J Clin Tuberc Other Mycobact Dis 12 (2018) 54–65

Contents lists available at ScienceDirect

J Clin Tuberc Other Mycobact Dis

journal homepage: www.elsevier.com/locate/jctube

Dynamics of tuberculosis in Wau, South Sudan during a period of armed conflict

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ARTICLE INFO

Keywords:
Tuberculosis
Armed conflict
Wau
South Sudan

ABSTRACT

Background: South Sudan has endured decades of armed conflict, with the most recent in 2016. This has left the health system and infrastructure overstretched by a myriad of infectious diseases like tuberculosis. Our study aimed at quantitatively and qualitatively documenting TB dynamics and challenges with access to health care during a period of civil unrest in Wau.

Materials & Methods: A cross sectional study was carried out between January and February 2016 at Wau Teaching Hospital (WTH). Sputum was randomly collected from 207 of the 1035 TB suspects and analyzed using Ziehl-Neelsen (ZN) and Fluorescent Microscopy (FM), Culture, Capilia MTBC Neo, and DST. The laboratory results and questionnaire metadata were used for descriptive statistics, logistic regression in R version 3.4.2. These results were presented along with results from a qualitative assessment of the situation at WTH.

Results: Of 207 TB suspects, 39 (18.8%) were positive on FM with bacilli growth on culture, later confirmed as *Mycobacterium tuberculosis* complex. Only 5.4% of the cases were resistant to Isoniazid. Age; 20–45 OR = 13 (95%CI = 2.4–25.6, p = 0.011), > 46 OR = 3 (95%CI = 0.5–58, p = 0.005) and raw milk consumption OR = 2.2 (95%CI = 0.37–42.48, p = 0.005) were associated with being TB positive. The qualitative evaluation reveals that gunfights in the surroundings of Wau influenced the number of patients attending WTH, with some travelling up to 545 km to seek medical attention.

Conclusion: We report a high prevalence of tuberculosis among patients who presented at WTH, with approximately 1 out of 5 individuals testing positive for tuberculosis. This is likely an underestimation given the challenges patients had to endure as they sought medical attention. Tuberculosis epidemiology is likely to be driven by individual and household factors, but further investigations are needed to fully understand the risk profile. The tools in use were adequate for TB diagnostics and we observed a remarkably low prevalence of drug resistance, a statistic that is worth preserving. We therefore call for action from all stakeholders.

1. Background

Tuberculosis (TB) is an infectious and chronic inflammatory disease caused by members of *Mycobacterium tuberculosis* complex (MTBC) [1,2]. TB caused by *Mycobacterium tuberculosis* is the 9th leading cause of death globally and in addition, the leading cause from a single infectious agent ranking above HIV/AIDS [2]. This disease accounts for about 9.6 million new cases and 1.5 million deaths annually, majority of who were from Africa, Asia and the former Soviet Union [2]. Factors such as: population density, poverty, malnutrition, armed conflict and imprisonment have for long been linked to the increased incidence of tuberculosis [3-5]. For example, some reports have suggested that armed conflict is most likely responsible for the current TB epidemic in Ukraine [4,6]. Indeed, a recent report using high definition molecular

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https://doi.org/10.1016/j.jctube.2018.06.001
Received 24 February 2018; Received in revised form 10 April 2018; Accepted 5 June 2018
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tools has revealed the legacy of armed conflict on tuberculosis spread [7]. A *Mycobacterium tuberculosis* strain which evolved in Afghanistan and then into the former Soviet Union has spread throughout Northern and Western Europe [7]. The protracted armed conflict in South Sudan is likely to present the same scenario for the Great Lakes region of Africa. The current World Health Organization (WHO) TB estimate for all forms of tuberculosis in South Sudan stands at 140 cases per 100,000 persons, and it is apparent that lack of data has made regular estimates for this country difficult [3,8]. In this case, data generation in such an unstable setting requires that the National Tuberculosis Program (NTP) remains fully functional. Countries like Afghanistan, whose NTP has remained functional during periods of armed conflict have demonstrated that it is possible to meet internationally set TB control targets [9]. Like Afghanistan, South Sudan is currently experiencing high levels of migrations [10]. Wau region is surrounded by three Internally Displaced Persons (IDP) camps within the United Nations Mission sites, "Unity" to the North East, Western Equatorial to the South West and the Lakes IDP camp, each accommodating ~ 0.55, 0.120 and 0.128 million, internally displaced persons respectively [11]. This mass migration has not only led to a complex humanitarian challenge exacerbated by famine [3], but it is most likely to give rise to a tuberculosis epidemic in the region [3]. In order to address this eminent public health problem, there is dire need to document the current dynamics of the disease and identify context specific challenges to its control. This study was therefore aimed at documenting the routine diagnostic capacity, drug susceptibility of isolated *Mycobacterium tuberculosis*, and identify potential drivers of TB. Furthermore, we qualitatively assessed the challenges of accessing TB management facilities in this area.

2. Materials and method

2.1. Study site

The city of Wau is located in the Northwestern part of South Sudan and is home to approximately 151,120 people. This population grew by 20% in the four years after its separation from Sudan [11]; the area is inhabited by the Balanda, Ndogo, Jur Chol, Dinka and Fellata Mbororo who are primarily agro- pastoralists [11,12].

The education, health and economic infra-structure is poor, and has further crumbled due to the recent armed conflict [11]. In fact, this city has been at the center of conflict for sometime and for this reason, it has been referred to as a garrison town acting as a base for Khartoum forces during the second Sudanese civil war in 1998 [11]. Wau Teaching Hospital is a 500-bed health facility, and it's the only hospital of this size in a 350-km radius serving approximately 3 million people [13] (Fig. 1 & S1A-B). The hospital has had a critical shortage of professional health cadres since only about 1.5 physicians and 2 Nurses/Midwifes are available for every 100,000 citizens [14]. The clinicians in this hospital deal mostly with common diseases such as: gastro-intestinal infection, malaria, and HIV/AIDS and tuberculosis [13].

2.2. Study design

This was a cross sectional study conducted at Wau Teaching Hospital between January and February 2016 at the peak of the current armed conflict in South Sudan. The study targeted suspected TB cases, defined as; "Any individual who had a persistent cough for at least two weeks and presented at the tuberculosis unit at the Wau Teaching Hospital" . We used both qualitative and quantitative methods to allow for metric-based analysis linked to nuanced social dynamics of tuberculosis in Wau. In this regard, a structured questionnaire was used to collect quantitative data from the randomly selected patients.

2.3. Sample size estimation

The sample size estimation of suspected tuberculosis cases at the Wau Teaching Hospital was computed based on 0.08–0.46% national average tuberculosis prevalence estimate reported [3,15]. However, we recognize that the regional average although unknown, would more likely be higher than the national average for some areas. Therefore, we assumed a range of 5–19% as reported in pastoral systems with similar settings [16]. With these assumptions, we expected to detect a minimum of 5 cases and a maximum of 19 cases if we randomly sampled 100 suspected TB cases at Wau Teaching Hospital. Here, we collected a sample from a sequence of five (from every fifth) suspected tuberculosis case at Wau teaching hospital. In addition to clinical history and biodata, a questionnaire was administered to each of the sampled suspected TB cases. Given the instability in the region at the time, we utilized a two months window to collect 207 samples from approximately 1035 suspected TB patients.

2.4. Sample collection and transportation

It is note worthy that some of the TB suspects had previously been on medication even in the absence of a confirmatory diagnosis. Three sputum samples (spot-morning-spot) were consecutively collected from 207 suspected TB cases. These included among others routine and referred suspected cases of TB from various areas in Greater Bahr El Ghazal. On the initial hospital visit, the patient was provided with a sterile wide-neck sputum container (see Figure S1) to provide a sputum specimen. The suspected TB case was also given another sputum container and instructed basing on standard early-morning sputum specimen collection for the next day's visit to the clinic [17].

