The Wellcome Trust Bovine TB Project in Ethiopia: The Bovine TB Project Team

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Abstract

The importance of bovine tuberculosis to the economy and public health is being assessed in Ethiopia to generate the evidence base for better control options. A cross sectional prevalence survey in cattle and human populations will be conducted. The influence of cattle genetic background on susceptibility to bovine TB will be studied. Prevalence and type of *M. bovis* in exotic and local breeds will be compared and the immunology, pathology and genetics of cattle susceptibility studied. The efficacy of neonatal BCG vaccination will be tested in a natural transmission setting and a protocol developed for such trials. The cost of bovine TB to the society will be estimated and a cost-benefit model of intervention developed. The project will strengthen local capacity to undertake mycobacterial typing and epidemiological modelling studies. [*Ethiop.J.Health Dev.* 2008;22(Special Issue):128-131]

Introduction

As part of its poverty reduction strategy, Ethiopia has recently embarked on a programme of increasing milk production through intensive breeding of exotic dairy cattle. Holstein-Friesians have been imported and distributed to farmers in many regions, including the Sellale and Holeta areas near Addis Ababa. Holstein-Friesian cows yield up to 5-7 times more milk per kg body weight than local Zebu breeds of *Bos indicus* ancestry (1). There is much evidence however that exotic breeds are more susceptible to bovine TB than local Zebu. The programme to increase milk may therefore have the inadvertent effect of increasing bovine TB.

Bovine tuberculosis is an important cause of economic loss in affected communities. It reduces milk yield and meat production in infected animals. *M. bovis* may be transmitted through aerosols, or through the consumption of raw milk or other contaminated products posing a serious threat to human health.

Bovine tuberculosis has been eradicated from most of the developed world through the test and slaughter strategy where infected animals are removed. The UK spends about 100 million pounds sterling every year implementing these measures (2). The high cost of the test and slaughter policy restricts its application for the control of bovine TB in developing countries. In addition, available resources are often directed at combating human TB caused by *M. tuberculosis*.

Ethiopia ranks 8th in the number of newly diagnosed TB cases globally (3). This is further aggravated by the spreading HIV epidemic. There is however little information on the contribution of *M. bovis* to this burden. The proportion of extra-pulmonary TB has been rising steadily over the last decade, currently accounting for 35% of all newly diagnosed cases (4). For comparison, the rates for Uganda and Kenya are 8% and 14% respectively (3). Over three-quarters of the extra-pulmonary cases affect lymph nodes, raising the possibility of orally acquired *M. bovis.*

A consortium involving researchers from Imperial College London, the Veterinary Laboratories Agency, Weybridge (UK), Trinity College Dublin (Ireland), Swiss Tropical Institute, Basel (Switzerland), International Livestock Research Institute, Nairobi (Kenya) and the Armauer Hansen Research Institute in Addis Ababa was established to study the problem of bovine TB in Ethiopia. The project was designed to measure the cost of bovine TB to Ethiopia by assessing its impact on livestock and human health. Based on the findings, an economic model will be developed to assess the likely effect of introducing Holstein cattle into the local herd and of implementing control measures for TB. Three questions are being addressed by the study: the magnitude and economic cost of the bovine TB problem in Ethiopia, the likely impact and economic cost of changes in the genetic background of the Ethiopian cattle herd, and cost-effective interventions to reduce the impact of bovine TB in Ethiopia.

Methods and expected outcomes

The project is funded by the Wellcome Trust as part of the Animal Health in the Developing World initiative and received approval from institutional ethical review committees and the National Ethical Review Committee of Ethiopia. In addition, an independent committee composed of veterinarians reviewed and approved the veterinary aspects of the proposal. The study is currently being undertaken in seven linked work packages (WP) that complement each other.

WP 1 - Cross-sectional survey of *M. bovis* in Ethiopian cattle (Stephen Gordon, Endalamaw Gadisa, Stefan Berg).

The objective of this WP is to obtain an overview of the population structure of *M. bovis* in Ethiopia and to determine which strains show a greater prevalence, and whether particular strains show association with certain cattle breeds. Samples are collected from suspected TB lesions during routine examination of cattle slaughtered in the abattoirs of Gondar, Woldeya, Ghimbi, Jinka and Butajira and cultured for mycobacteria in Addis Ababa. The isolates are characterized using molecular techniques, initially with spoligotyping and VNTR and subsequently

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with multi-locus sequence typing (MLST) and whole genome microarray analysis for selected isolates. Findings will be compared with results from other settings such as the UK where a control policy has been in place for over 50 years.

