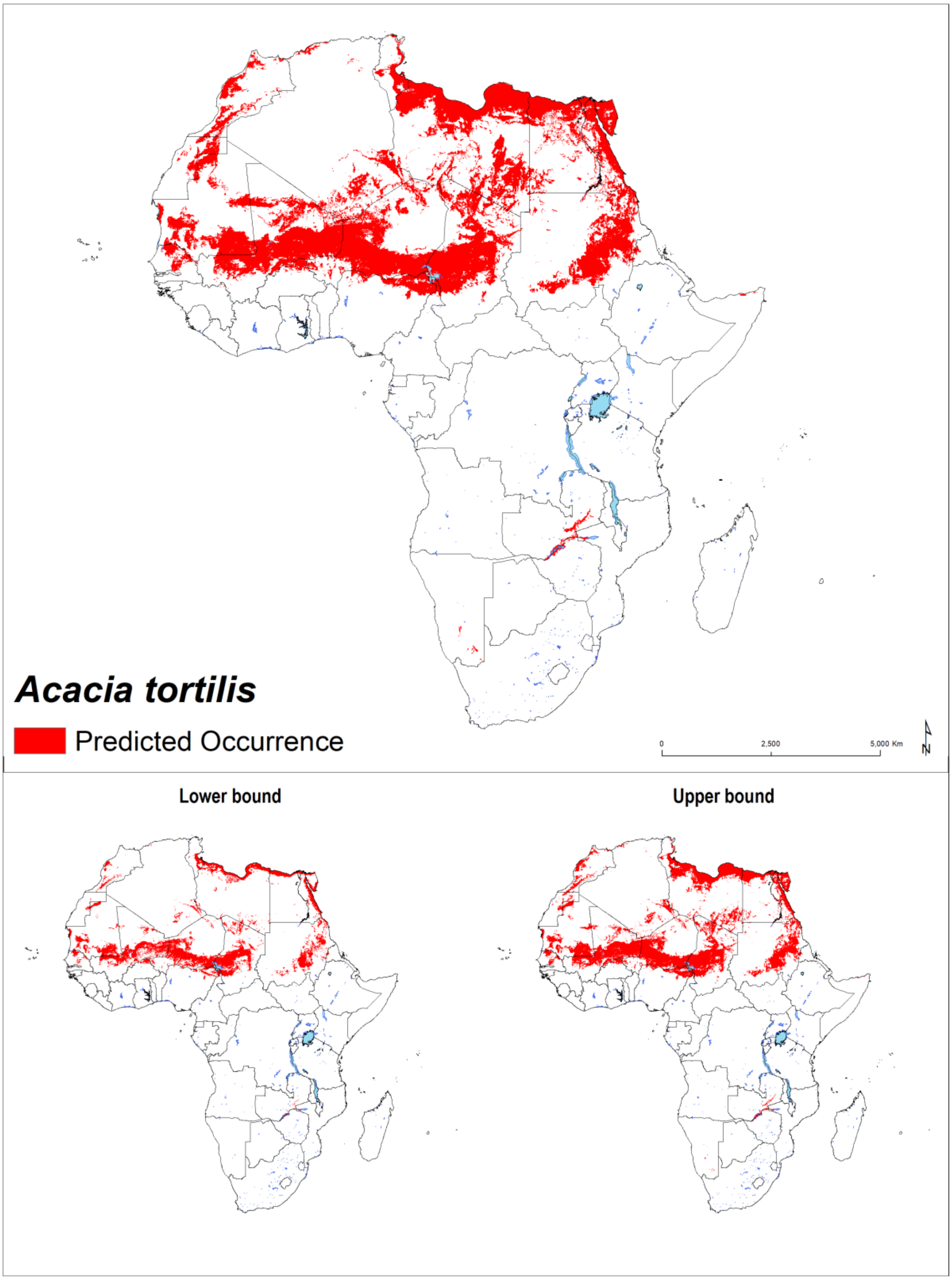

Figure 15S. Environmental suitability for *Balanites aegyptiaca* across Africa and prediction uncertainty (95% confidence interval).

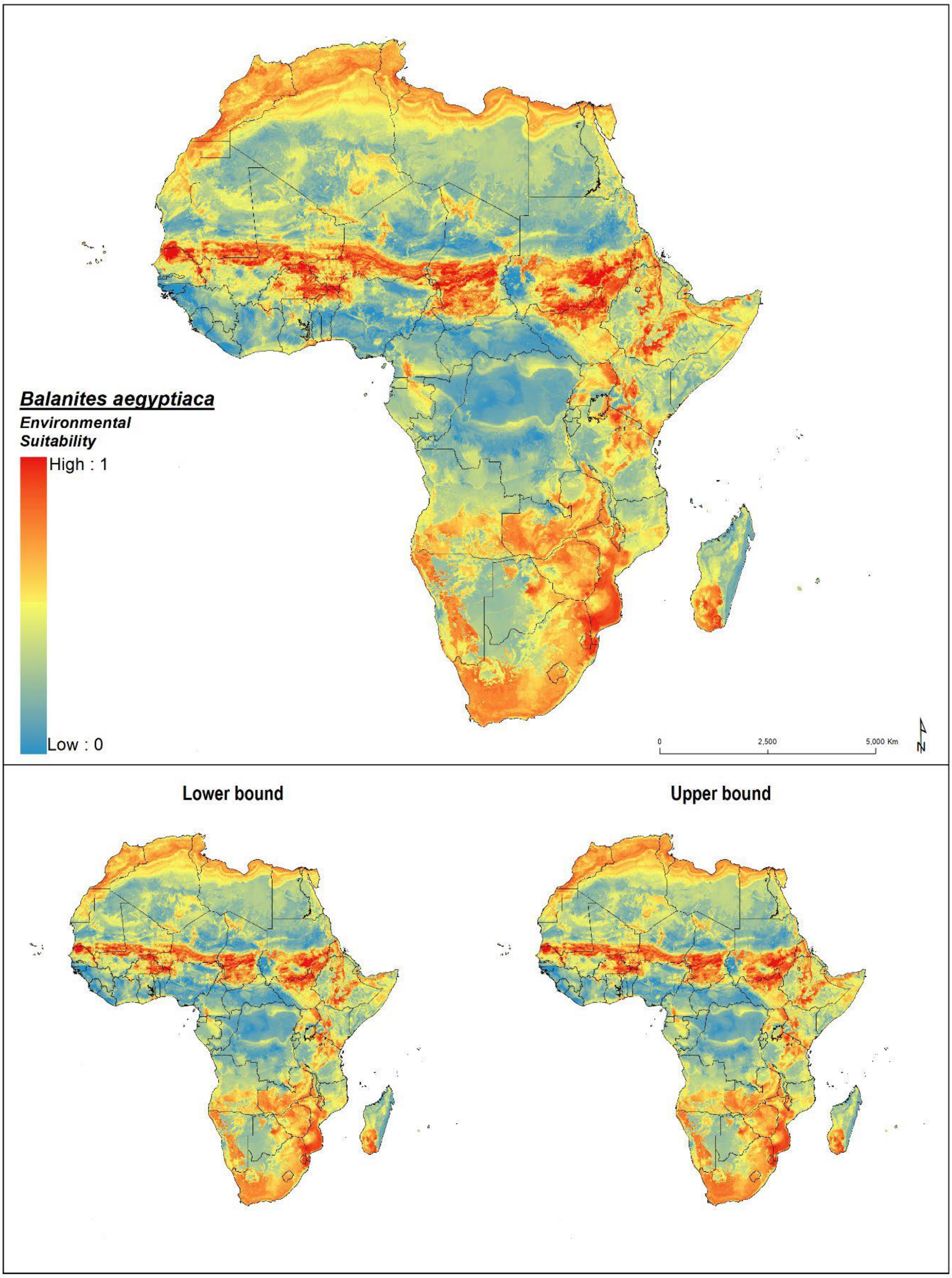

Figure 16S. Predicted occurrence for *Balanites aegyptiaca* across Africa and uncertainty.

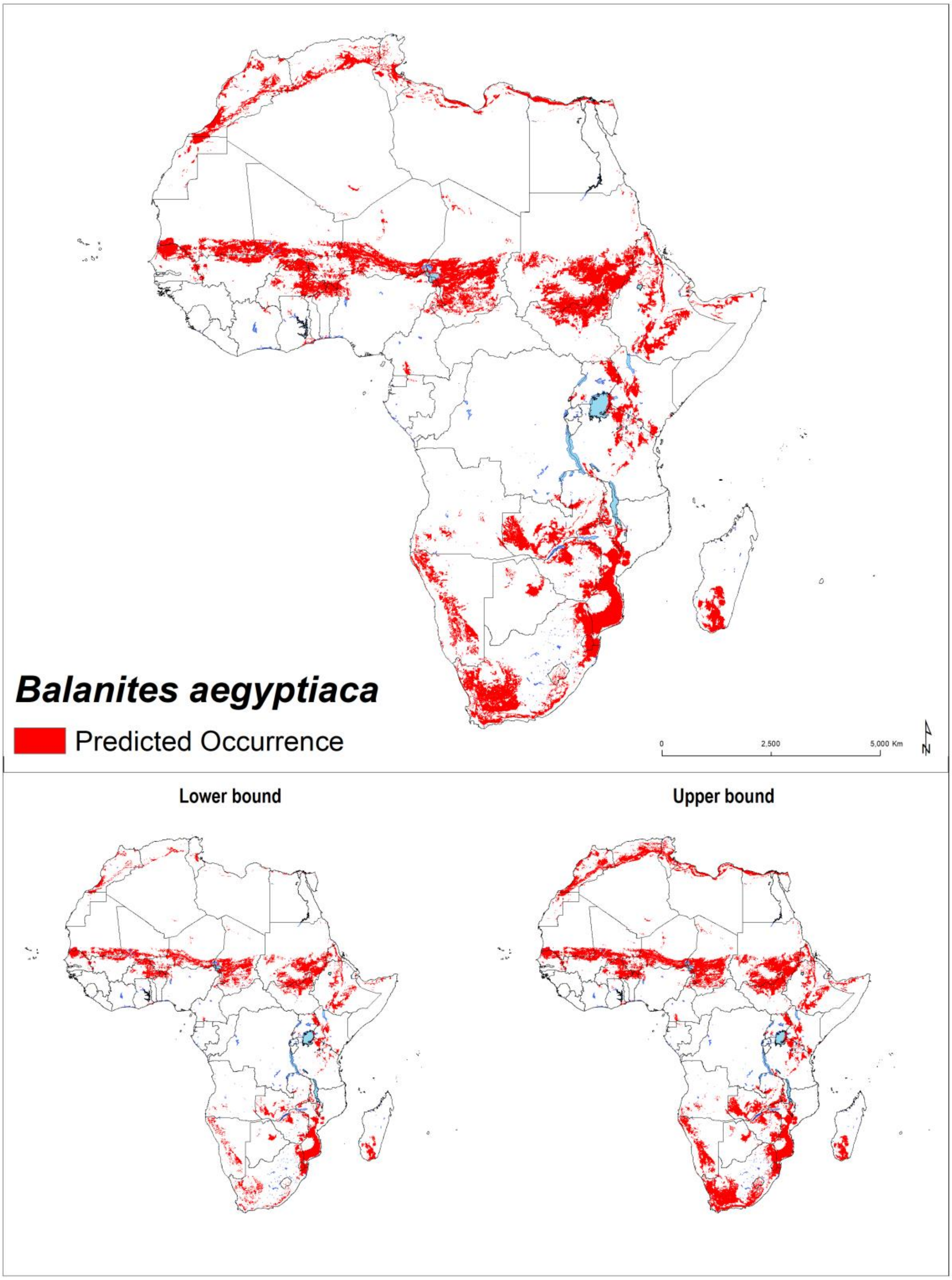

Figure 17S. Environmental suitability for *Faidherbia albida* across Africa and prediction uncertainty (95% confidence interval).