The third specimen was collected when the early-morning specimen was delivered to the laboratory. Here, fluorescent microscopy was done as described below, and the positive samples were kept at - 20 °C in the EPI (Expand Programme on Immunization Unit) refrigerators at Wau Teaching Hospital. Each tube was then packaged in a three-layer receptacle (zip lock sterile bags), maintained at least at 0 °C in an ice box, and transported by road and air transport to the Central Diagnostic Laboratory (CDL), College of Veterinary Medicine, Animal Resources and Biosecurity (COVAB), Makerere University. These were then stored at −80 °C before being delivered to World Health Organization Supranational Tuberculosis Reference Laboratory in Wandegaya, Kampala. The sample transportation by road and air from Southern Sudan was done in compliance with the Southern Sudanese public health Act on transportation of biological material and International Air Transport Association regulations www.iata.org/ads/issg.htm (IATA) respectively.

2.5. Fluorescent microscopy in Wau, South Sudan

Once samples were collected at the teaching hospital, they were then submitted to the in-house diagnostic laboratory for acid-fast microscopic testing (Figure S1C & S1D). A thin smear of the processed sputum material was heat fixed on the slide by the flaming of a Bunsen burner. This was then flooded with Auramine O-Rhodamine B solution and allowed to stand for 15 minutes. This was then followed by a 2-3-minute chlorine free water rinse until there was no more dye leaving the fixed slide. The slide was then flooded for a further 2 minutes with acid alcohol to remove any redundant stain, followed by rinsing with distilled water and then air-drying. At this point, potassium permanganate was added for 2 minutes followed by rinsing and air-drying [18,19]. Presence or absence of acid-fast bodies was determined by use of a fluorescent microscope at 20X, 40X objective magnification for field selection, and later using 100X oil immersion objective to observe the morphology of fluorescing organisms. Bacillary load was graded using standard acid-fast bacillus (AFB) scoring system according to the International Union against Tuberculosis and Lung Disease (IUATLD) and the WHO smear grading scale [17].

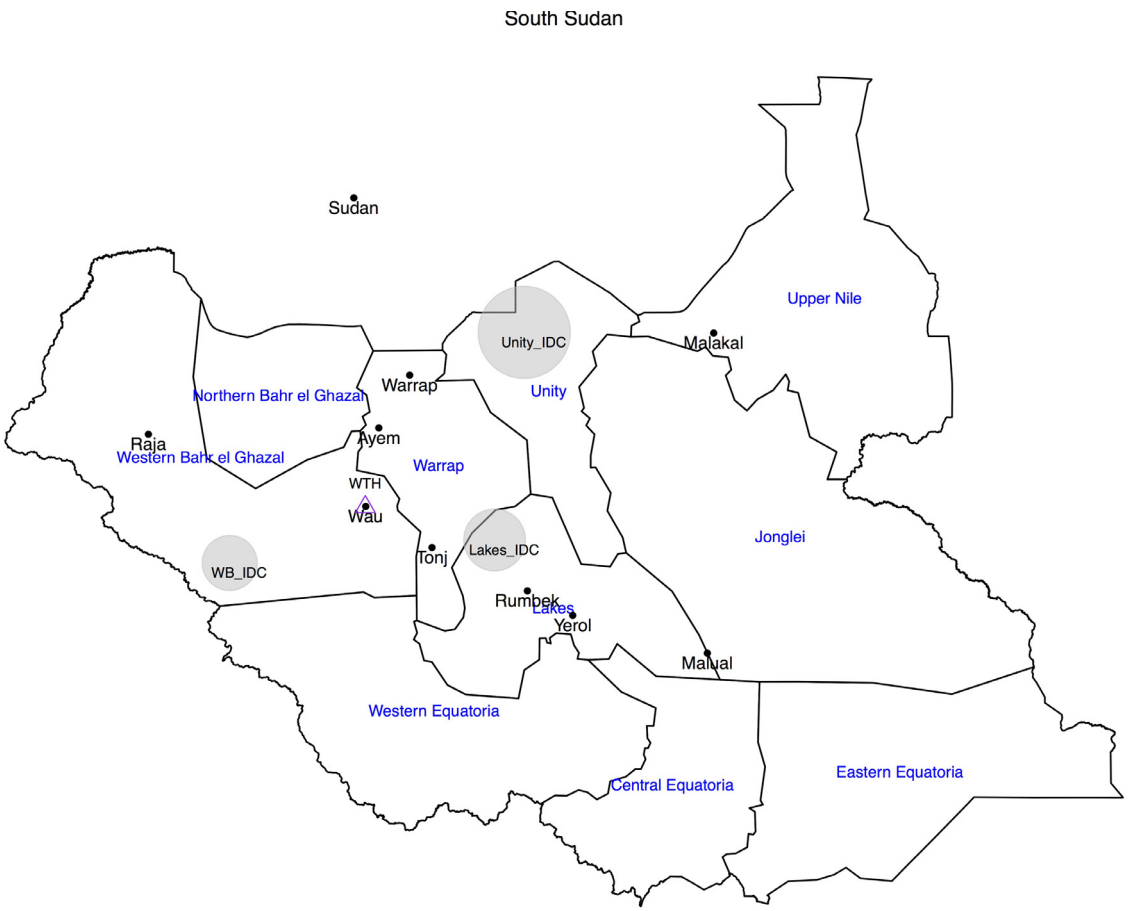


Fig. 1. Shows the Map of South Sudan. Labelled in blue, are the former 10 states Provinces. Each black point represents an area from where a suspected tuberculosis case that visited the Wau Teaching Hospital travelled from. The grey circular shapes represent the internally displaced camps surrounding Wau. Their size represents the number of people in the camp. WTH stands for Wau Teaching Hospital while IDC is internally displaced camp.

2.6. Mycobacteria laboratory diagnostics in Uganda

Sixty-five (65) samples, including all thirty nine (39) acid-fast bacilli positive samples and twenty six (26) randomly selected acid-fast bacilli, negative from Wau Teaching Hospital were then subjected to Ziehl–Neelsen (ZN) test in a BSL-2 (Biosafety Level 2) in College of Veterinary Medicine, Animal Resources & Biosecurity (COVAB) Makerere [20]. It should however be noted that all other specimen handling including opening/closing of tubes, pipetting, smear preparation, culture decontamination/concentration, culture plating and identification methods was done inside a BSL-3 at WHO Supranational Tuberculosis Reference Laboratory in Wandegeya, Kampala [20].

2.6.1. Ziehl–Neelsen acid fast microscopy

Smears were prepared for ZN according to the WHO recommended protocol from sputum material [17,20]. Briefly, the sample was spread evenly on a slide and left for 12–30 minutes to air dry. This was then heat fixed on a hot plate at 85 °C for about 3–5 minutes [17]. One percent (1%) carbol-fuchsin was added to the slide and heated with a Bunsen flame intermittently three times for 15 minutes. This was then rinsed using tap water after which 3% acid alcohol was added for 3 minutes. Methylene blue (0.3%) was then added for up to 1 minute and rinsed off using tap water. Positive and negative controls were included in the process. Slide examination was performed with light microscope at Å ~ 1000 magnification and the AFBs identified were graded according to the International Union against Tuberculosis and Lung Disease (IUATLD) and the WHO smear grading scale [17,20].