WP2: Cross-sectional survey of *M. bovis* in human lymph node TB (Abraham Aseffa, Howard Engers). Fine needle aspirate (FNA) material has recently been shown to be adequate for the diagnosis of TB with cytology, culture or polymerase chain-reaction (PCR), circumventing the need for the more invasive excision biopsy. In this WP, mycobacterial isolates from a total of 1300 FNA samples collected from patients with suspected TB lymphadenitis presenting at the hospitals in Gondar, Woldeya, Ghimbi, Jinka and Butajira are cultured and isolates characterized. The study will identify the proportion of *M. bovis* in cultures in FNA samples from cervical lymph nodes of patients with suspected TB lymphadenitis and will characterize the genetic diversity of *M. bovis* isolates from humans and compare these with isolates from cattle to establish a zoonotic link. The data will be linked to the epidemiological analysis in WP6. Data from patients consenting to be tested for HIV will be used to assess the influence of HIV on the risk of TB lymphadenitis. In this WP, the sensitivity of PCR in the diagnosis of TB lymphadenitis from FNA samples will be determined using culture as gold standard.

WP3 - Prevalence study of bovine TB in Sellale region (Gobena Ameni, Martin Vordermeier): In this WP, the prevalence of bovine TB in the different breeds of cattle kept on pasture in Sellale was determined (5). Single intradermal comparative tuberculin skin testing was performed on 925 Holstein cattle, 2578 Zebu breeds and 1921 crossbreeds. The prevalence of bovine TB in Zebu was 11.6% compared to 11.9% in cross breeds and 22.2% among Holstein cattle. Overall prevalence was 13.5%. The risk of bovine TB among Holstein cattle was found to be at least two times higher than among the Zebu kept on pasture, under identical filed husbandry management. The sensitivity of the INF-□ γ test using avian and bovine PPD, ESAT-6 and CFP-10 peptide cocktails as well as serological assays in comparison to the conventional tuberculin skin test was evaluated (6). A significantly higher IFN- γ response to the cocktail was observed among Holsteins compared to the Zebu, and among Holsteins kept indoors than Holstein cattle kept in a pasture. A higher lesion severity was also noted in Holstein cattle kept indoors compared to those kept in pasture. Cattle husbandry was thus found to be a predominant factor affecting the pathology of bovine TB and IFN-γ responses to mycobacterial antigens in Ethiopia.

WP4 - An analysis of the influence of cattle genetic background on susceptibility to bovine TB (Dan Bradley, Yonas Hirutu): Although there is evidence that *Bos indicus* cattle are more resistant to bovine TB than *Bos taurus*, a direct link between cattle susceptibility to *M. bovis* and genetic admixture component is yet to be established. Comprehensive individual admixture estimates will be analysed against epidemiological, susceptibility and *M. bovis* strain types to detect any significant differences between the two cattle lineages. A heritable component of susceptibility will be highlighted. Several candidate genes implicated in susceptibility to *M. tuberculosis* may play a similar role in *M. bovis* infection. Genetic variants unique to one or other bovine lineage will be ascertained using single nucleotide polymorphisms (SNPs). Physically linked clusters of tightly linked *Bos Taurus/Bos indicus* diagnostic SNP markers, selected from mapped data from the bovine genome project, will be used to assess the amount of recombination that has occurred between ancestral genomes to capture relative contribution of African and European components within the *Bos taurus* fraction of the genome. A set of informative SNP markers for tracking admixture in African cattle will thus be developed. Admixture will be estimated for the infected samples from the prevalence studies. The study is expected to provide information on the genetic basis for differences in susceptibility to bovine TB between the two cattle lineages and provide data on involvement of candidate genes in disease susceptibility.

WP5 - Experimental infection of cattle with *M. bovis* (Mboya Burudi, Richard Bishop): In this WP, a controlled experimental challenge model will be employed to generate comparative data on immune responses to BCG vaccination in Holstein and *Bos indicus* cattle and further define differences in genetic susceptibility between the two breeds. It will offer an experimental platform to support a genome-wide screen for loci that influence differential susceptibility to bovine TB. Groups of Holstein and *Bos indicus* cattle will be vaccinated with BCG or infected with the sequenced *M. bovis* field strain and their immune responses assessed regularly using various immunological assays including host gene arrays to study gene expression.

