Special Issue

on

Bovine Tuberculosis in Ethiopia
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139 Summary Of Working Group Discussions
Tuberculosis: A Global Overview

Douglas Young

Together with HIV/AIDS and malaria, tuberculosis (TB) is recognised as one of the most important threats to human health. There are around 9 million new cases of TB every year, resulting in up to 2 million deaths. Key issues in combating global TB include the emergence of strains of *Mycobacterium tuberculosis* that are resistant to available drugs, synergy with the HIV pandemic, and a historical shortage of funding for both research and disease control (1). In 2006, the Global Partnership to Stop TB launched a 10-year plan designed to address the aims set out in the UN Millennium Development Goals. The Global Plan to Stop TB envisages saving 14 million lives by effective treatment of 50 million people, and aims to cut the global burden of TB in half by the year 2015 (2).

A central element of the Global Plan is the need to develop improved tools for TB control; it is projected even the most efficient implementation of current control strategies will fail to meet the Millennium Development Goals in many parts of Africa. The Plan advocates investment of $9 billion in research and development for new tools. A series of initiatives have been put in place to promote the R&D goals (Figure 1). These include three public-private foundations directed towards development of new vaccines (the Aeras Global TB Vaccine Foundation (3), new drugs (the Global Alliance for TB Drug Development (4), and new diagnostics (FIND, the Foundation for Innovative Diagnostics (5). International consortia addressing complementary research efforts are supported by funds from the Bill & Melinda Gates Foundation, the European Union, the National Institutes of Health, and other national funding agencies.

The budget required to support the TB vaccine programme – including clinical trials, underpinning research, and maintenance of BCG vaccination – is approximately $3 billion over the next decade.

To date, clinical trials have been initiated for six candidates (6). Four of these are designed to boost the immune response established by neonatal BCG vaccination. These include two purified protein preparations delivered along with an adjuvant, and two viral vectors carrying genes for major *M. tuberculosis* antigens. Two further candidates were produced by modifying BCG strains to enhance their immunogenicity.

**Drugs:** Two strategies are being pursued for TB drug development (7). The first is based on a search for compounds with properties similar to those of existing drugs. These compounds inhibit biochemical functions essential for mycobacterial growth, and will replace drugs that have become ineffective in treatment of resistant organisms. In addition to controlling drug-resistant disease, it is anticipated that, using appropriate drug combinations, standard treatment times can be progressively reduced from the current 6-month minimum to 4 months or less.

A second strategy is to target non-replicating bacterial sub-populations that are able to persist during treatment with standard drugs and are thought to underlie the need for prolonged therapy. It is anticipated that drugs which kill non-replicating *M. tuberculosis* will allow a radical reduction in treatment times for cure of active disease, and may also be effective in rapid elimination of bacteria in individuals with latent infection. Research underpinning this strategy focuses on the need for a better understanding of the physiology of *M. tuberculosis* within human lesions. This is being addressed by analysing whole genome transcriptional profiles of *M. tuberculosis* in freshly resected tissue samples from individuals with active and latent infection. Targets shown to be essential for bacterial survival are then entered into high-throughput screens for drug discovery.

**Global, regional, local:** Global initiatives to combat TB have the advantage of attracting major funding opportunities and of bringing together the efforts of international experts. A potential drawback is that there may be differences in the problems that need to be addressed in different countries, and there may therefore be limitations in one-size-fits-all global solutions.

There is growing interest, for example, in possible differences between the strains of *M. tuberculosis*...
prevalent in different parts of the world (8). *M. tuberculosis* lineages can be viewed in terms of “ancient” and “modern” strains that may have adapted to succeed in lower and higher density human populations respectively. Differences in host-pathogen biology associated with different lineages may result in different epidemiological patterns that may affect the relative effectiveness of different control measures. Africa is characterised by the greatest diversity in terms of *M. tuberculosis* lineages.

Differences amongst human populations also have an impact on the epidemiology of TB. Age-related patterns of disease differ between Africa and Asia for example, with the peak incidence seen in young adults in most African countries displaced towards older age groups in Asia (9). There is an increased incidence of TB amongst older men in Tanzania and Nigeria, which is not seen in Kenya and South Africa: such differences may result from a combination of social and environmental influences. A high incidence of extra-pulmonary TB is seen in Ethiopia, but not in neighbouring Kenya (9). One factor that might influence this difference could be exposure to bovine TB through infected milk or meat.