2.6.2. Mycobacterium culture

The samples tested on *Ziehl–Neelsen* microscopy above were also cultured on Mycobacterium medium at the WHO supranational Tuberculosis Reference Laboratory in Wandegaya, Kampala as described elsewhere [17,20]. In brief, sputum samples were transferred into 50 ml centrifuge tubes and distilled water was added up to 10 ml, followed by the same volume of a solution of N-acetyl–L cysteine (NALC), NaOH solution (6% NaOH, 2.9% sodium citrate, and 0.5% NALC) and incubated at room temperature on the shaker for 20 minutes. This was then followed by a neutralization step with phosphate buffer (pH 6.8), followed by a concentration step by centrifugation at 3000RPM for 20 minutes. A 0.5 ml inoculation was deposited and spread on Lowenstein-Jensen (L-J) with glycerol and pyruvate media, the rest was frozen in 3 aliquots each. The media were incubated at 37 °C after inoculation, the earliest examination for culture growth was done in the 3rd and 5th day post inoculations, and this was done to monitor fast growing mycobacteria as well as contamination. These were then examined every week for a further seven weeks.

2.6.3. Mycobacterium tuberculosis complex identification

Samples that were positive for typical mycobacteria colony morphology [21], were then further tested on Capilia TB-Neo to identify *Mycobacterium tuberculosis* complex (MTBC) [22]. The Capilia TB-Neo kit is an adapted immune-chromatographic test that detects presence of MPB64 antigens specifically produced by *Mycobacterium tuberculosis* complex (MTBC). Therefore, any acid-fast and culture positive sample that tested negative on this test would provisionally be identified as Non-Tuberculous Mycobacteria. An 80–100 µL specimen was deposited into the specimen placing area of the test plate. Reading section of the

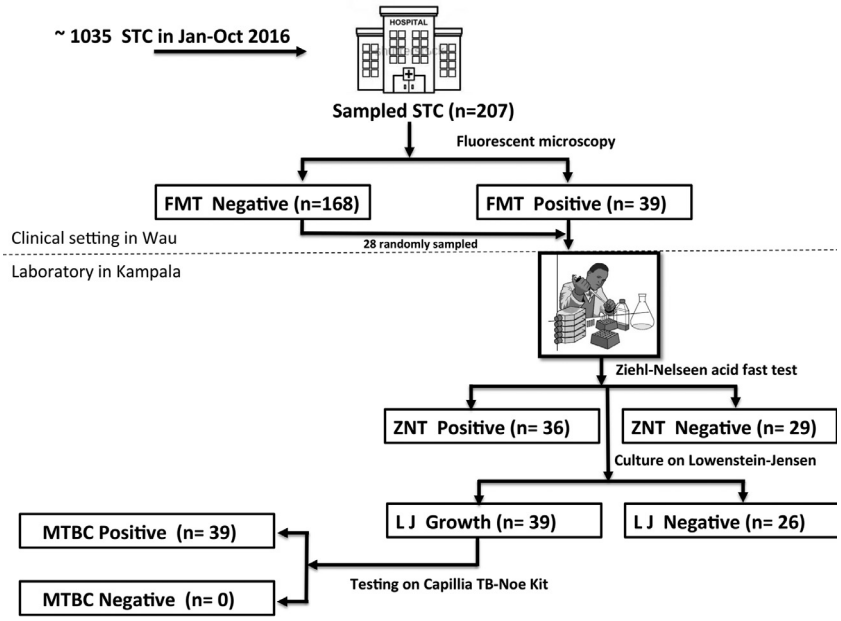


Fig. 2. Shows the diagnostic and molecular typing schematic flow for the collected samples from sampling to the point identification of Mycobacterium tuberculosis complex.

kit was observed on the test plate after 15 min and interpreted the result as follows: Positive, if a purple red line is observed in the reading areas of both [T] and [C]: Negative, if a purple red line was not observed in the reading area [T] but the color was observed in the reading area [C]. A test result was interpreted within 60 minutes after placing each specimen on the test plate [22,23].

2.7. Drug susceptibility testing (DST)

Isolated mycobacteria were tested for antimicrobial resistance using the HAIN MTBDRplus assay. This is a four step assay that involves DNA extraction from the bacteria cells, amplification of extracted DNA, Reverse hybridization of amplified DNA, then the evaluation and interpretation of results [1]. DNA was isolated using the GenoLyse method (Hain Lifesciences, Nehren, Germany). The obtained DNA template was amplified using the HAIN MTBDRplus kit, actual amplification was done in Bio-Rad thermocycler machine (Bio-Rad Laboratories Inc., Singapore). A reaction volume of 50 µl was used (5 µl of DNA template, 45 µl of mastermix 10 µL of AM-A to 35 µL of AM-B). The amplification profile run in the thermo cycler included; step one of Initial denaturation at 95 °C for 15 minutes, followed by step two of 20 cycles with denaturation at 95 °C for 30 seconds and annealing at 65 °C for 2 minutes, followed by step three of 30 cycles that included denaturation at 95 °C for 25 seconds, annealing at 50 °C for 40 seconds, and extension at 70 °C for 40 seconds. The amplification profile was concluded with a final extension at 70 °C for 8 minutes. Positive Control containing 5 µL of M. tuberculosis H37Rv Susceptible to both Rifampicin & Isoniazid was used in the assay. The Reverse hybridizations were done using Genotype MTBDRplus Hybridization protocol fully described in the kit (Hain Lifesciences, Nehren, Germany). The resultant hybridization stripes were evaluated and interpreted using the protocol described in the assay (Hain Lifesciences, Nehren, Germany). The stripes obtained from the hybridization were aligned along evaluation sheets to compare the CC and AC bands with respective lines on the sheet. The presence of the *rpoB* WT bands and KatG WT was observed as an indication for presence or absence of Rifampicin and Isoniazid resistance and susceptibility respectively (Hain Lifesciences, Nehren, Germany).

2.8. Data management

Clinical and metadata from the questionnaire were entered in an Excel Microsoft database, whereby each patient was assigned a unique number as part of the process of anonymizing our data. The clinical and laboratory tuberculosis testing done in Wau and Kampala was added to the database for each patient.