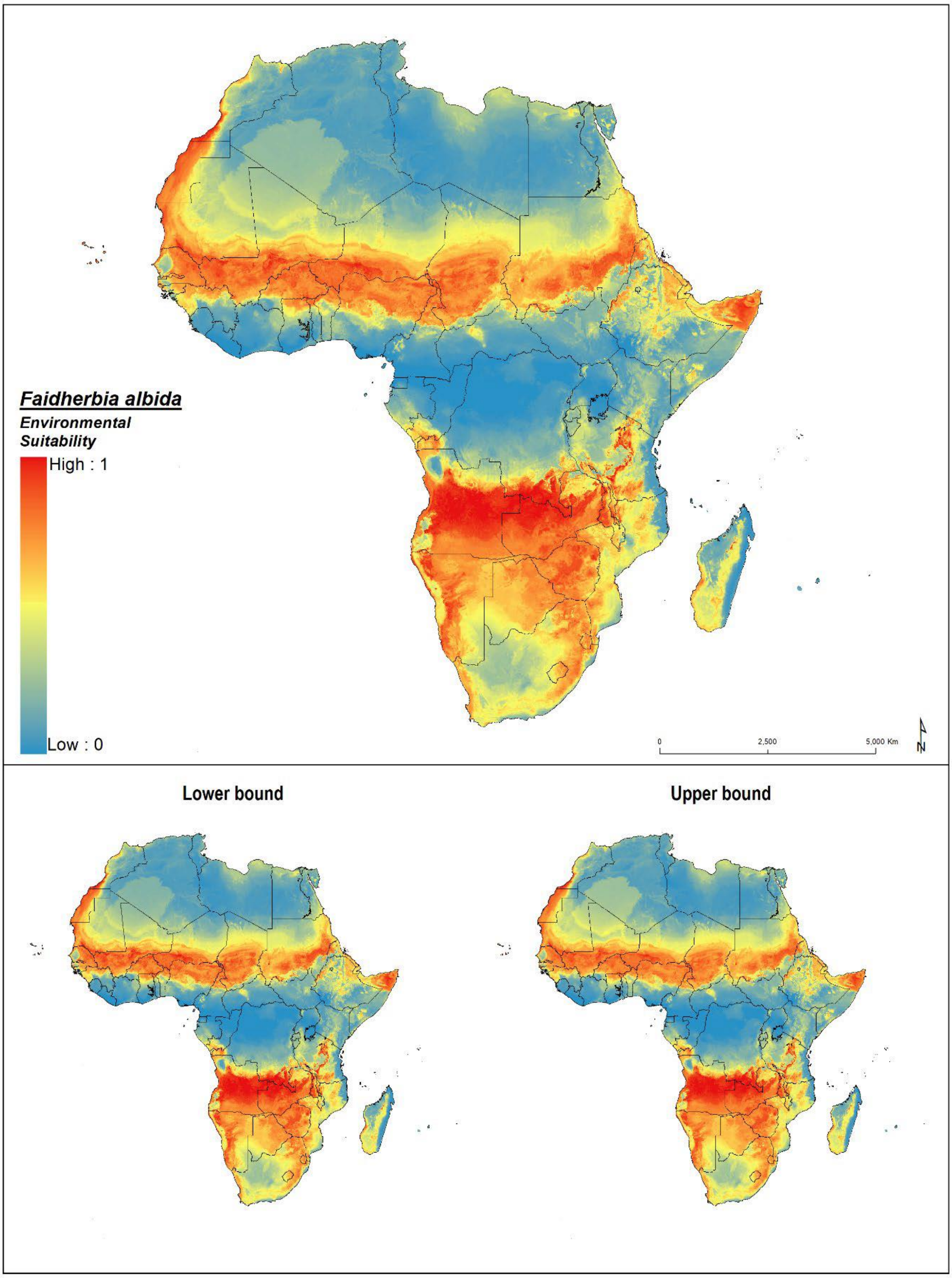

Figure 18S. Predicted occurrence for *Faidherbia albida* across Africa and uncertainty.

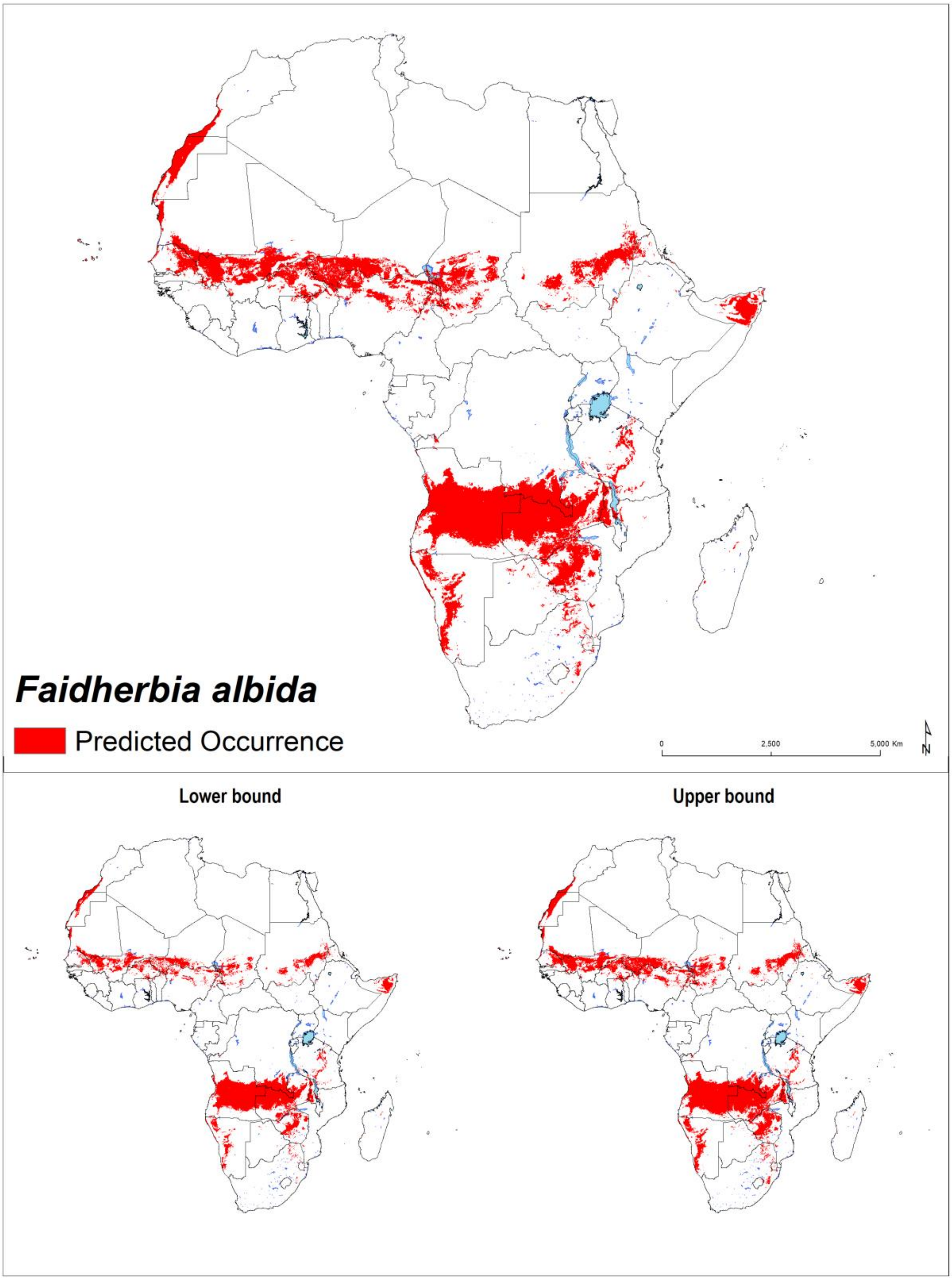

Figure 19S. Environmental suitability for *Dichrostachys cinerea* across Africa and prediction uncertainty (95% confidence interval).

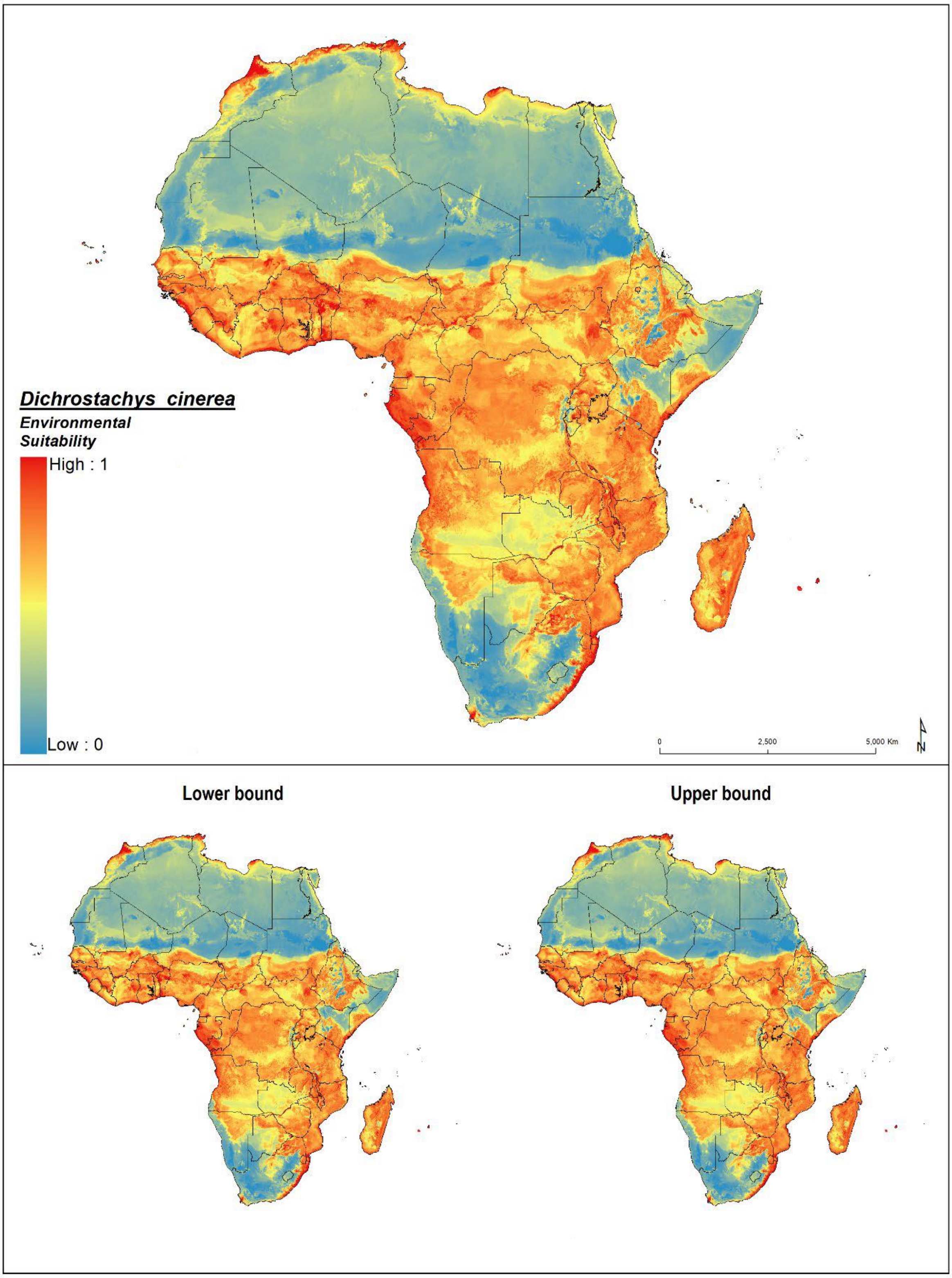

Figure 20S. Predicted occurrence for *Dichrostachys cinerea* across Africa and uncertainty.

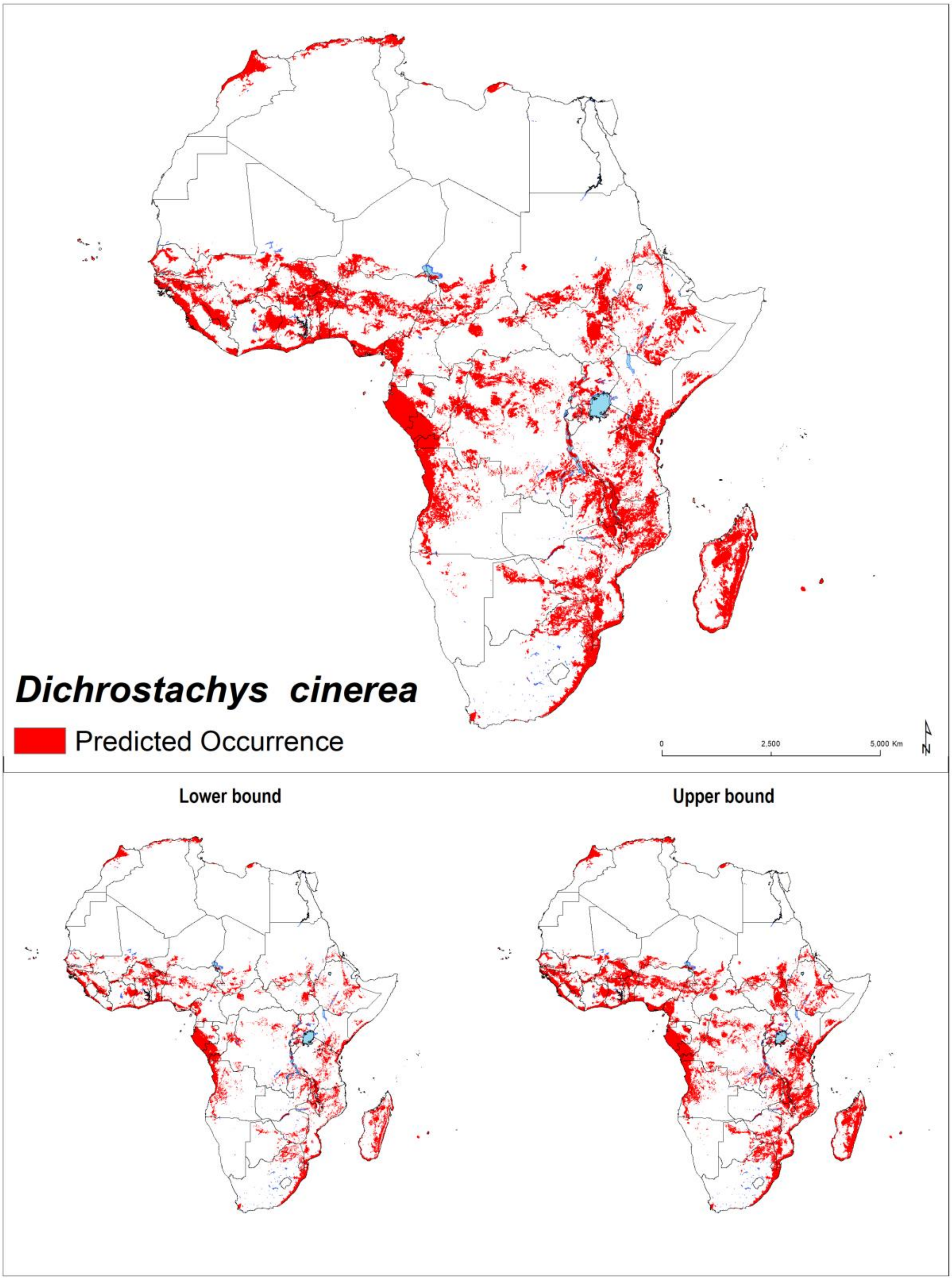

Figure 21S. Environmental suitability for *Euphorbia candelabrum* across Africa and prediction uncertainty (95% confidence interval).