**Bovine TB in the global framework:** The potential contribution of bovine TB to human disease was fiercely debated at the First British Congress of Tuberculosis in London in 1901. Robert Koch expressed a strong opinion that humans could not contract disease from the products of infected cattle and that control of bovine TB was irrelevant in addressing human disease. This position was equally strongly opposed by other experts, including Sir John McFadyean, Principal of the Royal Veterinary College in London, triggering a series of investigations over the next few years. While Koch maintained his viewpoint, he was opposed by the majority of scientists attending the International Tuberculosis Congress in Washington in 1908, paving the way for introduction of pasteurisation of milk, and test-and-slaughter policies to clear herds of infected animals. As a result, bovine TB is now rigorously controlled in all high-income countries. Retrospective estimates suggest that, prior to introduction of these control measures, as much as one third of human TB in the UK might have been caused by infection from cattle.

Bovine TB does not feature in the Global Plan to Stop TB. Should it? Do R&D efforts towards improved tools for human TB offer prospects for better control of bovine TB? Would this in turn have a beneficial impact on human disease? Britain spends almost £100 million per year in efforts to control bovine TB; there is no programme to control bovine TB in Ethiopia. Is there a case, from the perspective of human health or animal welfare, to try and control TB in Ethiopian cattle? While these are straightforward questions, with potentially important implications, we lack the baseline data required to provide them with a sensible answer. We have sparse anecdotal data on the prevalence of TB in Ethiopian cattle; there are indications that prevalence is related to farming conditions and to cattle breed. We have no information about the strains of *M. bovis* that are present in Ethiopia; are there African lineages of *M. bovis* analogous to the African lineages of *M. tuberculosis*? Does *M. bovis* make an important contribution to human TB in Ethiopia, particularly to the high incidence of extrapulmonary disease?

The Wellcome Trust programme to study Bovine TB in Developing Countries attempts to address these questions within the framework of an economic evaluation of the potential impact of disease control in Ethiopia. The aim of the Stakeholders Meeting is to set this study within the wider context of farmers, veterinarians, health care professionals and government officials as a means of bringing attention to this neglected area.
References

Development of cattle TB vaccines based on Heterologous prime-boosting strategies

Martin Vordermeier, R, Glyn Hewinson

Abstract
Development of a TB vaccine for cattle is a research priority in Great Britain. Two challenges need to be addressed. Firstly, vaccine strategies enhancing the efficacy of M. bovis bacille Calmette Guérin (BCG), currently the only potentially available TB vaccine, and secondly the development of a diagnostic test to be used alongside vaccination to differentiate vaccinated and infected animals (DIVA test). Significant progress in developing TB vaccines for cattle has been made over the last 7 years. Specifically: (i) DNA, protein, or viral subunit subunit vaccines used in combination with BCG have been shown to give superior protection against experimental challenge in cattle than BCG (heterologous prime-boost), (ii) neonatal BCG vaccination provides protection, (iii) prototype reagents that allow discrimination between vaccinated and infected animals have been developed; and (iv) and correlates of disease severity have been identified that can predict the success or failure of vaccination. The present overview provides details of some of these advances. [Ethiop.J.Health Dev. 2008;22(Special Issue):100-104]

Introduction
In 1996, an independent scientific committee reviewed the problem of bovine TB in GB. One of the recommendations put forward was that vaccination of cattle offered the best long-term solution for controlling TB in the National Herd. Cattle TB vaccination could also be an attractive and cost-effective control strategy in developing countries where other control strategies are difficult and expensive to implement. The development of novel vaccines against bovine TB has to some degree closely followed that of the human TB vaccine effort and there is significant alignment between the human and bovine TB vaccine programmes (1) and vaccines like recombinant viruses aimed at the development of human TB vaccines have been already tested in cattle (see below).

Mycobacterium bovis Bacille Calmette Guerin (BCG)
BCG is the most widely used human vaccine in the world. It was derived from a strain of M. bovis, which was isolated from a cow with tuberculous mastitis. Challenge experiments and field trials in cattle since 1919 have resulted in data showing a high degree of variability in the ability of BCG to protect cattle against infection with Mycobacterium bovis, the causative agent of bovine TB, almost some degree of protection was imparted in most of these studies (see (1-4) for reviews). Importantly, BCG vaccination sensitises animals to the tuberculin skin test, and vaccinated animals will therefore, at least for a significant period post-vaccination, test positive in the classical skin test. For this reason, test and slaughter-based control strategies based on tuberculin skin testing were favoured above BCG vaccination. More recent experimental studies with BCG have confirmed its potential to protect cattle to some degree against bovine TB by reducing disease severity and pathology (5,9). In addition, BCG vaccination was more effective when delivered to neonatal calves than to older animals (10,11). BCG has some of the qualities required for a veterinary vaccine (low costs, excellent safety profile), but it does not confer complete protection and therefore the aim of TB vaccine programmes is to improve its efficacy. However, the most promising vaccination strategies identified to date have mostly involved improving upon BCG vaccination rather than replacing it (see below). BCG remains the prototype, gold standard vaccine with which to judge the efficacy of any novel vaccine.