2.9. Quantitative data analysis

2.9.1. Summary statistics

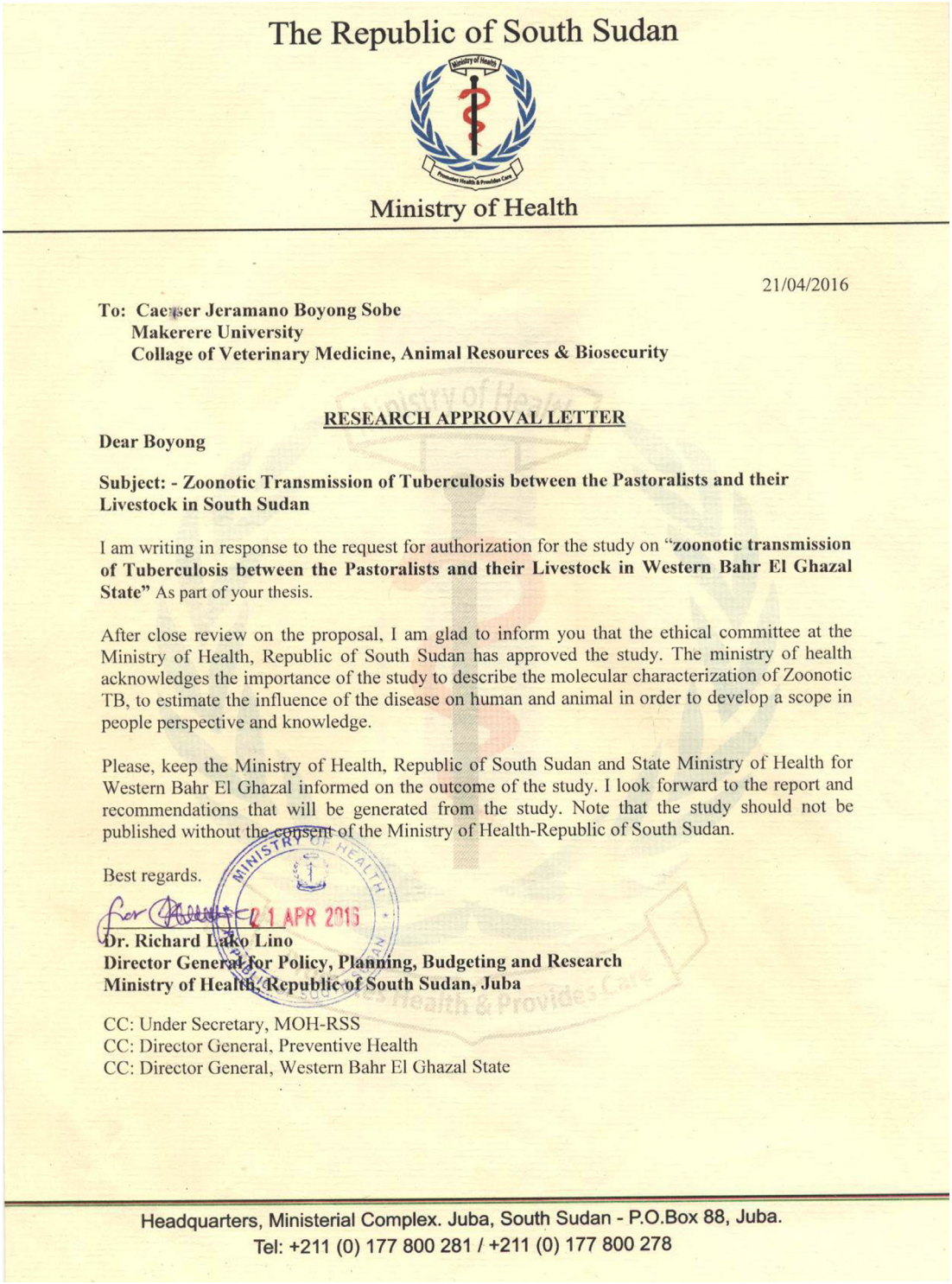
The dataset was then exported to R version 3.4.2. The data was summarized, and we conducted preliminary univariate associations using the Fischer's exact test in R statistics. From these comparisons, a univariate model was developed for the outcome variables of being positive or negative on the fluorescent acid-fast test conducted in Wau. The variables were assessed for their inclusion into the multivariable model i.e. variables with a p-value ≤ 0.2.

2.9.2. Logistic regression model for associations with presence of AFB

The outcome variable of being positive or negative on the florescent microscopy test in Wau against explanatory variables from the clinical biodata as well as metadata from the questionnaire were used to evaluate the association. The odds ratios, P values and confidence intervals have been presented in tables. A logistic regression model was built using the back selection of explanatory variables in the model, until we obtained a model stabilization with the lowest Akaike information criterion (AIC). Model validation was done using the standard Hosmer Lemeshow (HL) test and Areas under the curve (AUC).

2.9.3. Covariate patterns evaluation for TB prediction in Wau

A decision tree was built using the outcome variables for the logistic regression model developed as described above. This was built basing on the corresponding database of N = 207. First, the data set was transformed using “dplyr” package in R to reflect the possible covariate (patient factors) combinations and their corresponding binary outcomes. In this case, the binary outcome was success (Positive sample on fluorescent microscopy test) and failure (Negative sample on fluorescent microscopy test), and the number of trials is the sum of the two binary outcomes. The covariate patterns that corresponded to a zero



The Republic of South Sudan

Ministry of Health

21/04/2016

To: Caesar Jeramano Boyong Sobe
Makerere University
Collage of Veterinary Medicine, Animal Resources & Biosecurity

RESEARCH APPROVAL LETTER

Dear Boyong

Subject: - Zoonotic Transmission of Tuberculosis between the Pastoralists and their Livestock in South Sudan

I am writing in response to the request for authorization for the study on "**zoonotic transmission of Tuberculosis between the Pastoralists and their Livestock in Western Bahr El Ghazal State"** As part of your thesis.

After close review on the proposal, I am glad to inform you that the ethical committee at the Ministry of Health, Republic of South Sudan has approved the study. The ministry of health acknowledges the importance of the study to describe the molecular characterization of Zoonotic TB, to estimate the influence of the disease on human and animal in order to develop a scope in people perspective and knowledge.

Please, keep the Ministry of Health, Republic of South Sudan and State Ministry of Health for Western Bahr El Ghazal informed on the outcome of the study. I look forward to the report and recommendations that will be generated from the study. Note that the study should not be published without the consent of the Ministry of Health-Republic of South Sudan.

Best regards.

21 APR 2016

Dr. Richard Lako Lino
Director General for Policy, Planning, Budgeting and Research
Ministry of Health, Republic of South Sudan, Juba

CC: Under Secretary, MOH-RSS
CC: Director General, Preventive Health
CC: Director General, Western Bahr El Ghazal State

Headquarters, Ministerial Complex. Juba, South Sudan - P.O.Box 88, Juba.
Tel: +211 (0) 177 800 281 / +211 (0) 177 800 278

Fig. 3. Research Approval Letter from South Sudan Ministry of Health.

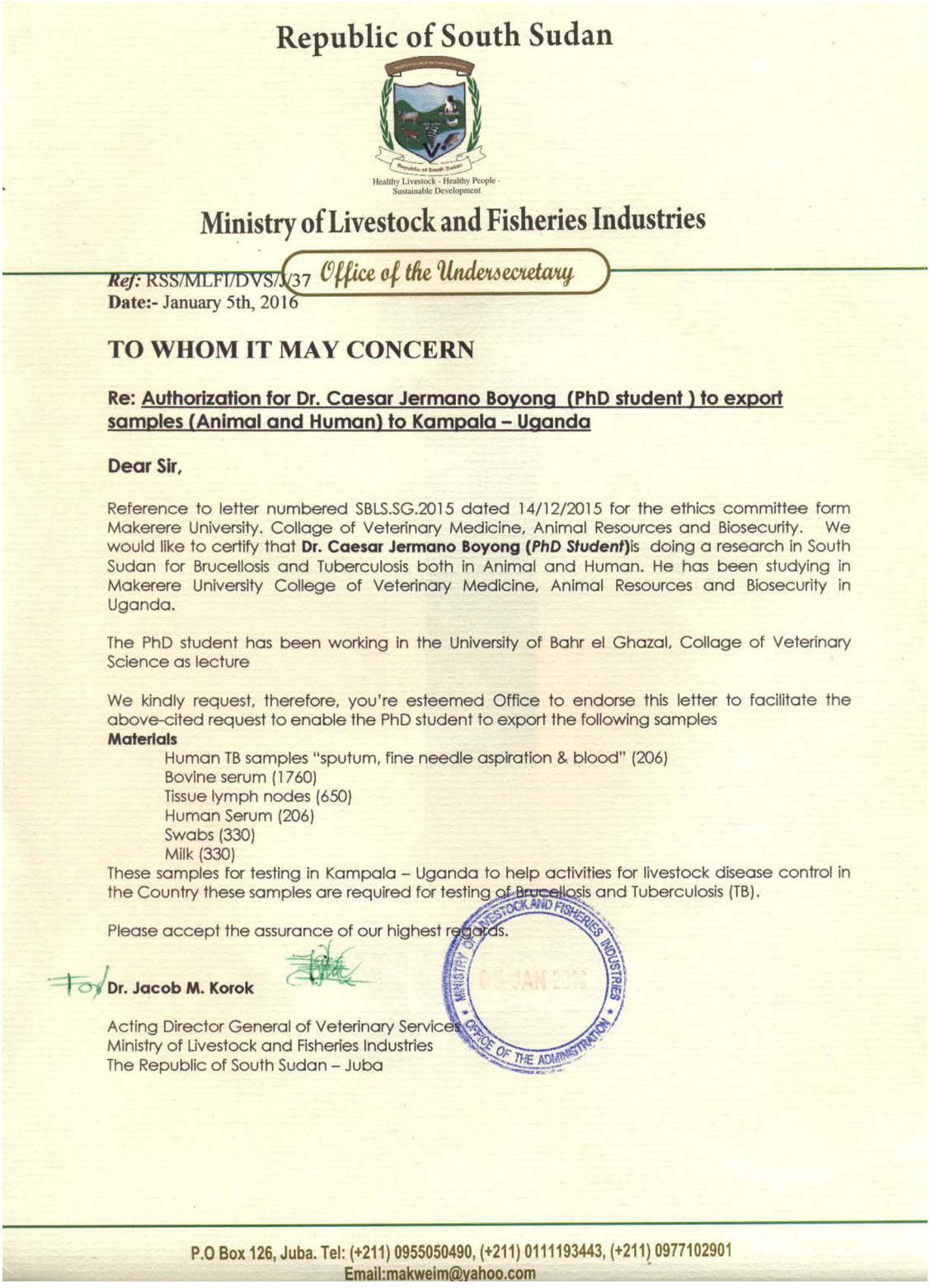
probability of occurrence were removed, and the remaining data was sorted in descending order i.e. the patterns with the highest probability of being positive on the fluorescent microscopy being on top.