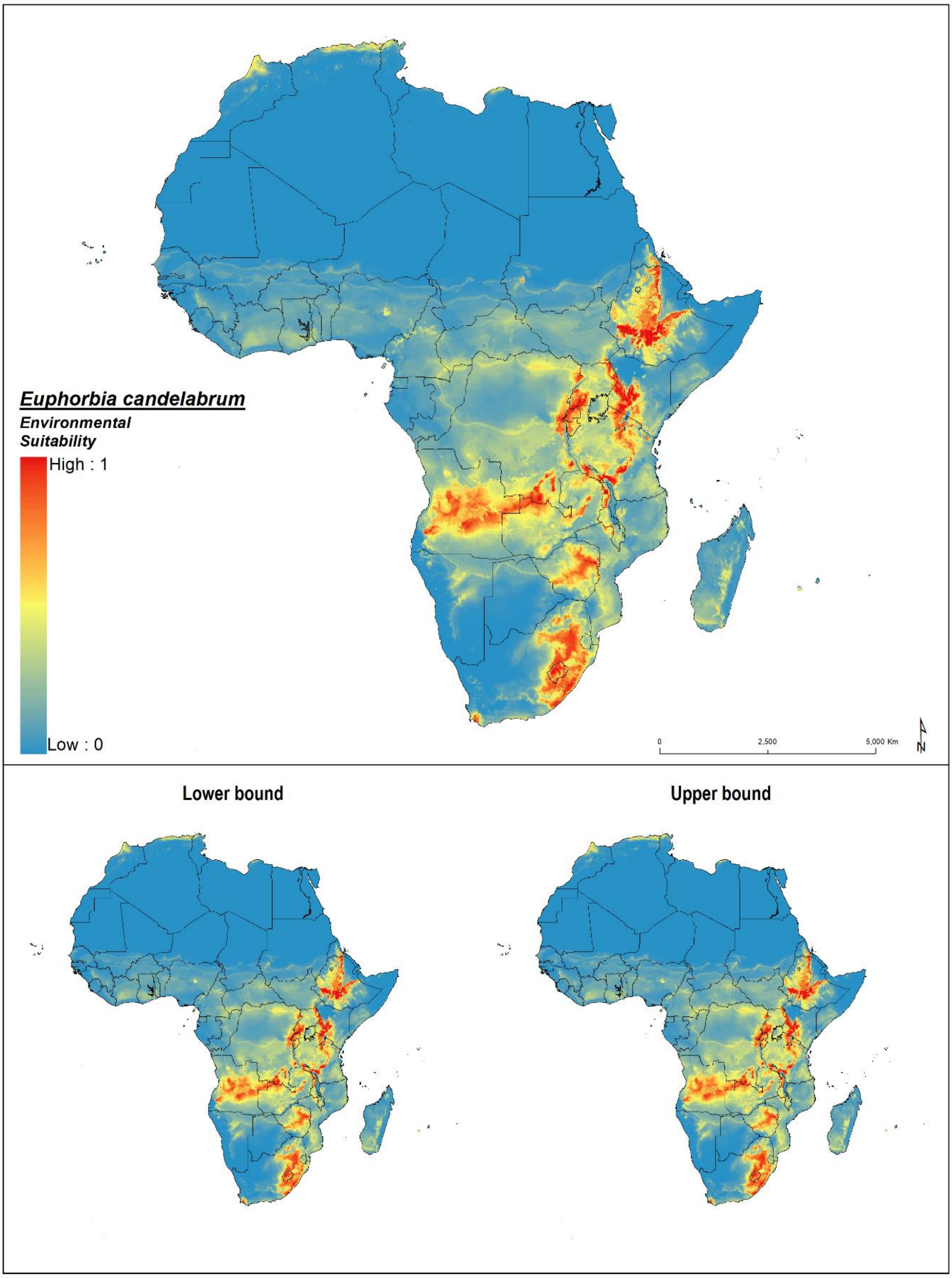

Figure 22S. Predicted occurrence for *Euphorbia candelabrum* across Africa and uncertainty.

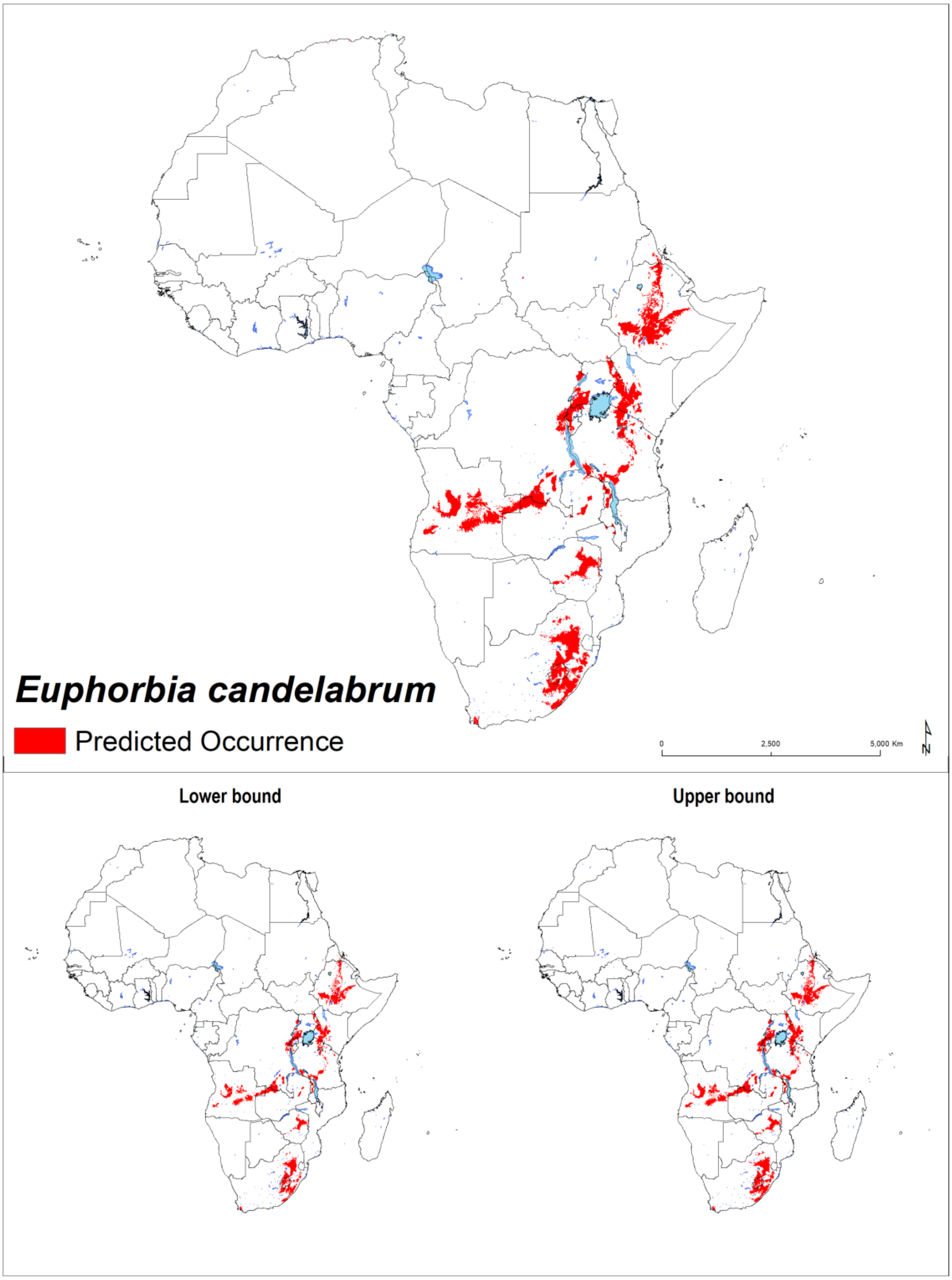

Figure 23S. Number of thorny vegetation species described in Sudan (11) potentially present by 5km x 5km area.

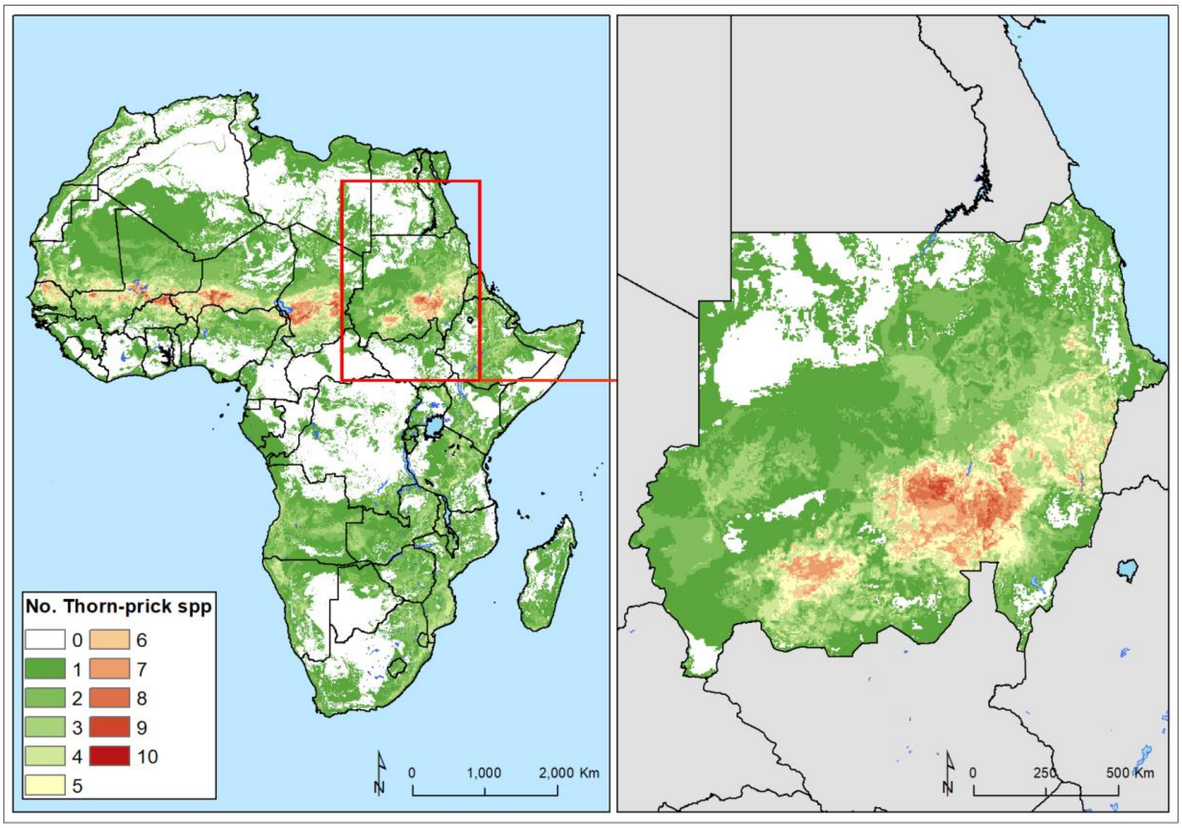

Table 1S. Thorny vegetation species (trees and shrubs) present in Sudan according to the Invasive Species Compendium (https://www.cabi.org/ISC), the ILDIS LegumeWeb (http://www.ildis.org/LegumeWeb/) and PROTAbase (https://www.prota4u.org/database/)