WP6 - Estimation of the costs to society of bovine TB and cost-benefit model of interventions (Jakob Zinnstag, Rea Tschopp, Getu Melese). The effect of infection on livestock productivity, on human health and human-health related costs including income loss will be assessed to estimate the cost of bovine TB to the Ethiopian society. The prevalence of infection in cattle and humans, the risk factors for the transmission of *M. bovis* in cattle and to humans and the efficacy of various

intervention strategies will be estimated to develop a mathematical transmission model for *M. bovis* in a similar way to that described for brucellosis by Zinnstag *et al* (7). Disease prevalence in cattle and humans will be estimated

based on the literature, abattoir survey data and data from human samples collected from cervical lymph nodes and pulmonary TB patients. Household surveys will be conducted on bovine TB cases and matched controls to determine risk factors. Analysis of the health benefits of intervention strategies will be computed in monetary terms for the agricultural (avoided losses) and public health sectors (avoided costs) as well as for the households of index cases (avoiding treatment cost, income loss, and coping cost). Cost-effectiveness of different intervention strategies will be assessed. Various alternatives exist for the control of bovine TB: different BCG vaccination scenarios, test and slaughter strategy and the pasteurization of milk. The transmission model for brucellosis will be modified and validated as far as possible from repeated cross sectional surveys. It is expected that an animal to human bovine TB transmission model linked to an economic assessment package capable of testing different control strategies will be generated. The study will provide information on the cost of bovine TB to human health and agricultural sectors. At the end, the most profitable and effective strategies to control bovine TB in Ethiopia will be described.

WP 7 - Evaluation of the impact of cattle vaccination on the development of bovine TB (Glyn Hewinson, Gobena Ameni): Vaccination is an attractive control strategy for bovine TB. A realistic estimate of vaccine efficacy under conditions that reproduce the natural route and dose of infection encountered in the field is a requirement to develop a cost-benefit analysis. The efficacy of neonatal BCG vaccination will be assessed by monitoring disease in a cohort of vaccinated and control sentinel calves introduced into the naturally infected donor herd at the Holeta farm of the National Artificial Insemination Centre (NAIC), Ministry of Agriculture and Rural Development. The vaccine test is carried out in cattle managed under husbandry conditions similar to the normal farming situation where these segregated cattle are left to graze during the day and kept in cattle sheds overnight. The study will determine the protective efficacy of neonatal BCG vaccination in Holstein cattle under intensive farming conditions in Ethiopia and establish a protocol for field evaluation of bovine TB vaccines in a natural transmission setting.

Capacity building: A major outcome of this project is strengthening the research capacity at AHRI. Priority has been given to develop expertise in molecular typing and capacity to carry out epidemiology and modelling research. This consortium project has so far enrolled four PhD students (three of whom are Ethiopian) in a "sandwich" training scheme. A fifth PhD candidate with an MD background will be enrolled at Addis Ababa University (AAU) soon. The AHRI TB laboratory has been upgraded with biosafety cabinets and a generator purchased for the partner laboratory at the Aklilu Lema Institute of Pathobiology, AAU. A small laboratory is being constructed at Holeta farm. The project has supported field practice and thesis writing for 15 final year DVM students so far. Training workshops have been conducted in Addis Ababa for veterinarians and medical professionals on epidemiology and genetics.

The project has already succeeded in initiating communication between human and animal health workers in Ethiopia. It is hoped that the established capacity will strengthen further collaborative research on zoonotic diseases of public health and economic importance in the future.

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References

1. Tadesse M, Dessie T**.** Milk production performance of Zebu, Holstein Friesian and their crosses in Ethiopia. Livestock Research for Rural Development 2003;15: 3.
2. NFU. Badgers & Bovine TB - The Facts. http://www.nfuonline.com/x9727.xml
3. World Health Organization. WHO Report 2006. Global Tuberculosis Control Surveillance, Planning, Financing. Geneva. Switzerland.
4. Wondimagegne G. Status of TB control programme in Ethiopia. Proceedings of the Third National TB Workshop. 21-22 December 2006. AHRI/ALERT, Addis Ababa, Ethiopia.
5. Ameni G, Aseffa A, Engers H, Young D, Hewinson G, Vordermeier M. Cattle husbandry in Ethiopia is a predominant factor affecting the pathology of bovine tuberculosis and gamma interferon responses to mycobacterial antigens. Clin Vaccine Immunol. 2006;13(9):1030-6.
6. Ameni G, Aseffa A, Engers H, Young D, Gordon S, Hewinson G, Vordermeier M. Both prevalence and severity of pathology of bovine tuberculosis are higher in Holsteins than in zebu breeds under field

cattle husbandry in central Ethiopia. Clin Vaccine Immunol. 2007;14:1356-61.

7. Zinsstag J, Roth F, Orkhon D, Chimed-Ochir G, Nansalmaa M, Kolar J, Vounatsou P. A model of animal-human brucellosis transmission in Mongolia. Prev. Vet. Med. 2005;69(1-2):77-95.