2.10. Qualitative data collection and analysis

In addition to this questionnaire, an observational approach and non-structured interview base on a snow balling strategy was used to generate qualitative data. This information was recorded in a daily journal written, and based on routine activities and interaction with patients, clinicians and the laboratory diagnostic team. This then forms the basis on which our qualitative evaluation of tuberculosis dynamics was done.

2.11. Ethical considerations

This study used biological material (sputum), non-invasively collected from suspected TB patients in Wau Teaching Hospital. The metadata, clinical and biodata collected as part of the sample collection were anonymized by assigning unique numbers to each patient. We



Republic of South Sudan

Healthy Livestock - Healthy People - Sustainable Development

Ministry of Livestock and Fisheries Industries

Office of the Undersecretary

Ref: RSS/MLFI/DVS/1/37
Date:- January 5th, 2016

TO WHOM IT MAY CONCERN

Re: Authorization for Dr. Caesar Jermano Boyong (PhD student) to export samples (Animal and Human) to Kampala – Uganda

Dear Sir,

Reference to letter numbered SBLS.SG.2015 dated 14/12/2015 for the ethics committee form Makerere University. Collage of Veterinary Medicine, Animal Resources and Biosecurity. We would like to certify that **Dr. Caesar Jermano Boyong (*PhD Student*)**is doing a research in South Sudan for Brucellosis and Tuberculosis both in Animal and Human. He has been studying in Makerere University College of Veterinary Medicine, Animal Resources and Biosecurity in Uganda.

The PhD student has been working in the University of Bahr el Ghazal, Collage of Veterinary Science as lecture

We kindly request, therefore, you're esteemed Office to endorse this letter to facilitate the above-cited request to enable the PhD student to export the following samples

Materials

Human TB samples "sputum, fine needle aspiration & blood" (206)
Bovine serum (1760)
Tissue lymph nodes (650)
Human Serum (206)
Swabs (330)
Milk (330)

These samples for testing in Kampala – Uganda to help activities for livestock disease control in the Country these samples are required for testing of Brucellosis and Tuberculosis (TB).

Please accept the assurance of our highest regards.

Dr. Jacob M. Korok

Acting Director General of Veterinary Services
Ministry of Livestock and Fisheries Industries
The Republic of South Sudan – Juba

MINISTRY OF LIVESTOCK AND FISHERIES INDUSTRIES * OFFICE OF THE ADMINISTRATION *

P.O Box 126, Juba. Tel: (+211) 0955050490, (+211) 0111193443, (+211) 0977102901
Email:makweim@yahoo.com

Fig. 4. Export permit from Ministry of Livestock and Fisheries Industries South Sudan.

received a Research Approval Letter from South Sudan Ministry of Health

(Fig. 3), Export permit from Ministry of Livestock and Fisheries Industries South Sudan (Fig. 4), and the Import permit from the Ministry of Agriculture Animal Industry and Fisheries Uganda (Fig. 5). Furthermore, the work received Institutional Ethical approval from Makerere University No. SBLS.SG.2015 (Fig. 6). Eligible samples were only collected from suspected TB patients presenting to TB and leprosy clinic at Wau Teaching Hospital, and agreed consent including under 18 Years of age, wherein their consent had been signed by a parent or legal guardian (Supplementary 1).

MINISTRY OF AGRICULTURE,
ANIMAL INDUSTRY AND FISHERIES
WEBSITE: www.agriculture.go.ug
DEPARTMENT OF LIVESTOCK HEALTH
AND ENTOMOLOGY
P. O. Box 513, ENTEBBE, UGANDA
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TELEPHONE: 256 041 320 627, 320166
FAX: 256-041-321047, 256-041-321010,
256-041-321255,320428

In any correspondence on
this subject please quote **LHE 46/172/405**

THE REPUBLIC OF UGANDA

30th October 2015

Ceaser Jermano Boyong Sobe (PhD - STUDENT)
C/O
College of Veterinary Medicine
Animal Resources and Biosecurity
Makerere University
P.O. Box 7062,
KAMPALA - UGANDA

IMPORT PERMIT FOR IMPORTATION OF LYMPH NODES, BLOOD CATTLE AND HUMANS FROM SOUTH SUDAN INTO UGANDA

Your request of 14th October 2015 refers; Permission is granted to you to import Lymph nodes, blood cattle and humans for bovine Tuberculosis Research and Training purposes from South Sudan to Makerere University Kampala, Uganda.

The following conditions must be met by the importer;

1. The samples must be handled in a laboratory (or laboratory suite) that operates at containment level 2 as defined by the OIE (OIE Terrestrial Manual 2008, Chapter 1.1.2 Biosafety and Biosecurity in the veterinary Microbiology Laboratory and Animal facilities.

2. The importer must destroy all received biological samples at the end of the study under supervision of the Commissioner Animal Health accordance to requirements for dangerous goods transportation.

3. The importer must confirm that the samples above will neither be used in any form or way for purposes associated with chemical, biological weapon nor will they be re- exported, resold or otherwise transmitted to another institute.

30/10/2015

COMMISSIONER ANIMAL HEALTH
MINISTRY OF AGRICULTURE,
ANIMAL INDUSTRY AND FISHERIES
P. O. Box 513, Entebbe - Uganda

Fig. 5. Import permit from the Ministry of Agriculture Animal Industry and Fisheries Uganda.

3. Results

3.1. Quantitative data

3.1.1. Summary statistics

Out of the 207 suspected TB cases at Wau Teaching Hospital (WTH) (Fig. 2), 61.8% (128/207) were males, predominately from the Dinka ethnic group 62.3% (127/207), whose main economic activity was agro-pastoralism 69.6% (144/207). Majority of the individuals who presented at WTH were residents of Wau town living in households with an average of six dwellers (Table 1).

4. The sample should be packaged in such away that there will be no leakage during transportation.

5. This import permission is valid for a period of one month from date of issue and is subject to cancellation should conditions necessitating doing so arise.