Species	Description	Synonims	Common names
Acacia tortilis subs spirocarpa	Perennial non-climbing tree	*Mimosa tortilis*	
Acacia mellifera	Perennial non-climbing tree	*Mimosa mellifera*	Black thorns
Faidherbia albida	Perennial non-climbing tree	*Acacia albida*	White Acacia
Acacia senegal		*Senegalia senegal, Acacia circummarginata, Acacia cufodontii, Acacia glaucophylla, Acacia kinionge, Acacia oxyosprion, Acacia rupestris, Acacia senegal (L.) Willd. subsp. modesta, Acacia senegal (L.) Willd. subsp. senegalensis, Acacia somalensis, Acacia spinosa, Acacia thomasii, Acacia volkii, Mimosa senegal*	
Acacia ehrenbergiana	Perennial non-climbing tree	*Acacia flava*	Salam
Acacia laeta	Perennial non-climbing tree		Daga
Balanites aegyptiaca	Tree	*Agialid aegyptiaca, Ximenia aegyptiaca*	
Dichrostachys cinerea	semi-deciduous to deciduous tree	*Cailliea glomerata, Dichrostachys glomerata, Dichrostachys nutans, Dichrostachys platycarpa, Mimosa cinerea, Mimosa glomerata, Mimosa nutans*	Acacia Saint Dominique
Euphorbia candelabrum	Succulent	*Euphorbia calycina N.E.Br.* *Euphorbia confertiflora Volkens* *Euphorbia murielii N.E.Br.* *Euphorbia reinhardtii Volkens*	
Acacia nilotica subs nilotica	Perennial non-climbing tree	*Acacia arabica, Acacia scorpioides, Mimosa arabica, Mimosa nilotica, Mimosa scorpioides*	
Acacia seyal	Perennial non-climbing tree	*Acacia fistula, Acacia flava, Acacia stenocarpa*	

Table 2S. Distribution of thorny vegetation species (trees and shrubs) present in Sudan across Africa according to the Invasive Species Compendium (https://www.cabi.org/ISC), the ILDIS LegumeWeb (http://www.ildis.org/LegumeWeb/) and PROTAbase (https://www.prota4u.org/database/)

Sub-region	Country	*Acacia flava*	*Acacia laeta*	*Acacia mellifera*	*Acacia nilotica*	*Acacia senegal*	*Acacia seyal*	*Acacia tortilis*	*Balanites aegyptiaca*	*Faidherbia albida*	*Dichrostachys cinerea*	*Euphorbia candelabrum*
Central Africa	Angola			■	■	■		■	■	■	■	
	Cameroon				■	■	■		■	■	■	
	CAR					■	■		■		■	
	Chad	■	■	■	■	■	■	■	■	■	■	
	Congo								■		■	
	DR Congo					■			■	■	■	■
	Equatorial Guinea								■			
	Gabon								■		■	
	Sao Tome & Principe										■	
Eastern Africa	Burundi								■		■	■
	Comoros										■	
	Djibouti	■		■	■		■	■	■		■	
	Eritrea		■	■	■	■		■	■	■	■	
	Ethiopia	■	■	■	■	■	■	■	■	■	■	■
	Kenya		■	■	■	■	■	■	■	■	■	■
	Madagascar										■	
	Malawi			■	■		■		■	■	■	
	Mauritius				■						■	
	Mozambique			■	■	■	■	■	■	■	■	
	Reunion										■	
	Rwanda					■			■		■	■

Sub-region	Country	*Acacia flava*	*Acacia laeta*	*Acacia mellifera*	*Acacia nilotica*	*Acacia senegal*	*Acacia seyal*	*Acacia tortilis*	*Balanites aegyptiaca*	*Faidherbia albida*	*Dichrostachys cinerea*	*Euphorbia candelabrum*
	Seychelles											
	Somalia											
	South Sudan											
	Tanzania (Mainland)											
	Tanzania (Zanzibar)											
	Uganda											
	Zambia											
	Zimbabwe											
Northern Africa	**Algeria**											
	Egypt											
	Libyan Arab Jamahiriya											
	Morocco											
	Sudan											
	Tunisia											
	Western Sahara											
Southern Africa	**Botswana**											
	Lesotho											
	Namibia											
	South Africa											
	Swaziland											
Western Africa	**Benin**											
	Burkina Faso											
	Cape Verde											

Sub-region	Country	*Acacia flava*	*Acacia laeta*	*Acacia mellifera*	*Acacia nilotica*	*Acacia senegal*	*Acacia seyal*	*Acacia tortilis*	*Balanites aegyptiaca*	*Faidherbia albida*	*Dichrostachys cinerea*	*Euphorbia candelabrum*
	Côte d'Ivoire		■		■	■	■		■		■	
	Gambia				■	■			■	■	■	
	Ghana				■	■	■		■	■	■	
	Guinea				■				■		■	
	Guinea-Bissau				■				■	■	■	
	Liberia								■		■	
	Mali	■	■		■	■	■	■	■	■	■	■
	Mauritania	■			■	■	■	■	■	■	■	
	Niger	■	■		■	■	■	■	■	■	■	
	Nigeria		■		■	■	■	■	■	■		
	Senegal				■	■	■	■	■	■	■	
	Sierra Leone								■		■	
	Togo				■		■		■	■	■	

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Appendix 8 – Paper 5 Estimating the burden of mycetoma in Sudan for the period 1991-2018 using a model-based geostatistical approach

PLOS NEGLECTED TROPICAL DISEASES

RESEARCH ARTICLE

Estimating the burden of mycetoma in Sudan for the period 1991–2018 using a model- based geostatistical approach

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Abstract

Mycetoma is widespread in tropical and subtropical regions favouring arid areas with low humidity and a short rainy season. Sudan is one of the highly endemic countries for mycetoma. Estimating the population at risk and the number of cases is critical for delivering targeted and equitable prevention and treatment services. In this study, we have combined a large dataset of mycetoma cases recorded by the Mycetoma Research Centre (MRC) in Sudan over 28 years (1991–2018) with a collection of environmental and water and hygiene-related datasets in a geostatistical framework to produce estimates of the disease burden across the country. We developed geostatistical models to predict the number of cases of actinomycetoma and eumycetoma in areas considered environmentally suitable for the two mycetoma forms. Then used the raster dataset (gridded map) with the population estimates for 2020 to compute the potentially affected population since 1991. The geostatistical models confirmed this heterogeneous and distinct distribution of the estimated cases of eumycetoma and actinomycetoma across Sudan. For eumycetoma, these higher-risk areas were smaller and scattered across Al Jazirah, Khartoum, White Nile and Sennar states, while for actinomycetoma a higher risk for infection is shown across the rural districts of North and West Kurdufan. Nationally, we estimated 63,825 people (95%CI: 13,693 to 197,369) to have been suffering from mycetoma since 1991 in Sudan,51,541 people (95% CI: 9,893–166,073) with eumycetoma and 12,284 people (95%CI: 3,800–31,296) with acti- nomycetoma. In conclusion, the risk of mycetoma in Sudan is particularly high in certain restricted areas, but cases are ubiquitous across all states. Both prevention and treatment services are required to address the burden. Such work provides a guide for future control and prevention programs for mycetoma, highly endemic areas are clearly targeted, and resources are directed to areas with high demand.

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Author summary

Mycetoma is a chronic infection that is recently been labelled as a neglected tropical dis- ease (NTD) by the World Health Organization (WHO). The epidemiological features of mycetoma are not well described. The prevalence of the disease are globally underesti- mated. In Sudan, there are few studies reporting prevalence. In order to estimate myce- toma burden, we combined a large dataset of mycetoma cases recorded by the Mycetoma Research Centre (MRC) in Sudan with a collection of environmental and water and hygiene-related datasets in a geostatistical framework to produce estimates of the disease burden across the country. Our geostatistical models fitted based on the eumycetoma and actinomycetoma cases recorded by the MRC in Sudan between 1991 and 2018, have shown a spatially heterogeneous and distinct distribution of both mycetoma forms across this endemic country. According to our predictions, most of the eumycetoma cases would have occurred around the Khartoum area and Al Jazirah state and most of the actinomy- cetoma cases would have concentrated in the rural North and West Kurdufan states. Our findings here have several implications for clinical and public health practices. Both pre- vention and treatment services are required to address the burden. Such work provides a guide for future control and prevention programs for mycetoma, highly endemic

Introduction

Mycetoma is a chronic debilitating infection designated a neglected tropical disease (NTD) by the World Health Organization (WHO) [1,2]. It mainly presents as a painless swelling with sinus tracts that discharge granules encapsulating the causative organisms [3]. The infection mainly affects the lower extremities, but any body parts can be involved [2,4,5]. Mycetoma presents in two forms according to the causative agent; eumycetoma caused by fungal agents and actinomycetoma caused by a group of filamentous bacteria [6–9]. Young adult males are mostly affected by this condition, although all age groups and gender are susceptible [10,11]. Affected communities are usually underprivileged with poor access to education and safe sani- tation [12,13]. Farmers and shepherds are the most commonly exposed due to the nature of their occupations and interaction with the environment [14,15].

Mycetoma is widespread in tropical and subtropical regions favouring arid areas with low humidity and a short rainy season [16–18]. It has been reported from Africa, South and Cen- tral America, and Asia [19]. Sudan is one of the highly endemic countries for mycetoma with a massive impact on patients, the community, and the health system. Most mycetoma cases in Sudan are caused by the fungal form (eumycetoma), and the primary causative agent is *Madurella mycetomatis* [20,21]. The medical literature highlights the role of thorn pricks in the pathogenesis of mycetoma by stressing the association of mycetoma occurrence with trauma, especially from acacia trees thorns. Frequently, thorns are identified impeded in myce- toma lesions during surgery, supporting this theory. However, it is still unclear whether the trauma creates a portal of entry for pathogenic organisms resident in soil or the acacia thorns are infected, causing direct inoculation [21,22].

The epidemiological features of mycetoma are not well described. The incidence and preva- lence of the disease are globally underestimated, and most of the reported cases are based on hospital records and short prevalence surveys conducted locally [23,24]. Since the middle of the last century, much effort was made to estimate the mycetoma burden in Sudan accurately. Abbott's study in 1952 estimated the prevalence of mycetoma to be 4.6 per 100,000 inhabitants based on a cohort of 1,231 mycetoma patients admitted to hospitals throughout the country [25]. More recently, in 2014, a large meta-analysis conducted by van de Sande *et al*. estimated that the mycetoma prevalence in Sudan was 1.81 cases per 100,000 inhabitants, although the authors acknowledged this could be much higher in some villages [26].

Estimating the population at risk and number of cases cases is critical for delivering targeted and equitable prevention and treatment services, planning control and elimination programs, and implementing tailored case-finding and surveillance. Estimates of number of cases and population at risk are typically obtained through routine disease surveillance (secondary data sources), house-to-house case searches or large-scale surveys. For diseases of low prevalence such as mycetoma, routinely collected data is not reliable enough to produce estimates of the disease burden since it underestimates their actual impact and distribution. On the other hand, although house-to-house and large-scale surveys can deliver more accurate estimates, they tend to be unfeasible because of their high cost and logistic needs. Nowadays, geospatial modelling techniques for predicting the distribution and burden of diseases have proven criti- cal to produce reliable estimates, especially in low-income countries with limited resources [27–30]. In this study, we have combined a large dataset of mycetoma cases recorded by the Mycetoma Research Centre (MRC) in Sudan over 28 years (1991–2018) with a collection of environmental and sanitation-related datasets in a geostatistical framework to produce esti- mates of the disease burden across the country. Number of cases estimates were generated sep- arately for the bacterial (actinomycetoma) and fungal (eumycetoma) forms of mycetoma.

Materials and methods

Records of mycetoma cases

The data included in this study were extracted from the patient database compiled by the Mycetoma Research Centre (MRC) at Soba University Hospital in Khartoum (Sudan) from 1991–2018. This centre was established in 1991 to manage mycetoma patients attending from all states of Sudan, and more than 10,000 cases have been registered at the centre since it opened [25,31].

The variables extracted from the database included patients' demographic characteristics, details of their clinical presentation and diagnosis, and their location of origin using patients' addresses, which were then used to find the most accurate location, namely geographical coor- dinates, for all the recorded mycetoma cases (Fig 1).