Cattle models to test TB vaccines
The fact that cattle TB vaccines can be experimentally tested for efficacy directly in the target species is a big advantage over human vaccine development. However, to be able to compare vaccines tested in different laboratories it is important to use standardised infection models. The most commonly used experimental infection model infects calves via the intratracheal route (Table 1) (7,8). This is a robust model resulting in pathology mainly in the lower respiratory tract thereby closely reflecting the pathology seen in the majority of infected cattle. Its advantages are that almost 100% of infected animals produce productive disease with reproducible location and severity thus requiring relatively small group to detect significant protection. Its short duration (3-4 months post-infection) also make it attractive. Potential disadvantages are that, due to the relatively high infection doses required to achieve infection and disease in most animals, the immune system can be overwhelmed and potentially effective vaccines could be classified as non-effective. To overcome some of these limitations, we have developed a vaccination model where transmission of disease is facilitated by in-contact with naturally infected cattle (Table 1). The advantages are that a natural route and infective dose is used, which is unlikely
to overwhelm the immune system, and that data generated in this model will be highly relevant to the actual field situation to guide the design of field trials. The disadvantages are larger group sizes necessary to achieve the required statistical power, due to the lower infection rates compared to intratracheal infection. Encouragingly, our preliminary experiments conducted in GB have demonstrated a relatively high transmission rate (>50% based on immunological conversion of in-contact animals to interferon-gamma (IFN-γ) test positivity and mycobacterial culture, Vordermeier et al., unpublished data) potentially allowing smaller group sizes. As part of the Wellcome Trust project ‘Bovine tuberculosis in the developing world’ (Animal Health in the developing world initiative), a similar in-contact transmission experiment is at the moment being conducted in Ethiopia to test vaccine efficacy after neonatal BCG vaccination. Vaccines giving promising results may then be tested in larger field trials, which would likely require large numbers of cows that would run for a considerable time.

Table 1: Examples of cattle models to test vaccines

<table>
<thead>
<tr>
<th>Model</th>
<th>Advantages</th>
<th>Disadvantages</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Experimental challenge:</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intratracheal model</td>
<td>‘Few’ animals required (n ≤10-12/group): 100 % infection rates of control animals</td>
<td>Immune system may be overwhelmed without giving vaccine a chance (high challenge dose: 1-5000 CFU)</td>
</tr>
<tr>
<td></td>
<td>Short duration (3-4 months): highly standardised, synchronised infection, defined infection strain, defined disease kinetics and pathology</td>
<td></td>
</tr>
<tr>
<td>‘Field experiment’</td>
<td></td>
<td></td>
</tr>
<tr>
<td>In contact transmission</td>
<td>Natural route and infective dose: data highly relevant for trial designs</td>
<td>More animals required (&gt;20/group): low infection rates of controls</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Long in-contact period (12 months): no synchronised infection, disease kinetics not defined, pathology less defined</td>
</tr>
<tr>
<td>Field trial</td>
<td>Real-life situation: routes, doses, management</td>
<td>Very large numbers required (n = 100-1000s)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Long and expensive (years)</td>
</tr>
</tbody>
</table>

Recent progress in developing cattle TB vaccines that are better than BCG vaccines

Several strategies have been implemented to improve the efficacy of BCG, namely the use of subunit vaccines in the form of DNA vaccines, protein subunit vaccines administered with a suitable adjuvant, live recombinant vaccines like attenuated recombinant viruses expressing mycobacterial antigens, or recombinant BCG expressing additional antigens not, or under-expressed in BCG. Another possible strategy involves the development of rationally attenuated *M. bovis* strains (see (12-14) for reviews. The practicality of these strategies have been greatly facilitated by the elucidation of the genome sequences of *M. bovis*, *M. tuberculosis*, and *M. bovis* BCG (Pasteur) (15-17).