COMMISSIONER ANIMAL HEALTH
MINISTRY OF AGRICULTURE,
ANIMAL INDUSTRY AND FISHERIES
P. O. Box 513, Entebbe - Uganda

Kasirye Martin (Dr)
For: COMMISSIONER ANIMAL HEALTH

c.c.: Asst. Commissioner Veterinary Inspections and Regulations
" Customs officer (EIA)
" Senior Veterinary Inspector – EIA
" PVO Diagnostic and Epidemiology

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Fig. 5. (*continued*)

3.1.2. TB prevalence and drug susceptibility

The fluorescent microscopy (FM) conducted at WTH detected 39 TB cases, which is approximately 18.8% TB prevalence among individuals who attended WTH during the study period (Table 1). There were twice more males with TB than females, majority of who were from Dinka and Balanda ethnic groups resident in Wau town. The univariate analysis indicates an association between being a TB case and gender, ethnicity, location of residence, as well as milk consumption preferences (Table 1). The findings from the laboratory in Uganda show that 36 of 39 cases on FM were also positive on ZN. However, culture results show that all the 39 cases identified using FM in Wau grew on Mycobacteria selective media and confirmed as *Mycobacterium tuberculosis* complex using Capilia MTBC Neo (Fig. 2). Only two cases were identified as resistant to Isoniazid (Table 4). This represents a 5.4% (2/37) prevalence of Isoniazid resistance among the TB cases at Wau teaching hospital during the study period. All the resistant individuals were adults from the Dinka Ethnic group. On the other hand, the result represents a 94.6% and 100% susceptibility to Isoniazid and Rifampicin respectively.

3.1.3. Factors associated with TB prevalence in Wau

Table 2 shows results of a logistic regression model, it shows that an individual in the age category 20–45 and > 46 had odds of 13 and ~3 for being a TB case when compared to those in age category 10–19 years respectively. Milk consumption also appears to be associated with being a TB case, Individuals who consumed boiled milk had odds of 2.2 for being a TB case when compared to those who did not consume milk.

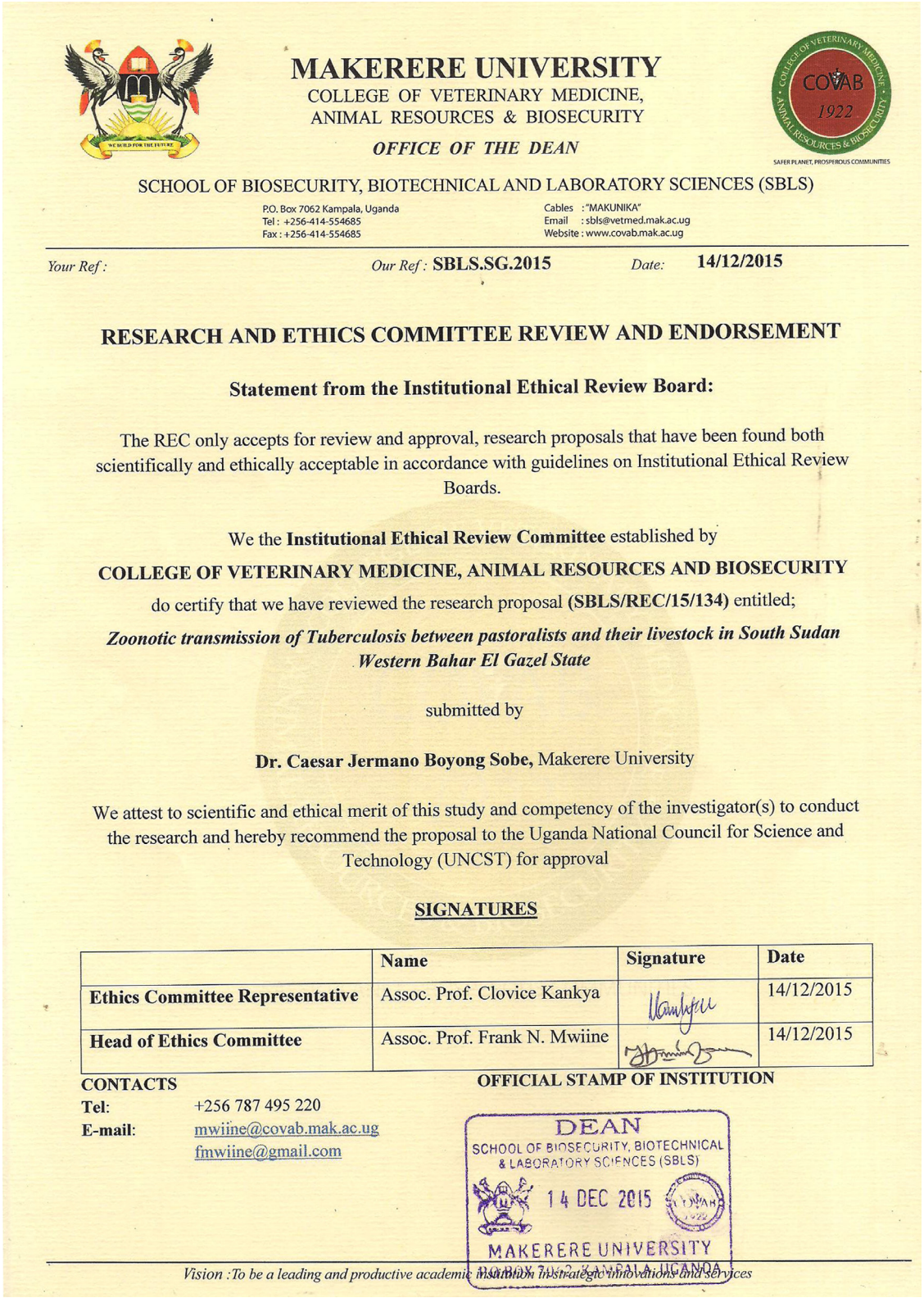
3.1.4. Covariate patterns for TB diagnostics

Table 3 shows the covariate patterns and the corresponding probability of being a TB case in Wau. Approximately, six percent 5.8% (3/51) of the covariate patterns had a probability of 100% being positive on the FM test. However, nine of these occurred once. Three covariate patterns above 50%, with each occurring once had a 66.7% probability of being TB positive using the FM test. Up to 61.2% (38/62) of the covariate patterns had a probability of 0, and these are not shown in Table 3.

3.2. Qualitative results

3.2.1. Tuberculosis at Wau Teaching Hospital (WTH)

A sample collection day usually started with a routine early morning fifteen-minute commute from the University of Bahr El Ghazal campus to Wau teaching hospital. At the WTH, a work-day started with a briefing from the clinician on the expected caseload for that day. This forecast was based on the previous day's updates on the armed conflict status i.e.; if the day before had relatively few sporadic gun battles within the 350-kilometer radius, the numbers were likely to be high, usually in excess of 15 cases. The opposite was true when there had been sustained gun fighting the previous day. The Leprosy and Tuberculosis clinic (see Figure S1-A) managed all cases of leprosy, tuberculosis and Buluri ulcer in this area. Suspected TB cases visiting this WTH were usually waiting to be seen by a physician at 08:30, and the physician would usually start seeing the patients between 09:30 and 11:00. Safety-wise, we observed that protective gear was not used by clinicians, laboratory technicians and the suspected cases. The suspected TB patients were seen by a clinician for an average of twenty-five minutes, after which almost all were requested to provide a sputum samples to the laboratory for the acid-fast diagnosis (see Figure S1- C & D). As part of this history taking, the clinician would also administer the questionnaire for our study to the patient with their consent. Outside in the waiting room through brief chats with suspected TB cases, we learnt that some had walked for hours and some for days from villages as far as Tonj, Raja, Kuajok, through dangerous and unstable territories to reach Wau Teaching Hospital. Once the test came back as positive from the diagnostic technician, the clinician then started these cases on a regiment of anti-TB drugs that were provided free of charge with support from USAID. These first line anti-TB treatment included Ethambutol and Rifampicin (see Figure S1- E & F). It should be noted that most of the cases had been on treatment but some had defaulted due to lack of drug replenishment or they could not return earlier because of the insecurity. One of the cases from the nearby police unit had



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RESEARCH AND ETHICS COMMITTEE REVIEW AND ENDORSEMENT

Statement from the Institutional Ethical Review Board:

The REC only accepts for review and approval, research proposals that have been found both scientifically and ethically acceptable in accordance with guidelines on Institutional Ethical Review Boards.