Explanatory variables

Geostatistical models based on the counts of actinomycetoma and eumycetoma cases were constructed separately using two independent variables potentially associated with the occur- rence and distribution of mycetoma: environmental suitability and an indicator of poor hygiene conditions [32]. We used gridded surfaces of predicted environmental suitability for both types of mycetoma as modelled previously (S1 Fig) [17]. Environmental suitability for mycetoma across Sudan was modelled using a combination of linear regression and machine learning algorithms. These algorithms were used to identify the environmental risk factors associated with the occurrence of mycetoma cases, and subsequently established the likelihood for the occurrence of mycetoma based on the values recorded for each environmental factor at

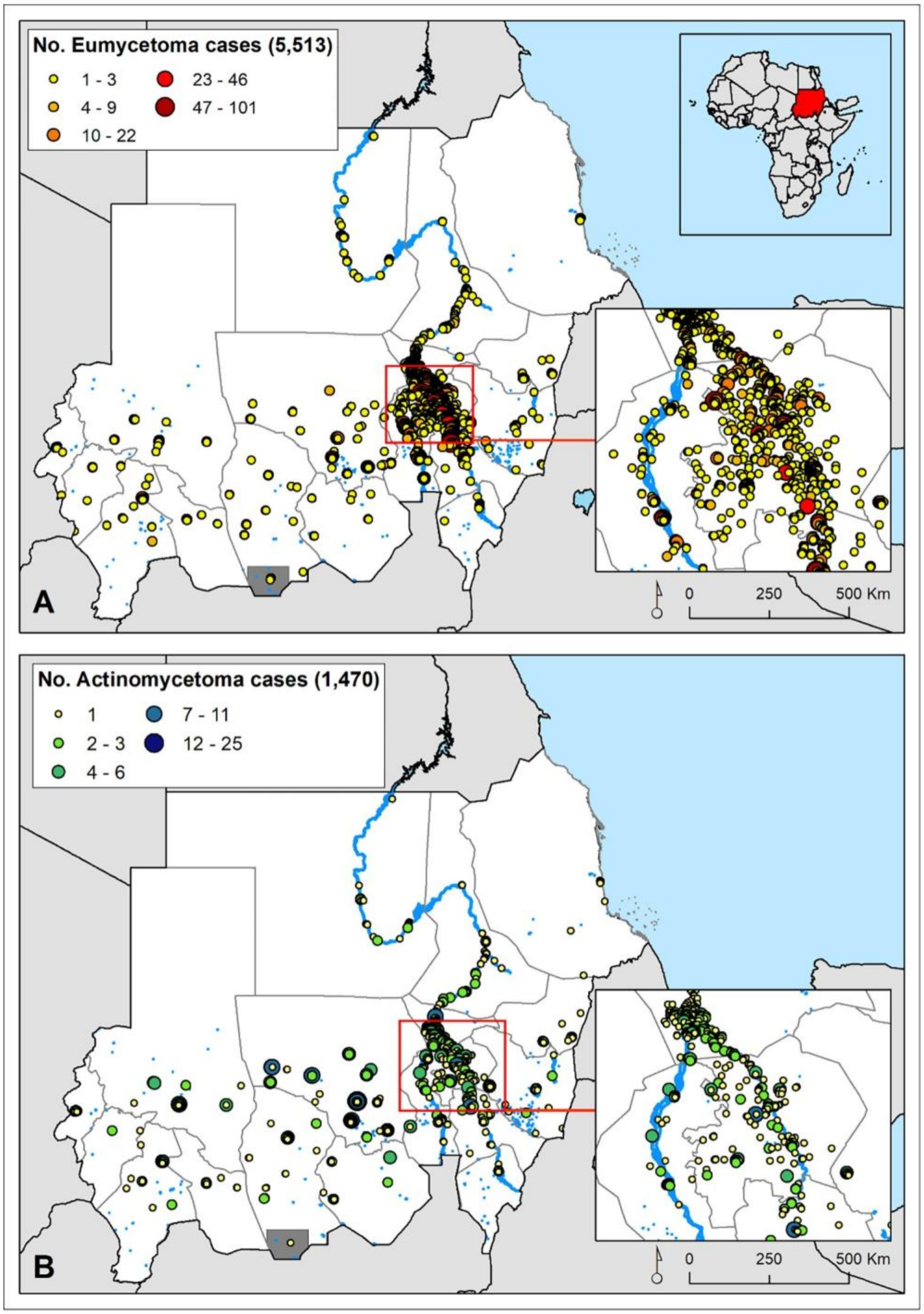

Fig 1. Distribution of eumycetoma (A) and actinomycetoma (B) cases recorded by the Mycetoma Research Centre from 1991 to 2018.

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every area of 1sq-km. We also obtained a continuous estimate of household access to unim- proved sanitation services by WHO/UNICEF Join Monitoring Program by 2017. As defined by this program, an "improved" sanitation facility is one that “safely sep- arates excreta and wastewater from human contact either by safe containment and disposal in situ or by safe transport and treatment off-site. Unimproved latrine, open defecation, well without a pump and surface water are considered unimproved sources of sanitation. [33]. (S2 Fig) This gridded map displaying the estimated percentage of households using unimproved sanitation is based on a Bayesian geostatistical model developed using a collection of environmental and socio- economic data and data from 600 sources across more than 88 low-income and middle- income countries (LMICs) [32].

Geostatistical modelling to estimate mycetoma burden

The records of mycetoma cases and modelled environmental suitability and accessibility to unimproved sanitation were combined within a geostatistical framework. The records of geo- located mycetoma cases were aggregated on a spatial grid of 1 sq-km resolution across the country. Thus, we modelled counts of mycetoma cases per 1 km^2 for 1991–2018 and took the estimated gridded population at the same spatial resolution for 2020 into account. Due to the lack of reliable census data for all the populated areas across Sudan, we obtained gridded con- tinuous estimates of the total population for 2020 from the WorldPop project [34,35]. Because of the patchy distribution of the population in Sudan, we opted for using the constrained ver- sion of modelled estimates at 100 metres resolution [36,37]. This method only generates esti- mates within areas containing built settlements (S3 Fig). We aggregated the population estimates to a grid of 1 sq-km resolution to match the geographical aggregation for mycetoma cases and the covariates.

We developed geostatistical models to predict the number of actinomycetoma and eumy- cetoma cases in areas considered environmentally suitable for the two mycetoma forms, as delineated by a previous modelling exercise across Sudan (S4 and S5 Figs) [17]. We let myce- toma risk depend on the predicted environmental suitability and household level utilization of unimproved sanitation values obtained in the previous step. We included spatial random effects to account for spatial variation in mycetoma cases between locations of origin that was not explained by the explanatory variables, and independent random effects to account for potential overdispersion. We validated the models using a variogram-based procedure that tests the compatibility of the adopted spatial structure with the data. More details are provided in S1 Text. The analysis was carried out using the R package *PrevMap v 1.5.3* [38], which implements geostatistical models' parameter estimation and spatial prediction using Monte Carlo Maximum Likelihood [39]. This model was applied to produce predictions of the number of eumycetoma and actinomycetoma cases since 1991 at 1km^2 spatial resolution and probability maps of exceeding a 50 per 10,000 people and 5 per 10,000 people cases thresholds for eumycetoma and actinomycetoma, respectively. The reason two different threshold were used eumycetoma is more common in Sudan compared to actinomycetoma. We checked the validity of the assumed covariance model for the spatial correlation using the Monte Carlo algorithm and empirical semi-variogram as described in S7 Fig. Addition- ally, maps of 95% confidence intervals for the number of cases were generated for each 1 sq- km grid location.

We used the raster dataset (gridded map) with the population estimates for 2020 down- loaded from the WorldPop project to compute the potentially affected population since 1991. An output raster dataset computing the estimated number of eumycetoma and actinomyce- toma cases per grid cell was obtained by multiplying the 1km^2 raster dataset of predictive num- ber of cases with the corresponding grided map with the total population estimated per 1 sq- km pixel for built-up areas. The same procedure was used to estimate the uncertainty range of the affected population using the gridded maps of 95% confidence interval (CI) for predicted number of cases. These raster datasets were then used to extract the aggregate number of peo- ple with mycetoma and uncertainty range by administrative area (district and states).

Results

Mycetoma records: General description

The modelled data included 7,812 unique points obtained from patients seen at the MRC in the period 1991–2018, and they came from all eighteen states of Sudan. The study included 5,513 patients (79%) with confirmed eumycetoma and 1,470 patients (21%) with actino-myce- toma. Most of the mycetoma patients were from Al Jazirah State (34.4%) and Khartoum State (14.5%) (Fig 1). Further details of the patients' geographical distribution have been published elsewhere [17].

Predicted number of mycetoma cases and burden estimation

The relative risk of eumycetoma was over 4-fold higher in the localities around the Khartoum area, most of them in the state of Al Jazirah, than was expected across Sudan for the study period, whereas those at higher risk of actinomycetoma were from the North Kur-dufan state (S6 Fig). The geostatistical models confirmed this heterogeneous and distinct distribution of the estimated number of eumycetoma and actinomycetoma cases across Sudan (Figs 2 & 3). For eumycetoma, these higher-risk areas were smaller and scattered across Al Jazirah, Khar- toum, White Nile and Sennar states, while for actinomycetoma a higher risk for infection is shown across the rural districts of North and West Kurdufan. The data also clearly showed the exceedance probability of number of cases rate of 50 per 10,000 inhabitants and 5 per 10,000 inhabitants for eumycetoma and actinomycetoma, respectively (Fig 4).
Nationally, we estimated 63,825 people (95%CI: 13,693 to 197,369) to have been suffering from mycetoma since 1991 in Sudan (Tables 1 & 2): 51,541 people (95%CI: 9,893–166,073) with eumycetoma and 12,284 people (95%CI: 3,800–31,296) with actinomycetoma. Five

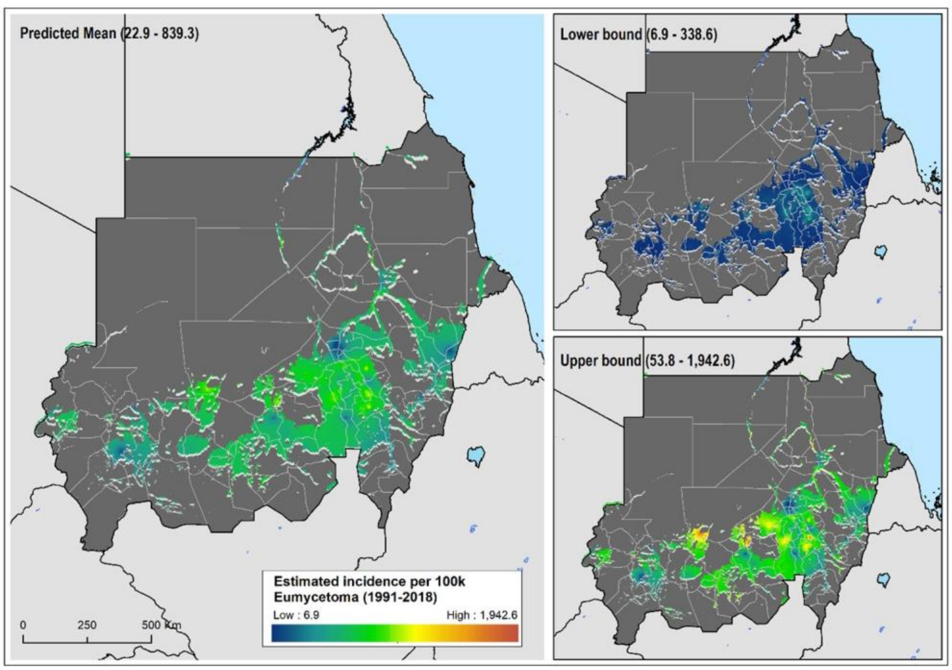

Fig 2. Estimated eumycetoma cases per 100,000 inhabitants between 1991 and 2018; mean predicted number of cases and, lower and upper 95% CI bounds. Areas considered environmentally unsuitable for the occurrence of eumycetoma as predicted by environmental model have been excluded.

https://doi.org/10.1371/journal.pntd.0010795.g002

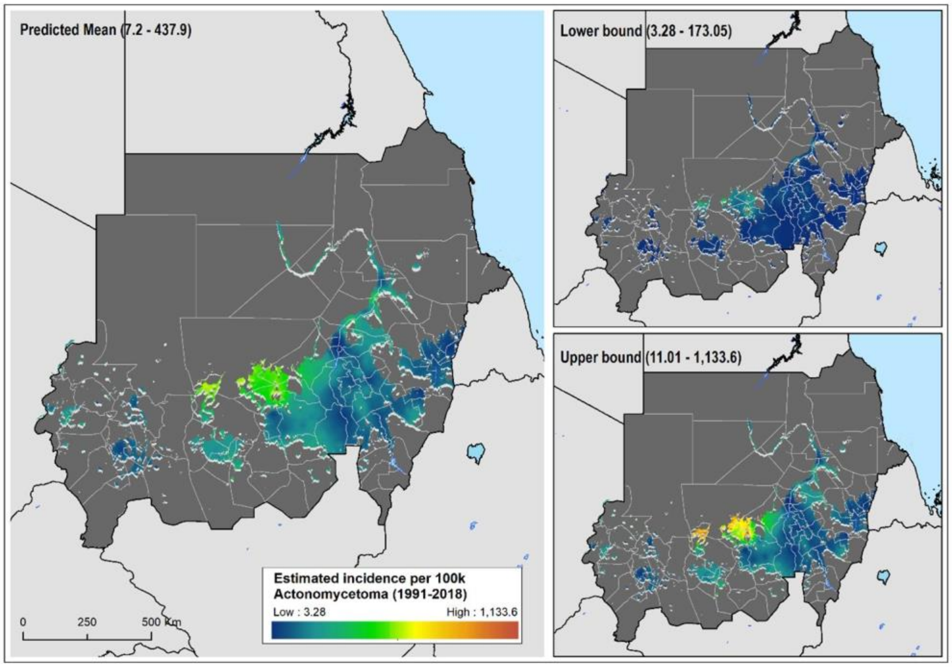

Fig 3. Estimated actinomycetoma cases per 100,000 inhabitants between 1991 and 2018; mean predicted number of cases and, lower and upper 95% CI bounds. Areas considered environmentally unsuitable for the occurrence of actinomycetoma as predicted by environmental model have been excluded.

https://doi.org/10.1371/journal.pntd.0010795.g003

regions (Al Jazirah, White Nile, North Kurdufan, Khartoum and Sennar) would have contrib- uted over 66% of the total number of cases expected in the period 1991–2018. The greatest pro- portion (22%) of people affected by any form of mycetoma resided in the Al Jazirah state, although when differentiating by type of mycetoma, most of actinomycetoma cases would have occurred in the North Kurdufan state. The remaining thirteen states were predicted to have had less than 1,000 cases of mycetoma during the study period (Fig 5). S1 and S2 Tables provide district level estimates of the number of expected eumycetoma and actinomycetoma cases respectively.