Recent results in cattle have also shown that the most effective vaccination strategies against bovine TB have been based on priming the immune system with BCG followed by boosting with subunit vaccines (*heterologous prime-boost strategy*) containing protective antigens that are present in BCG. Heterologous prime-boost immunisation strategies involve using two different vaccines, each expressing the same antigen.

Heterologous prime-boost strategies based on DNA vaccines. DNA vaccines can be useful as part of heterologous prime-boost protocols. We tested heterologous prime-boost protocols in cattle based on priming the immune response with a cocktail of 3 DNA vaccines encoding the mycobacterial proteins, HSP65, HSP70 and APA (which were not protective by themselves), followed by boosting with BCG (Table 2). This induced significant enhancement of protection in six parameters used to determine vaccine efficacy, compared to BCG which induced significant protection in only 2/6 of these parameters (6). Subsequent experiments showed that superior protection to BCG could be achieved with this combination of vaccines irrespective of whether the DNA vaccines or BCG were used for the priming immunisation (18) (Table 2).

Heterologous prime-boost strategies based on protein subunits. Conceptually, protein subunits are very attractive. However, in contrast to DNA vaccines, protein subunits are unlikely to induce cellular immune responses in the absence of an adjuvant. Therefore, a high priority for the development of protein subunit vaccines is the identification of adjuvants that enhancing the development of cellular immune responses in cattle. A recent important development has been the definition of CpG motifs as adjuvant units within DNA vaccines.
(see (19) for review). Synthetic oligonucleotides containing such CpG motifs can be synthesised to produce short immuno-stimulatory sequences (CpG ODN), which can be added to vaccine formulations to enhance immunogenicity. Therefore, M. bovis culture filtrate proteins (CFP) were used in conjunction with such CpG ODN as cattle TB vaccines and they significantly enhanced the cellular immune responses of CFP. Importantly, significant protection was also seen in animals vaccinated with CFP plus CpG ODN, although the protective efficacy was inferior to that observed after BCG vaccination (20).

Based on these findings, further prime-boost experiments were performed in cattle using culture filtrate proteins delivered in the presence of CpG containing ODN to boost primary immune responses induced by BCG. Groups of cattle were vaccinated with either BCG, with BCG and CFP plus CpG at the same time followed by two CFP/CpG boosts. The results indicated that boosting BCG with CFP in CpG gave superior protection than vaccination with BCG alone. (Table 2) (21).

<table>
<thead>
<tr>
<th>Study N</th>
<th>Vaccine</th>
<th>Antigen</th>
<th>Adjuvant/ live vector</th>
<th>Comment</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>DNA/BCG</td>
<td>DNA vaccine cocktail: HSP65, HSP70, Apa</td>
<td>None (‘in-built’ adjuvant activity of DNA vaccines)</td>
<td>BCG Pasteur, 6 months old calves</td>
<td>6</td>
</tr>
<tr>
<td>2</td>
<td>DNA/BCG or BCG/DNA</td>
<td>As above</td>
<td>None (‘in-built’ adjuvant activity of DNA vaccines)</td>
<td>BCG Pasteur, neonatal calves</td>
<td>21</td>
</tr>
<tr>
<td>3</td>
<td>BCG/protein</td>
<td>M. bovis CFP</td>
<td>Emulsigen/bovine specific CpG ODN (ODN2007)</td>
<td>BCG Pasteur, 6 months old calves</td>
<td>21</td>
</tr>
<tr>
<td>4</td>
<td>BCG/MVA85A</td>
<td>Ag85A</td>
<td>Attenuated vaccinia virus (modified vaccinia Ankara strain, MVA)</td>
<td>6 months old calves. BCG SSI (freeze-dried)</td>
<td>22, and unpublished data</td>
</tr>
<tr>
<td>5</td>
<td>BCG/Ad85A</td>
<td>Ag85A</td>
<td>Attenuated, replication-deficient human adenovirus type 5</td>
<td>6 months old calves. BCG SSI (freeze-dried)</td>
<td>24, and published data</td>
</tr>
</tbody>
</table>

Heterologous prime-boost strategies based on recombinant viruses. Some of the advantages of live attenuated viruses over protein subunit vaccines are better induction of strong cellular immunity, and potentially lower production costs and simplified batch release test protocols. The first Phase I human trial of a new TB vaccine was based on a heterologous prime-boost strategy involving boosting BCG-mediated immunity with an attenuated vaccinia virus expressing Ag85A of M. tuberculosis (MVA85A) (22).