We the **Institutional Ethical Review Committee** established by

COLLEGE OF VETERINARY MEDICINE, ANIMAL RESOURCES AND BIOSECURITY

do certify that we have reviewed the research proposal **(SBLS/REC/15/134)** entitled;

Zoonotic transmission of Tuberculosis between pastoralists and their livestock in South Sudan Western Bahar El Gazel State

submitted by

Dr. Caesar Jermano Boyong Sobe, Makerere University

We attest to scientific and ethical merit of this study and competency of the investigator(s) to conduct the research and hereby recommend the proposal to the Uganda National Council for Science and Technology (UNCST) for approval

SIGNATURES

	Name	Signature	Date
Ethics Committee Representative	Assoc. Prof. Clovice Kankya		14/12/2015
Head of Ethics Committee	Assoc. Prof. Frank N. Mwiine		14/12/2015

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Fig. 6. Institutional Ethical approval from Makerere University.

previously been treated with anti-TB drugs a few months before but had returned with more severe and persistent cough suggesting resistance to the first line of anti-TB drugs. After two months of sample collection, the samples were packaged and transported to Kampala as described in the materials and methods section. The transportation of these samples was extremely complicated, the first challenge was maintaining the cold chain, given the extremely high temperatures at that time of the year. Secondly, securing air transport meant that our journey to Juba had to be permitted by security unit at Wau airport. Finally, and most challenging of all, was the process of shipping documentation for biological material from Juba airport to Entebbe. Despite these challenges, our team managed to retrieve the samples for further analysis in

Table 1
Summary of statistics for the sampled suspected TB cases At Wau teaching hospital.

Variable	Level	Prevalence %(n = 39)	Total (N = 207)	P-value*
Gender	Female	11.4 (9)	79	0.043
	Male	23.4 (30)	128	1
Ethnicity	Dinka	18.1 ([)	127	0.000
	Jur Chol	58.3 (7)	12	–
	Balanda	12.0 (6)	50	–
	Dango	0.0 (0)	14	–
	Others	75 (3)	4	–
Location	Wau	15.9 (26)	164	0.068
	Warrap	22.6 (7)	31	–
	Yerol	14.3 (1)	7	–
	Others	100(5)	5	–
Occupation	Farmers	15.3 (22)	144	0.068
	Police	19.2 (5)	26	–
	Prisoners	25 (2)	8	–
	Soldier	35.7 (5)	14	–
	Student	33.3 (5)	15	–
Family Size	1 (1–3)	30.6 (11)	36	–
	2 (4–6)	15.1(22)	146	0.079
	3 (7–9)	24.0 (6)	25	–
Direct Contact with Animals	No	15.8 (15)	95	–
	Cattle	17.6 (9)	51	0.382
	Sheep	19.4 (6)	31	–
	Goat	30.0 (9)	30	–
Water Sources	River	23.1 (3)	13	–
	Tanks	45.5 (5)	11	–
	Streams	16.9 (31)	183	0.061
Consumption of Milk	None	1.8 (1)	55	–
	Raw Milk	16.3 (8)	49	0.000
	Soured Milk	8.3 (1)	12	–
	Boiled	31.9 (29)	91	–
Products of Animal Origin#	Non	8.7 (6)	69	–
	Manure	34.2 (27)	79	–
	Hides	8.6 (3)	35	0.000
	Blood	12.5 (3)	24	–
Treatment Status	Non	0.0 (0)	21	–
	Other antibiotics	0.0 (0)	39	–
	TB Treatment	100 (39)	71 147	0.016
Education Status	Non	25.7 (26)	101	0.014
	Elementary	7.9 (5)	63	–
	Intermediate	33.3 (3)	9	–
	High School	14.7 (5)	34	–

* Fischer P value,
# Contact with products of animal origin, location others includes Rumbek,lol,Sudan,NBG, Tonj,UNS. Ethnicity others includes; Kerash, Shuluk, Darfurian and Zande

Uganda.

4. Discussion

The third sustainable development goal aims to limit the impact of diseases like: tuberculosis, Malaria and HIV/AID globally by 2030 [24]. Similarly, the World Health Organization's End Tuberculosis Strategy endorsed by member states in 2014 set an ambitious goal of ending tuberculosis by the year 2035 [1]. It is however most likely that armed conflicts will continue to present a challenge to these national and international TB control efforts. This is because health consequences of displacing people and the breakdown of social and health services greatly enhances the risk of transmission of tuberculosis [5]. There are remarkably few studies documenting the dynamics of tuberculosis in areas experiencing armed conflict, which inherently limits effective resource allocation for control. Therefore, this study aimed at documenting TB routine diagnostic capacity, drug susceptibility of recovered *Mycobacterium tuberculosis,* as well as identifying potential drivers in this area. Furthermore, we qualitatively assessed the challenges of accessing TB management facilities in this area.

Table 2
Logistic regression model for the association between selected variables and results from fluorescent microscopy test at Wau teaching hospital.

Variable	Level	Odds ratio (CI = 95%)	P-value
Age	10–19	1	
	20–45	13.35 (2.404 - 25.6)	0.011
	> 46	2.95 (0.49 - 58.96)	0.005
Gender	Male	1	–
	Female	1.89 (0.760 −5.06)	0.182
Family Size	1 (1–3)	1	–
	2 (4–6)	1.93 (0.65 - 6.05)	0.241
	3 (7–9)	2.23 (0.46 - 11.01)	0.311
Source of Income	Independent	1	–
	Dependent	1.55 (0. 57 - 4.27)	0.387
Water Source	River	1	–
	Tanks	3.30 (0. 38 - 31.45)	0.280
	Streams	1.21 (0. 24 - 7.03)	0.817
Milk Consumption	Not consuming	1	–
	Raw Milk	7.93 (0.126 - 15.58)	0.062
	Soured Milk	2.47 (0. 72 - 8.41)	0.578
	Boiled	2.21 (0. 37 - 42.48)	0.005

AIC 175.55, AUC = 0.82, HL (df = 8) p = 0.065

4.1. Tuberculosis prevalence estimate in Wau

We estimate that one in five patients who present at the Wau teaching hospital were infected with the pathogen that causes tuberculosis. The estimates in this study are higher than it has been reported in similar settings in Somalia and Ukraine [3,7,25]. We observed that majority of the cases were male, this however could be due to the skew in gender distribution in our data. However, because the patients were randomly selected, this could also be a reflection of the difference in the health seeking behavior between females and males. Indeed, males have been shown to be more likely to seek health services than their female counter parts [26]. The similar gender based health seeking behavior has also been shown in Asian countries like Bangladesh [27]. The area with the second highest number of TB cases was Warrap ~ 111 kms west of Wau town. In fact, Wau and Warrap accounted for 84% of the tuberculosis cases in this study. This suggest that these two townships could be hotspots for tuberculosis, and such a status quo would be driven by population density and compounded by poor health care systems [10]. Wau town is predominantly inhabited by the Dinka ethnic group who account for a significant proportion of our study population [10,13]. Therefore, it is not surprising that majority of the TB cases encountered in this study were from the Dinka ethnic group. It is however worth noting that 61% (24/39) of the TB cases had been in contact with livestock. Similarly, this could be because our sample is dominated by the Dinka ethnic group whose occupation is pastoralism.

From a diagnostic point of view, the tools used at Wau Teaching Hospital to detect *Mycobacterium* in sputum are adequate. This assessment is based on the result that all the thirty-nine detected cases using fluorescent microscopy at WTH were also found to contain *Mycobacterium* using the downstream diagnostics conducted in Uganda. Likewise, all 26 randomly sampled negatives on fluorescent microscopy in WTH were also found to be negative on the downstream diagnostics. The quality of such diagnostic tools is highly dependent on the skills and experience of the diagnostician, as well as the workload [7]. On this basis, other NTP centers could benefit from these skills and experience through training by the diagnostic technical team at Wau.