In terms of validating the geostatistical models fitted, the variogram-based procedure con- ducted using the data simulated from the models led us to conclude that the observed data are compatible with the assumptions of an exponential correlation function and that the underlying spatial structure has been accounted for by the spatial fixed and random effects (S7 and S8 Figs).

Discussion

To our knowledge, this is the first analysis to use geostatistical modelling to estimate the burden of mycetoma. The novelty of the current analysis is that we combined mycetoma case data with a collection of environmental factors to estimate the burden of mycetoma across Sudan. We have also provided the associated uncertainty interval to identify areas where further data collec- tion is required to improve the estimates. Given the difference in aetiology and potential risk factors for each type of mycetoma, we have fitted separate models for eumycetoma and actino- mycetoma. Our analysis can serve as a framework to estimate the global burden of mycetoma.

Our geostatistical models fitted based on the eumycetoma and actinomycetoma cases recorded by the MRC in Sudan between 1991 and 2018, have shown a spatially heterogeneous and distinct distribution of both mycetoma forms across this endemic country. According to

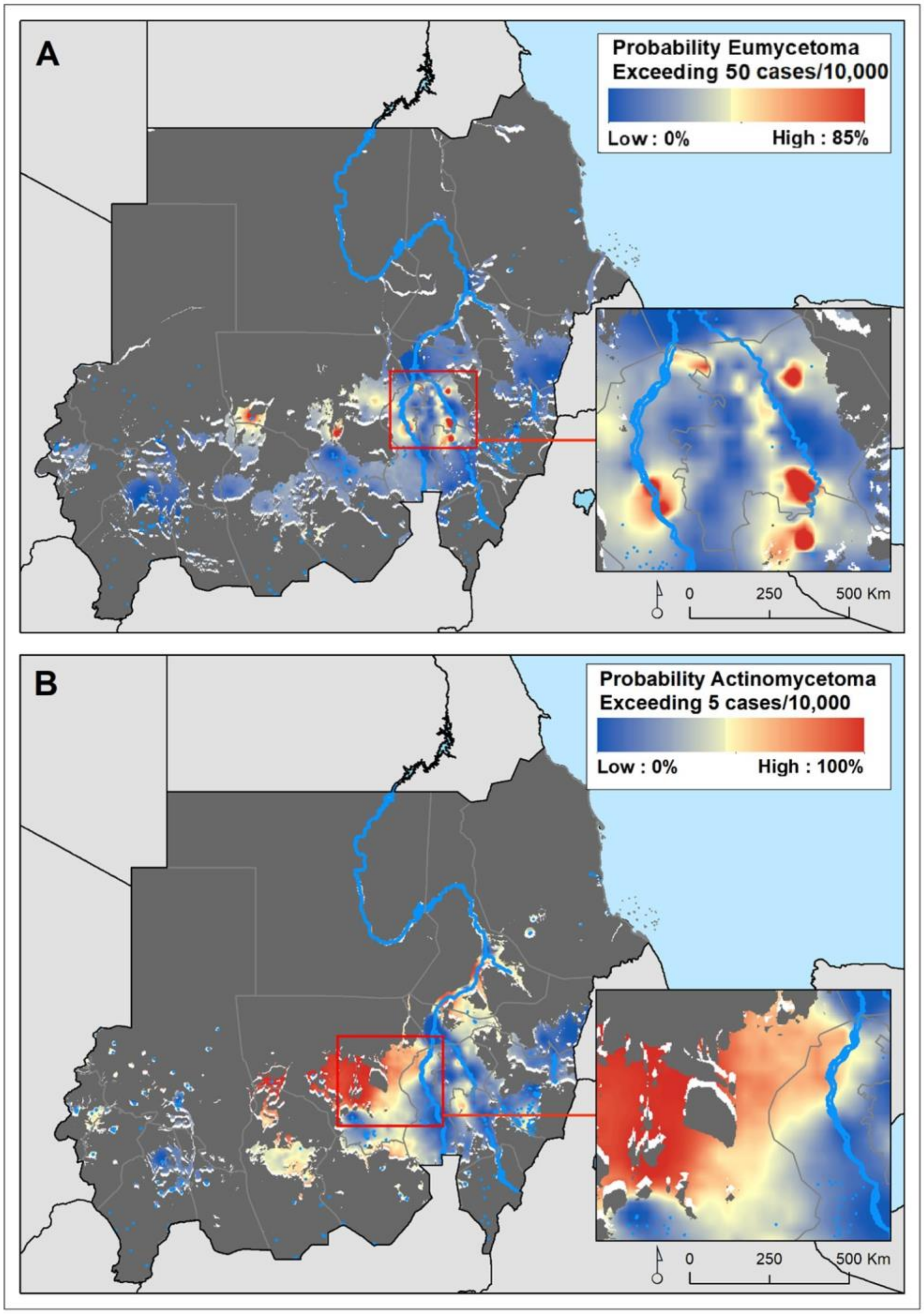

Fig 4. Maps displaying the probability of exceeding 50 cases and 5 cases per 1,000 inhabitants, for eumycetoma and actinomycetoma respectively, since 1991 in Sudan.

https://doi.org/10.1371/journal.pntd.0010795.g004

our predictions, most of the eumycetoma cases would have occurred around the Khartoum area and Al Jazirah state, and most of the actinomycetoma cases would have concentrated in the rural North and West Kurdufan states. It has also showed that the number of mycetoma cases in Sudan, so far mostly estimated through hospital records and local prevalence surveys [25,26], is likely to be sizeably underestimated.

Table 1. Estimation of eumycetoma cases by state in Sudan between 1991 and 2018.

State	Area predicted suitable (sq-km)	Estimated Eumycetoma Cases			
		No.	%	95% CI	
				Lower Bound	Upper Bound
Al Jazirah	22,307	12,263	23.8%	2,979	34,452
Al Qadarif	21,786	1,910	3.7%	297	6,689
Blue Nile	4,030	543	1.1%	88	1,854
Central Darfur	3,894	255	0.5%	31	970
East Darfur	12,964	1,046	2.0%	129	3,984
Kassala	28,963	3,205	6.2%	430	11,799
Khartoum	18,113	5,139	10.0%	1,231	14,837
North Darfur	18,683	1,163	2.3%	158	4,266
North Kurdufan	52,628	4,680	9.1%	696	16,582
Northern	6,696	989	1.9%	159	3,396
Red Sea	8,101	792	1.5%	102	2,999
River Nile	27,403	2,731	5.3%	464	9,176
Sennar	28,373	5,046	9.8%	1,110	15,271
South Darfur	13,169	1,547	3.0%	224	5,599
South Kurdufan	16,397	713	1.4%	96	2,62
West Darfur	5,983	506	1.0%	73	1,834
West Kurdufan	34,959	1,850	3.6%	238	6,910
White Nile	40,855	7,163	13.9%	1,388	22,834
Total	**365,304**	**51,541**		**9,893**	**166,073**

https://doi.org/10.1371/journal.pntd.0010795.t001

Table 2. Estimation of actinomycetoma cases by state in Sudan between 1991 and 2018.

State	Area predicted suitable (sq-km)	Estimated Actinomycetoma Cases			
		No.	%	95% CI	
				Lower Bound	Upper Bound
Al Jazirah	22,995	1,801	14.7%	719	3,794
Al Qadarif	33,525	489	4.0%	129	1,341
Blue Nile	2,341	118	1.0%	33	318
Central Darfur	2,850	89	0.7%	16	289
East Darfur	5,661	184	1.5%	45	543
Kassala	16,028	367	3.0%	103	978
Khartoum	15,910	1,351	11.0%	580	2,882
North Darfur	9,765	449	3.7%	113	1,291
North Kurdufan	69,740	2,826	23.0%	747	7,714
Northern	4,934	527	4.3%	139	1,392
Red Sea	2,549	123	1.0%	32	352
River Nile	23,565	869	7.1%	260	2,220
Sennar	25,218	729	5.9%	247	1,722
South Darfur	8,896	357	2.9%	91	1,014
South Kurdufan	9,498	161	1.3%	33	496
West Darfur	4,368	143	1.2%	40	39
West Kurdufan	22,912	545	4.4%	117	1,650
White Nile	42,463	1,156	9.4%	356	2,907
Total	**323,218**	**12,284**		**3,800**	**31,296**

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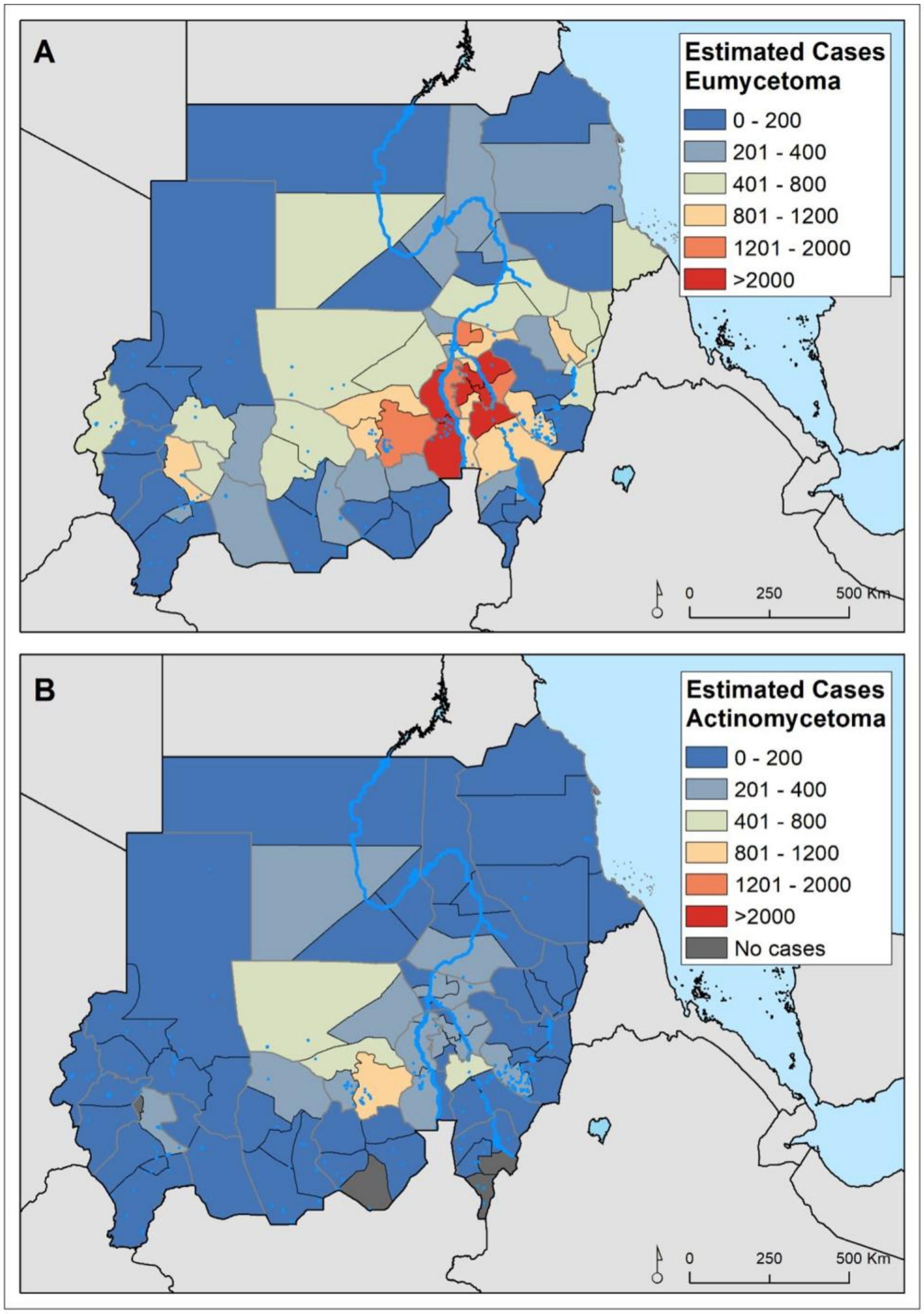

Fig 5. Estimated number of people that have eumycetoma (A) and actinomycetoma (B) between 1991 and 2018 as predicted by the fitted model-based geostatistical models.

https://doi.org/10.1371/journal.pntd.0010795.g005

Al Jazirah and North Kurdufan states showed high estimates of mycetoma cases, which can be attributed to the nature of the economic activities of the population residing those areas.

