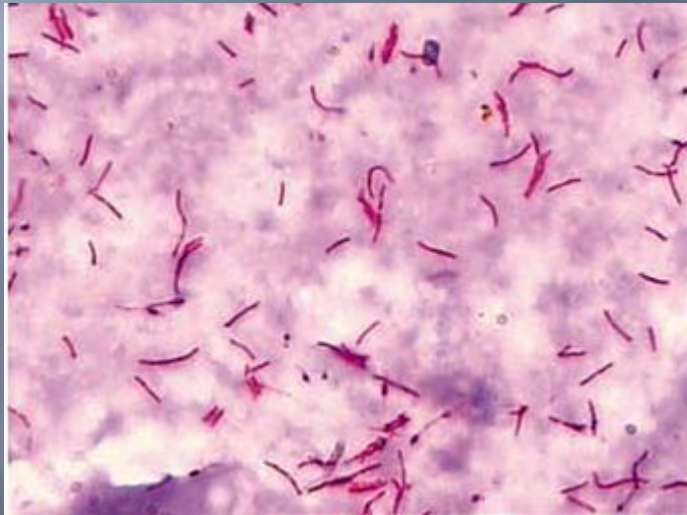# PREVALENCE, MOLECULAR CHARACTERIZATION AND DRUG SUSCEPTIBILITY OF ZOONOTIC RECURRENT AND CO-INFECTIOUS *MYCOBACTERIUM BOVIS* AND *MYCOBACTERIUM TUBERCULOSIS* IN WESTERN KENYA

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INTRODUCTION

- *Mycobacterium tuberculosis* and *Mycobacterium bovis* infect animals and humans

- Bovine tuberculosis has been on the increase in developed countries and continues to occur in developing countries

- Bovine tuberculosis is an infectious disease of cattle mainly caused by Mycobacterium bovis and characterized by progressive development of tubercles in any tissue or organ of the body.

- Africa, approximately 85% of cattle and 82% of the human population live in areas where the disease is prevalent

- Humans can easily become infected with bovine tuberculosis if their immune system is already battling an infection or else suppressed.

- MDR-TB (isoniazid and rifampicin),XDR-TB (isoniazid , rifampicin + fluoroquinolone or aminoglycosides)

- The epidemic of HIV and AIDS in developing countries is making *M. bovis* and *M tuberculosis* infection a serious public health threat

- *Mycobacterium tuberculosis* (MTB) has been assumed to be clonally simple, and a tuberculosis (TB) case was traditionally thought to be infected by a single MTB strain. (MIRUVNTR) loci typing PCR)

- *Mycobacterium tuberculosis* and *Mycobacterium bovis* poses major risks to the health and welfare of global human and animal populations.Human populations are directly at risk from infection and indirectly at risk through the impact on their food supply.

Current status of BTB/MTB

Country	Population	BTB-Livestock	MTB-livestock/Humans	New MTB-Humans	EST New TB/HIV cases	Current MDR-TB
World				**9.4 m**	**15%**	5.8%
Africa				3 .9M		
Kenya	37,538,000			132,357	47.9%	1.9%
Uganda	30,884,000	6.0%	3.1%(m bovis	101785	38.7	0.5

JUSTIFICATION

- A lot is known about this disease such as the causative agent and disease symptoms of bovine tuberculosis

- No current information is available in Kenya, since Kenya, Namibia, the Seychelles and Zimbabwe were declared free of bovine tuberculosis, but that was two decades ago. .

- The recurrence may be due to re-infection from infected patients, co-infection with a multi Drug-resistant *Mycobacterium tuberculosis* complex (MTC) or zoonotic reinfection

- The choice of Busia as a study area is ideal due to the complex effects of HIV/AIDS and high TB burden.

- Busia, a very poor, densely populated area whose communities mix crop growing with livestock raising on small plots of land .