Table 3
Covariate patterns and their TB predictive probability at Wau Teaching Hospital.

Age	Gender	Family Size	Income Source	Milk Consumption	Success	Trials	Probability of TB
20–45	Female	4–7	Dependent	Raw	1	1	1
20–45	Male	1–3	Dependent	Raw	2	2	1
< 46	Male	<8	Independent	Boiled Milk	1	1	1
20–45	Female	4–7	Independent	Boiled Milk	2	3	0.667
< 46	Female	4–7	Dependent	Boiled Milk	2	3	0.667
< 46	Male	<8	Dependent	Boiled Milk	2	3	0.667
20–45	Male	4–7	Independent	Boiled Milk	5	9	0.556
10–17	Male	1–3	Independent	Boiled Milk	1	2	0.5
20–45	Female	4–7	Dependent	Boiled Milk	3	6	0.5
20–45	Male	1–3	Dependent	Boiled Milk	4	8	0.5
20–45	Male	<8	Dependent	Raw	2	4	0.5
< 46	Female	4–7	Independent	Boiled Milk	1	2	0.5
< 46	Male	4–7	Independent	Boiled Milk	1	2	0.5
< 46	Male	4–7	Dependent	Boiled Milk	1	2	0.5
< 46	Male	<8	Dependent	Soured Milk	1	2	0.5
< 46	Male	4–7	Independent	Raw	2	6	0.333
20–45	Male	1–3	Independent	Boiled Milk	2	7	0.286
20–45	Male	4–7	Dependent	Boiled Milk	2	8	0.25
< 46	Male	1–3	Independent	Boiled Milk	1	4	0.25
< 46	Male	1–3	Dependent	Boiled Milk	1	4	0.25
< 46	Male	4–7	Independent	Raw	1	8	0.125
20–45	Male	4–7	Independent	Raw	1	13	0.077

Table 4
Shows the drug susceptibility profile using MTBD plus assay on the culture positive samples.

Variable	Level	Rifampicin (rpoB)		Isoniazid (KatG)	
		S	R	S	R
Sex	Male	28	0	27	1
	Female	9	0	8	1
Age	Young	1	0	1	0
	Adult	30	0	28	2
	Old	6	0	6	0
Occupation	Farmer	23	0	22	1
	Soldier	6	0	6	0
	Police	4	0	4	0
	Street vendor	1	0	1	0
	Student	3	0	2	1
Ethnicity	Balanda	5	0	5	0
	Dinka	22	0	20	2
	Jur Chol	7	0	7	0
	Others	3	0	3	0

Note that two samples were not tested so N = 37, R = Resistant and S = Susceptible

4.2. Factors associated with TB prevalence in Wau

The findings show a statistically significant relationship between TB and age. Individuals who were between the age of 20–45 and those above 46 years were 13 and 2.9 times more likely to be TB cases respectively when compared to individuals under the ages of 10–19 years. This finding is in an agreement with reports elsewhere [2,28,29] which reaffirms the notion that tuberculosis mostly affects people in the most productive ages of their life and possibly linked with HIV. It should be noted that although the summary statistics had shown a skewed distribution of TB cases towards the male gender, our model did not find this relationship statistically significant. The model however showed a significant association between consumption of milk with being a TB case. An individual who consumed boiled milk was twice more likely to be a TB case than those who did not consume milk. This finding could suggest that most of the cases would be infected with *Mycobacterium tuberculosis* as opposed to *M.bovis*. It could also indirectly represent the milk consumption behavior in urban centers where majority of suspected cases reside.

However, we cannot rule out the possibility of zoonotic tuberculosis given that consumption of raw milk also emerged marginally significant. This however cannot be confirmed by this report, given that we could not type the strains beyond the current molecular techniques due to inadequate resources.

4.3. Covariate patterns and TB case triage

Decision trees have been used elsewhere [30] to inform diagnostic algorithms, they are a representation of covariate patterns behind the logistic regression model developed. The predictive value of a combination of variables can be exploited at clinical level to aid triaging of cases in Wau Teaching Hospital. For example; a financially dependent female between 20–45 years of age coming from a house hold with 4–7 inhabitants and consumed raw milk had 100% probability of having tuberculosis. This covariate pattern however occurred once among the 207 suspected TB cases, but the same pattern with consumption of boiled milk instead of raw milk occurred three times, and two of those times it corresponded to a TB case. The findings of this analysis suggest that a high house occupancy is associated with a high probability of being a TB case. Such an approach has the utility of retrospectively identifying suitable combinations of variables which can aid clinical triaging at Wau Teaching Hospital.

4.4. Access to tuberculosis management facilities

The young nation of Southern Sudan has been experiencing armed conflict since 2013 which has lead to displacement of millions of people (See Fig. 1). In this study, although sixty-six percent of the suspected tuberculosis cases were residents of Wau Greater region, more than a third of the cases came from towns 100–545 Km from Wau town. Even by car, these would be considered large distances for a patient to travel to seek TB medical attention. Indeed, our qualitative data revealed that some of these individuals had walked these great distances to Wau. In some cases, the patients indicated that their journeys were interrupted by gunfights. It is therefore most likely that only those who could brave the long journey and the insecure conditions made it to this health facility. This suggests that the actual estimate of tuberculosis might be higher than what we have reported. The most striking aspect about these findings is that some TB cases indicated that they had previously been on treatment which they did not complete because it was not possible to travel to WTH for replenishment. This failure to adhere to treatment regiment is reported to be the biggest cause of resistance to

TB drugs elsewhere [27,30]. However, our drug susceptibility profiles show that majority of the cases were still treatable with the first line of TB drugs i.e. 94.6% and 100% susceptibility to Isoniazid and Rifampicin. In contrast with other countries like Ukraine, Georgia and Syria that have experience protracted armed conflicts [3,31,32], the findings show a remarkably low prevalence of resistance. Whether this is a direct result of a functional TB service provision as reported about Afghanistan, this cannot be answered with the data in our study. Therefore, further studies ought to be designed in this area to understand this trend.

4.5. Limitations of the study

Security constrains greatly limited the number of cases that could be sampled in this study which inherently reduced the statistical power and the robustness of the inferences drawn from this data. Furthermore, the limited resources meant that we could not type our samples beyond the current molecular granularity. In the absence of additional resources, the authors decided to preserve the topicality of this work. However, upon acquisition of resources, these samples will be analyzed using more advanced diagnostic and typing tools.

5. Conclusions

We report a high prevalence of tuberculosis among patients who presented at WTH, with approximately 1 among 5 individuals testing positive for tuberculosis. This is probably an underestimation, given the challenges patients had to endure seeking medical attention. Tuberculosis epidemiology is probably driven by individual and household factors, but further investigations are needed to fully understand the risk profile. The tools in use were adequate for TB diagnostics and we observed a remarkably low prevalence of drug resistance, a statistic that is worth preserving; we therefore call all stakeholders to action.

Supplementary 1

Informed consent form to the participants

Conflict of interest

Authors declare no competing interests.

Acknowledgment

The authors would like to thank all the brave staff at Wau Teaching Hospital in South Sudan who have kept the tuberculosis management operational, despite the insecure conditions. We thank the staff of both the Central Diagnostic Laboratory, College of Veterinary Medicine, Animal Resources and Biosecurity – Makerere University; and National Tuberculosis Referral Laboratory NTBRL – Kampala, who assisted in sample processing and culture. We would like to acknowledge the Norwegian Government for support through the NORHED (CAPAZOMANENTICO project No. UGA13-0031).

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.jctube.2018.06.001.

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