Most of the people there are sustained by arable farming or animal grazing, which increase the risk of contact with mycetoma causative organisms. Al Jazirah state had the biggest agriculture scheme in Sudan and North Kurdufan state is one of the biggest states in the production of gum Arabic.

Overall, the estimated number of eumycetoma cases is four times higher than actinomyce- toma in Sudan and across the states. This is in agreement with what has been reported through clinical observations and reports where the number of eumycetoma dominates [25]. Nonethe- less, there is no difference in proportions of areas suitable for the occurrence of both myce- toma forms. One six (17%) and 19% of the landmass of Sudan is suitable for the occurrence of actinomycetoma and eumycetoma respectively. For both types of mycetoma high environ- mental suitability was predicted in North Kurdufan and White Nile states.

The localities that had higher estimates of eumycetoma cases include, North Jazirah, Khar- toum Bahri, Um Rawaba, Sennar and Ad Douiem. These localities have large populations, that manly depend on farming as an occupation. For actinomycetoma North Kurdufan state accounted for most of the cases and again Um Rawaba had the highest estimates.

Our modelling approach is not without limitations. First, we must assume there might be underlying geographical and temporal biases in the recorded number of mycetoma cases in the country, as all the records were collected by the Mycetoma Research Centre, which is located in Khartoum, since its inception in 1991. From the exploratory analysis of this dataset, presented elsewhere [4], the number of mycetoma cases confirmed by the MRC has steadily increased since 1991 and the vast majority are coming from rural and peri-urban areas near the Khartoum area (Fig 1). In order to account for this potential geographical bias, we adjusted the number of cases by the estimated population density in 2020, although there still remain some uncertainty on where the infection may have occurred due to most cases being diag- nosed in advance stages of the disease. Neither was it possible to account for any existing tem- poral variation due to the uncertainty on when the infection took place, severe cases are more likely to be diagnosed in health facilities. Second, we did not account for any individual or household related risk factors such as work and social activities, that may also have driven the distribution and intensity of transmission [24]. We however included an estimate of poor sani- tation coverage, assuming a higher risk in more deprived areas with limited access to protected sanitation. Lastly patients address might be not appropriate indicator for point of infection in contagious diseases as well as mobile communities. Nonetheless, in our case the population under study are less mobile, the incubation period for mycetoma infection is not clearly defined, hence, the location of origin or address could be an indicator of the source of the infection.

Our findings here have several implications for clinical and public health practices. Our analysis indicated that only slightly less than a quarter of the landmass of Sudan is suitable for the occurrence of mycetoma. Nonetheless, the cases are ubiquitous across all states of Sudan which can be attributed to the population movement in Sudan. This implies that clinicians working in Sudan should have high index of suspicion for mycetoma for people presenting with swelling and sinuses discharging grains. Expanding diagnostic and treatment services to at least high burden states is required to address the existing cases. On the other hand, public health interventions focusing on prevention should focus on states and areas suitable for the occurrence of mycetoma. To prevent this disabling disease, geographically targeted public health education and social mobilization are required. Mycetoma can be prevented by encour- aging safe farming practices like wearing shoes that decrease the chance of trauma, organizing interaction with animals and building 'human-friendly' animal cages that avoid the use of thorny tree branches. The burden presented here warrants a mycetoma control program in Sudan, which should coordinate the treatment and prevention of the disease.

In conclusion the risk of mycetoma in Sudan is particularly high in certain restricted areas, but cases are ubiquitous across all states. Both prevention and treatment services are required to address the burden. Such work provides a guide for future control and prevention programs for mycetoma, highly endemic areas are clearly targeted, and resources are directed to areas with high demand. Moreover, medical personnel will have high levels of suspicion for myce- toma in areas with high burden and case will be adequately diagnosed and managed within their communities without the need to seek medical treatment in centralized medical facilities, in turn reducing the financial burden for mycetoma patients. Specialized mycetoma manage- ment centres can be established in communities where mycetoma is endemic which provide early case detection and management of mycetoma patients.

Disclaimer

The views expressed in this publication are those of the author(s) and not necessarily those of the NIHR or the Department of Health and Social Care" from the Financial Disclosure Statement.

Supporting information

S1 Text. Formulation and validation of geostatistical Poisson model.
(PDF)

S1 Fig. Maps of environmental suitability for eumycetoma (A) and actinomycetoma (B) The results of the Monte Carlo validation procedure for the actinomycetoma model.
(PDF)

S2 Fig. Estimated percentage of households accessing unimproved sanitation by 2017The results of the Monte Carlo validation procedure for the eumycetoma model.
(PDF)

S3 Fig. Map displaying estimated total population for Sudan in 2020 based on constrained methods Bar plot showing the increasing trends of mycetoma cases in Sudan since 1991 to 2018.
(PDF)

S4 Fig. Predicted occurrence of actinomycetoma form of mycetoma and uncertainty range across Sudan.
(PDF)

S5 Fig. Predicted occurrence of eumycetoma form of mycetoma and uncertainty range across Sudan.
(PDF)

S6 Fig. Relative risk estimated at district level for eumycetoma and actinomycetoma based on cases recorded by the Mycetoma Research Centre (Khartoum) during the period 1991– 2018 in Sudan.
(PDF)

S7 Fig. The results of the Monte Carlo validation procedure for the actinomycetoma model.
(PDF)

S8 Fig. The results of the Monte Carlo validation procedure for the eumycetoma model.
(PDF)

S9 Fig. Bar plot showing the increasing trends of mycetoma cases in Sudan since 1991 to 2018.
(PDF)

S1 Table. Estimation of eumycetoma cases by district in Sudan since 1991.
(PDF)

S2 Table. Estimation of actinomycetoma cases by district in Sudan since 1991.
(PDF)

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Methodology: Rowa Hassan, Jorge Cano, Claudio Fronterre.

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Visualization: Jorge Cano.

Writing – original draft: Rowa Hassan, Kebede Deribe.

Writing – review & editing: Rowa Hassan, Jorge Cano, Claudio Fronterre, Sahar Bakhiet, Ahmed Fahal, Kebede Deribe, Melanie Newport.

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Appendix 9 - Supplemental materials for Paper 5 **-** Estimating the burden of mycetoma in Sudan for the period 1991-2018 using a model-based geostatistical approach

Supplementary file

Figure 1. The results of the Monte Carlo validation procedure for the actinomycetoma model. The solid line is the observed variogram and the shaded area corresponds to the 95% bandwidth. The results lead us to conclude that the data are compatible with the assumption of an exponential spatial correlation function.

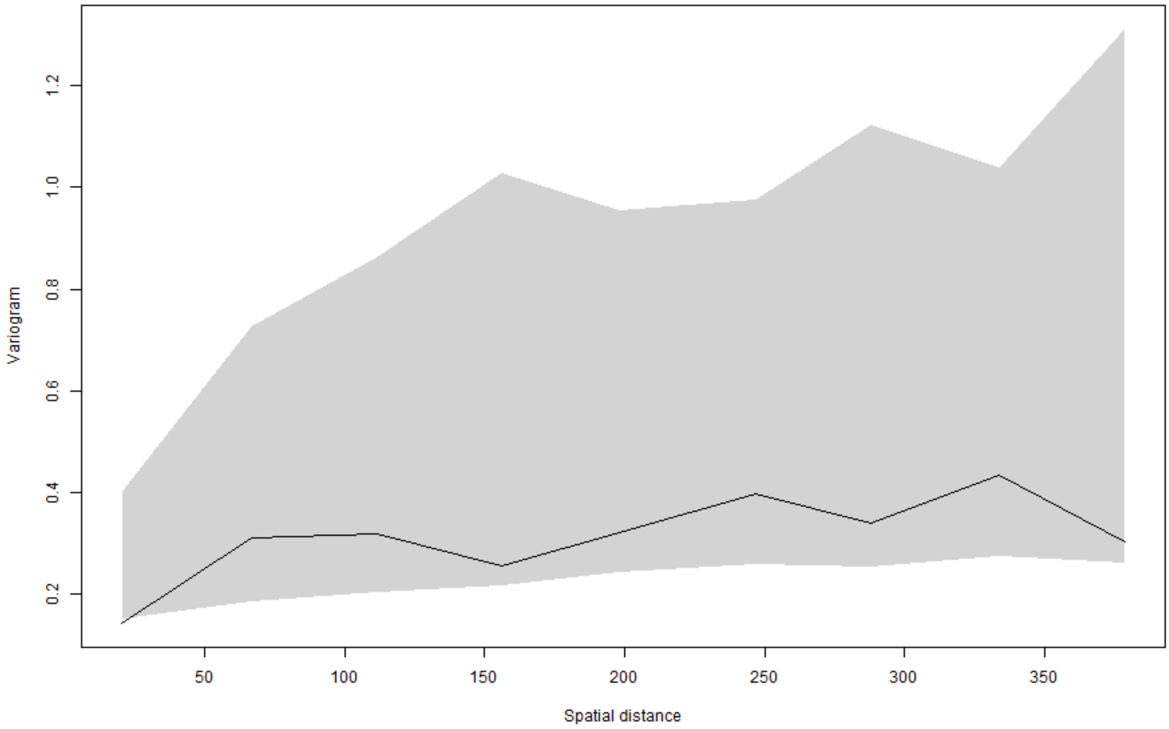

Figure 2. The results of the Monte Carlo validation procedure for the eumycetoma model. The solid line is the observed variogram and the shaded area corresponds to the 95% bandwidth. The results lead us to conclude that the data are compatible with the assumption of an exponential spatial correlation function.