- This study intend to conduct current research of bovine tuberculosis with respect to prevalence , molecular characterization , clonal complexity and drug susceptibility of zoonotic tuberculosis so as to inform the relevant health authority to curb and prevent the spread of Zoonotic tuberculosis from animals to humans and vice versa

Hypothesis

- **Hypothesis**
- *Mycobacterium bovis* and *Mycobacterium tuberculosis* infections in animals and humans in Busia Kenya are prevalent, genetically heterogeneous and drug resistant to TB drugs

- **MAIN OBJECTIVE**
- **To determine prevalence, genetic heterogeneity and drug susceptibility of *M. bovis* and/or M. *tuberculosis* isolates from animals and humans in Busia.**

- **SPECIFIC OBJECTIVE**
- To determine genetic heterogeneity of *M. bovis* and M. *tuberculosis* isolates in Busia district, Kenya.
- To determine drug resistance profiles of M. *tuberculosis* and *M. bovis* isolates.
- To conduct DNA sequencing of genes encoding for drug resistance and Multidrug-resistant TB in *M. bovis* and M. *tuberculosis* isolates.
- To determine prevalence of the latent *M. bovis and M. tuberculosis* infection in animals and humans in Busia.

MATERIALS AND METHODS

- **Study Site** Busia District
- **Study design** Cross sectional study
- **Study Population** Patients and Cattles with at least one positive culture for *M. tuberculosis* and *M. bovis* in Busia
- **Sample size.** Patients presenting with pulmonary TB symptoms n=216
- Animal presenting with TB symptoms n=383

MATERIALS AND METHODS

- Sample Collection and processing

Sputum samples will be collected from patients in the field and forwarded to national referral laboratory

Pre-treatment of Sputum Sample, 4% sodium hydroxide - a decontaminant

- stained first by the Ziehl-Neelsen

- Inoculation, Incubation and Reading of culture Biochemical identification tests

MATERIALS AND METHODS

- Samples collection from cattle
- clinical signs utilized will be symptoms of lymphadenopathies, loss of body weight and/or production loss, intermittent pyrexia, udder infection, and dry cough

MATERIALS AND METHODS

- Samples will be collected from each animal, namely, fine needle aspirate (FNA) from the prescapular lymph node (PSLN), citrated blood, milk, pharyngeal swab, urine, rectal pinch and fecal sample.

- Inoculation, Incubation and Reading of culture Biochemical identification tests

- Identification of mycobacteria isolated from bovine samples

identification

- *Mycobacterium tuberculosis complex* includes the four *species M. tuberculosis, M. bovis,* M. africanum, and M. microti.
- glycerol favour the growth of *M. tuberculosis* but inhibit the growth of both *M. bovis* and *M. africanum* Ogawa egg medium (Kudoh medium) without glycerol but containing 0.5% sodium pyruvate   this is supposed to encourage the growth of *M. bovis* and *M. africanum*

- Species level identification of isolates will be done by standard biochemical tests (niacin production, nitrate reduction, catalase and aryl sulfatase activity, Tween hydrolysis, thiopen-2-carboxylic acid hydrazide sensitivity)

MATERIALS AND METHODS

- Drug susceptibility testing for *M tuberculosis* and *M bovis* phenotypic

- Molecular biology techniques

Deoxyribonucleic acid (DNA) extraction ,

Mycobacterialinterspersed repetitive unit-variable number of tandem repeat (MIRUVNTR) loci typing PCR

Drug resistance-related genes PCR amplification reaction

DNA sequencing of drug resistance-related genes

MATERIALS AND METHODS

- Statistical Analysis
- Data entry and analysis will be performed using SPSS statistical package for social science version 10 (SPSS, Inc., Chicago, IL, USA).
- The quantitative data will be presented as a mean and standard deviation and the qualitative data will be presented as number and percentage
- chi-square (χ^2) will be used to find the association between row and column variables of qualitative data
- Pearson's correlation coefficients will be used to estimate the relationships of bovine tuberculosis in human and prevalence of bovine tuberculosis with animals.

Ethical considerations

- This study is a study within a study (SSC NO. 1701), the study is part of a Welcome-trust funded research project currently studying Epidemiology of zoonotic infections amongst livestock and their keepers in Western Kenya in collaboration with KEMRI, ILRI and University of Edinburgh (SSC NO. 1701).