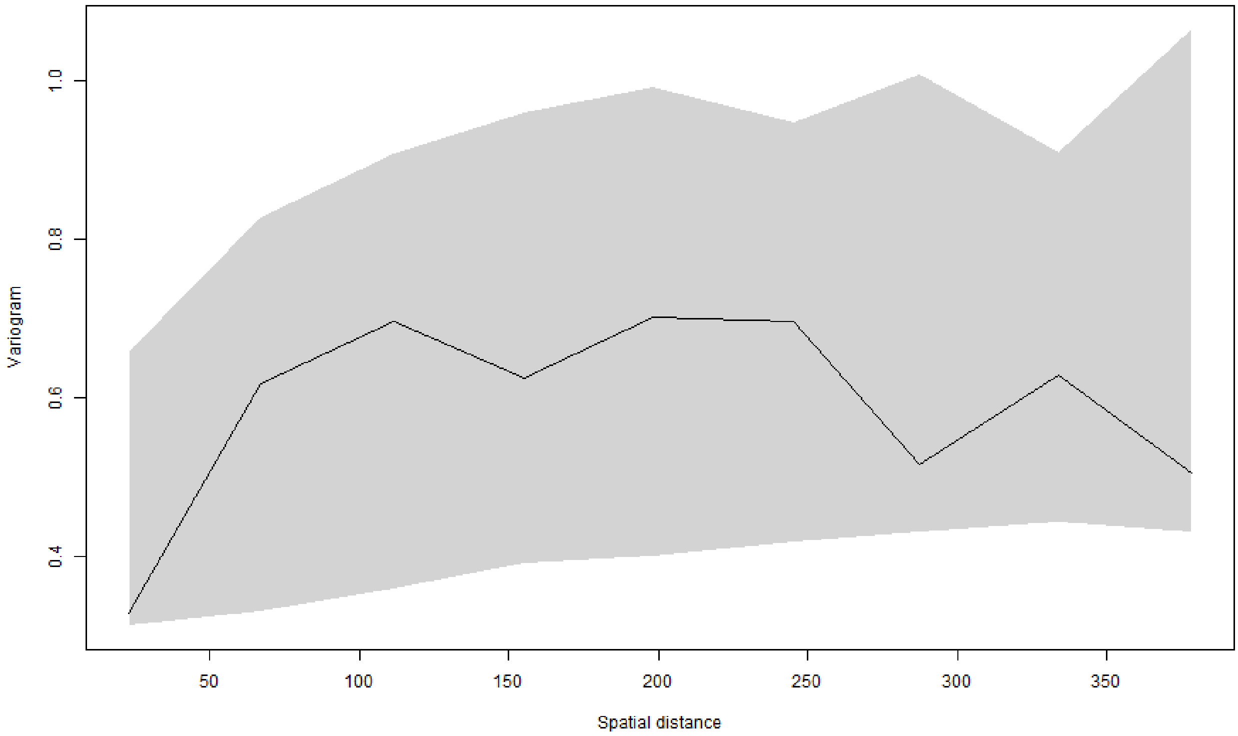

Table 1. Estimation of eumycetoma cases by district in Sudan since 1991

State	District	Area predicted suitable (sq-km)	Estimated Eumycetoma Cases		
			No.	95% CI	
				Lower Bound	Upper Bound
Al Jazirah	Al Kamlin	1,618	1,144	305	3,063
Al Jazirah	Al Mahagil	3,610	2,008	470	5,754
Al Jazirah	East al Gazera	3,393	1,241	289	3,561
Al Jazirah	North al Gazera	2,950	2,400	630	6,444
Al Jazirah	Sharq al Gazera	5,072	2,177	515	6,222
Al Jazirah	South al Gazera	2,863	1,054	210	3,241
Al Jazirah	Um Al Gura	2,801	2,239	560	6,167
Al Qadarif	Al Faw	6,596	169	22	637
Al Qadarif	Al Fushqa	7,153	426	57	1,570
Al Qadarif	Al Gadaref	801	170	37	499
Al Qadarif	Al Galabat	2,065	180	22	684
Al Qadarif	Al Rahd	5,171	965	159	3,299
Blue Nile	Ad Damazin	2,703	318	53	1,079
Blue Nile	Al Kurumik	157	10	1	38
Blue Nile	Al Roseires	823	181	30	607
Blue Nile	Baw	346	34	4	130
Blue Nile	Geissan	1	-	-	-
Central Darfur	Mukjar	478	62	7	243
Central Darfur	Zallingi	3,416	193	24	727
East Darfur	Al Deain	4,192	265	38	976

State	District	Area predicted suitable (sq-km)	Estimated Eumycetoma Cases		
			No.	95% CI	
				Lower Bound	Upper Bound
East Darfur	Nyala	8,772	781	91	3,008
Kassala	Al Gash	10,386	746	90	2,834
Kassala	Hamashkorieb	3,161	775	84	3,067
Kassala	Kassala	3,149	504	84	1,690
Kassala	Nahr Atbara	7,891	937	140	3,323
Kassala	Seteet	4,376	243	32	885
Khartoum	Karary	1,578	301	48	1,040
Khartoum	Khartoum	346	608	196	1,459
Khartoum	Khartoum Bahri	4,936	1,324	313	3,841
Khartoum	Omdurman	1,574	842	224	2,298
Khartoum	Sharg En Nile	7,585	1,104	206	3,548
Khartoum	South Khartoum	981	640	169	1,718
Khartoum	Um Badda	1,113	320	75	933
North Darfur	Al Fasher	5,823	601	91	2,118
North Darfur	Kabkabiya	255	130	14	500
North Darfur	Kutum	1,632	22	4	73
North Darfur	Mellit	3,395	146	16	573
North Darfur	Um Kadada	7,578	264	33	1,002
North Kurdufan	Bara	8,245	1,192	198	4,105
North Kurdufan	Jebrat al Sheikh	17,284	654	82	2,448
North Kurdufan	Sheikan	7,590	815	113	2,963

State	District	Area predicted suitable (sq-km)	Estimated Eumycetoma Cases		
			No.	95% CI	
				Lower Bound	Upper Bound
North Kurdufan	Sowdari	7,363	521	69	1,892
North Kurdufan	Um Rawaba	12,146	1,498	234	5,174
Northern	Addabah	623	54	7	198
Northern	Dongola	1,470	524	89	1,749
Northern	Merawi	2,797	321	51	1,107
Northern	Wadi Halfa	1,806	90	12	342
Red Sea	Halayeb	2,407	23	2	92
Red Sea	Port Sudan	509	290	48	995
Red Sea	Sinkat	1,578	12	1	48
Red Sea	Tokar	3,607	467	51	1,864
River Nile	Abu Hamad	4,082	353	45	1,319
River Nile	Ad Damer	1,769	187	35	603
River Nile	Al Matammah	2,539	580	111	1,819
River Nile	Atbara	8,357	658	105	2,292
River Nile	Berber	3,380	298	47	1,022
River Nile	Shendi	7,276	655	121	2,121
Sennar	Ad Dinder	6,469	911	155	3,065
Sennar	Sennar	9,458	3,301	810	9,416
Sennar	Singa	12,446	834	145	2,790
South Darfur	Buram	743	63	8	242
South Darfur	Id El Ghanem	1,640	180	23	672

State	District	Area predicted suitable (sq-km)	Estimated Eumycetoma Cases		
			No.	95% CI	
				Lower Bound	Upper Bound
South Darfur	Kas	111	16	2	63
South Darfur	Nyala	10,013	1,081	166	3,828
South Darfur	Tulus	662	207	25	794
South Kurdufan	Abu Jubaiyah	2,351	90	14	313
South Kurdufan	Dilling	6,802	310	40	1,156
South Kurdufan	Kadugli	1,791	36	6	122
South Kurdufan	Rashad	5,420	276	36	1,026
South Kurdufan	Talodi	33	1	-	4
West Darfur	Al Geneina	5,983	506	73	1,834
West Kurdufan	Abyei	375	33	6	106
West Kurdufan	As Salam	5,212	191	26	697
West Kurdufan	En Nuhud	12,846	786	101	2,943
West Kurdufan	Ghebeish	9,743	628	79	2,361
West Kurdufan	Lagawa	6,783	212	26	803
White Nile	Ad Douiem	8,766	2,240	455	7,011
White Nile	Al Gutaina	8,035	1,711	352	5,204
White Nile	Al Jabalian	6,586	947	177	3,035
White Nile	Kosti	17,468	2,265	404	7,584
Total		**365,304**	**51,541**	**9,893**	**166,073**

Table 2. Estimation of actinomycetoma cases by district in Sudan since 1991

State	District	Area predicted suitable (sq-km)	Estimated Actinomycetoma Cases		
			No.	95% CI	
				Lower Bound	Upper Bound
Al Jazirah	Al Kamlin	1,613	142	65	270
Al Jazirah	Al Mahagil	3,554	277	104	599
Al Jazirah	East al Gazera	3,938	220	90	457
Al Jazirah	North al Gazera	2,950	357	158	692
Al Jazirah	Sharq al Gazera	5,278	329	127	715
Al Jazirah	South al Gazera	2,861	188	59	466
Al Jazirah	Um Al Gura	2,801	288	116	595
Al Qadarif	Al Faw	9,503	49	9	160
Al Qadarif	Al Fushqa	7,734	86	19	250
Al Qadarif	Al Gadaref	2,759	65	26	141
Al Qadarif	Al Galabat	2,493	26	5	83
Al Qadarif	Al Rahd	11,036	263	70	707
Blue Nile	Ad Damazin	1,673	77	22	208
Blue Nile	Al Roseires	659	41	11	110
Blue Nile	Baw	9	-	-	-
Central Darfur	Mukjar	946	31	4	115
Central Darfur	Zallingi	1,904	58	12	174
East Darfur	Al Deain	1,750	60	21	145
East Darfur	Nyala	3,911	124	24	398
Kassala	Al Gash	3,755	61	14	175
Kassala	Hamashkorieb				

State	District	Area predicted suitable (sq-km)	Estimated Actinomycetoma Cases		
			No.	95% CI	
				Lower Bound	Upper Bound
		816	23	5	70
Kassala	Kassala	2,932	97	35	222
Kassala	Nahr Atbara	4,913	131	37	347
Kassala	Seteet	3,612	55	12	164
Khartoum	Karary	1,231	107	35	274
Khartoum	Khartoum	346	126	75	199
Khartoum	Khartoum Bahri	3,433	390	172	795
Khartoum	Omdurman	1,104	222	112	404
Khartoum	Sharg En Nile	7,705	307	89	845
Khartoum	South Khartoum	981	123	62	219
Khartoum	Um Badda	1,110	76	35	146
North Darfur	Al Fasher	4,664	196	59	519
North Darfur	Kabkabiya	1,107	128	26	386
North Darfur	Kutum	1,901	43	12	122
North Darfur	Mellit	1,096	62	13	195
North Darfur	Um Kadada	997	20	3	69
North Kurdufan	Bara	6,435	284	94	700
North Kurdufan	Jebrat al Sheikh	15,944	364	82	1,080
North Kurdufan	Sheikan	13,377	758	185	2,149
North Kurdufan	Sowdari	9,899	559	144	1,543
North Kurdufan	Um Rawaba	24,085	861	242	2,242
Northern	Addabah				

State	District	Area predicted suitable (sq-km)	Estimated Actinomycetoma Cases		
			No.	95% CI	
				Lower Bound	Upper Bound
		869	131	31	369
Northern	Dongola	2,030	235	67	595
Northern	Merawi	1,848	151	39	396
Northern	Wadi Halfa	187	10	2	32
Red Sea	Halayeb	10	-	-	-
Red Sea	Port Sudan	393	67	24	145
Red Sea	Sinkat	1,711	13	2	49
Red Sea	Tokar	435	43	6	158
River Nile	Abu Hamad	2,094	82	14	271
River Nile	Ad Damer	1,197	49	17	112
River Nile	Al Matammah	2,938	225	77	512
River Nile	Atbara	6,255	183	46	526
River Nile	Berber	2,985	84	25	211
River Nile	Shendi	8,096	246	81	588
Sennar	Ad Dinder	5,267	151	45	382
Sennar	Sennar	9,719	418	158	922
Sennar	Singa	10,232	160	44	418
South Darfur	Buram	429	16	3	50
South Darfur	Id El Ghanem	1,289	44	9	134
South Darfur	Nyala	6,495	228	66	619
South Darfur	Tulus	683	69	13	211
South Kurdufan	Abu Jubaiyah				

State	District	Area predicted suitable (sq-km)	Estimated Actinomycetoma Cases		
			No.	95% CI	
				Lower Bound	Upper Bound
		1,524	17	3	59
South Kurdufan	Dilling	2,782	44	8	138
South Kurdufan	Kadugli	220	1	-	4
South Kurdufan	Rashad	4,972	99	22	295
West Darfur	Al Geneina	4,368	143	40	393
West Kurdufan	Abyei	69	1	-	2
West Kurdufan	As Salam	4,264	54	11	162
West Kurdufan	En Nuhud	8,058	291	68	856
West Kurdufan	Ghebeish	7,149	160	31	501
West Kurdufan	Lagawa	3,372	39	7	129
White Nile	Ad Douiem	9,832	394	112	1,030
White Nile	Al Gutaina	8,035	295	108	654
White Nile	Al Jabalian	6,361	143	47	343
White Nile	Kosti	18,235	324	89	880
Total		**323,218**	**12,284**	**3,800**	**31,296**

Table 3. Monte Carlo maximum likelihood estimates and associated 95% confidence intervals for the eumycetoma model

Parameter	Estimate	95% CI
Intercept	-6.567	(-6.815, -6.32)
Environmental Suitability (eumycetoma)	-0.165	(-0.251, -0.079)
Unimproved sanitation	-0.031	(-0.119, 0.058)
$\log(\sigma^2)$	-0.157	(-0.409, 0.094)
$\log(\phi)$	4.106	(3.393, 4.82)
$\log(\tau^2)$	-1.955	(-2.647, -1.263)

Table 4. Monte Carlo maximum likelihood estimates and associated 95% confidence intervals for the actinomycetoma model

Parameter	Estimate	95% CI
Intercept	-6.87	(-7.242, -6.505)
Environmental Suitability (actinomycetoma)	-1.15	(-1.685, -0.624)
Unimproved sanitation	-0.34	(-0.73, 0.059)
$\log(\sigma^2)$	-0.17	(-0.552, 0.207)
$\log(\phi)$	4.47	(4.014, 4.924)
$\log(\tau^2)$	-3.08	(-3.874, -2.289)

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Appendix 3 – Paper 1 Clinical epidemiological characteristics of mycetoma in Eastern Sennar locality, Sennar State, Sudan.

Appendix 4 – Paper 2 Role of socioeconomic factors in developing mycetoma: results from a household survey in Sennar State, Sudan.

Appendix 8 – Paper 5 Estimating the burden of mycetoma in Sudan for the period 1991-2018 using a model-based geostatistical approach.

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Appendix 5 – Paper 3 Individual Risk Factors of Mycetoma Occurrence in Eastern Sennar Locality, Sennar State, Sudan: A Case-Control Study

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Appendix 6: Paper 4 - Modelling the spatial distribution of mycetoma in Sudan

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