So far, in a collaborative study with the group of Professor Adrian Hill at Oxford University, we have performed immunogenicity studies of the BCG/MVA85A heterologous prime-boost regimen in cattle. Prime-boost protocols using recombinant MVA85A and BCG in either combination resulted in significantly higher frequencies of Ag85-specific IFN- secretion cells than the viral vectors or BCG used alone. The most promising combination was BCG priming followed by one MVA85A boost (23). Similarly, we have shown that a prime boost protocol applied to cattle that consisted of BCG priming followed by heterologous boosting with a recombinant adenovirus expressing the same antigen, Ag85A, (Ad85A) developed by Professor Xing’s group at McMaster University, Toronto, Canada (24) resulted in superior antigen-specific IFN- responses as well as improved central T cell memory compared to BCG vaccination alone (24). Furthermore, in a recent challenge experiment using the intratracheal infection route, we could demonstrate that both MVA85A and Ad85A when used to boost BCG-induced immunity conferred significant protection, superior to BCG vaccination alone (Table 2).

Thus, significant advances have been made to develop prototype vaccine strategies that can enhance BCG vaccination efficacy based on DNA, protein and viral subunit vaccination. Further work is required to determine which of these approaches is the most effective, and how efficacy determine in experimental challenge experiments will translate into field efficacy. Thus, these candidates will be tested in the model involving in contact challenge as described above. In addition, it will be necessary to define, in addition to Ag85A, further protective antigens that can then be used as subunit vaccine candidates. This work is on-going, but further discussion is beyond the scope of this review.

Differential diagnosis of infected from vaccinated individuals, and correlates of protection.

In order to use a vaccine as part of a control strategy for bovine TB, discrimination between infected and uninfected vaccinated animals (so-called DIVA test) is a pre-requisite so that test and slaughter control strategies can be carried out alongside vaccination regimens. Over...
the last decade, encouraging progress has been made to make the implementation of a DIVA strategy alongside effective cattle TB vaccination likely. Conceptually, antigens whose genes are expressed in M. bovis yet absent from BCG constitute candidates for DIVA reagents. The antigens CFP-10 and ESAT-6 have been shown to be useful as diagnostic reagents to discriminate between BCG vaccinated and M. bovis-infected cattle (5,25-28) and constitute a prototype DIVA reagent. Both proteins are encoded by genes located on the RD1 region of the M. bovis genome that is deleted from the genomes of all strains of BCG (29-31). The genomes of M. tuberculosis, M. bovis and BCG Pasteur have now been sequenced (15-17) and systematic comparative genome comparison have been performed to identify further cattle DIVA antigens (25,32-33).

TB vaccine development would be greatly facilitated by the definition of immunological correlates/surrogates of protection. Although some progress has been made, for example by defining ESAT-6 and CFP-10-induced IFN- responses as inverse correlate of protection (5), further surrogates still await closer definition. It is beyond the scope of this manuscript to present these advances in detail and I refer to recent reviews for more detailed discussion (13, 14, 34, 35).

**Conclusion**

Significant progress has been made in the development of TB vaccines for cattle: Subunit vaccines based on DNA, proteins or viral subunits used in combination with BCG have resulted in better protection against experimental challenge with M. bovis than BCG vaccination on its own. BCG vaccination of neonates has also proved to be highly protective. DIVA reagents that allow discrimination between vaccinated and infected animals have been developed. Finally, correlates of disease severity are being actively sought that can predict the success or failure of vaccination hopefully in future shortening experimental protocols.

**Acknowledgements**

The authors were funded by the Department for Environment, Food and Rural Affairs, United Kingdom, and the Wellcome Trust.

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*Ethiop.J.Health Dev.* 2008;22(Special Issue)


“One health”: The potential of closer cooperation between human and animal health in Africa

Jakob Zinsstag, Marcel Tanner

Abstract

Emerging zoonoses affect livestock and humans, which calls for closer cooperation between animal and public health. Conceptually ideal, such cooperation is difficult to achieve and the causative agents of outbreaks are often confused. A lack of awareness may very likely be due to limited capacity and resources for available for diagnosis and surveillance of zoonoses, but also owing to the clinical perspective that focuses on the patients and much less on their surroundings. Consequently governments often neglect zoonotic diseases, reflecting the separation between human and veterinary medicine. The present paper explores the concept of closer cooperation initially coined as “one medicine” and presents examples of its application and future potential emphasising the African context. Zoonoses are certainly the most prominent example of a compulsory interaction between human and animal health. The interaction of humans and animals in Africa is inextricably linked and hence needs a thorough rethinking of institutions, legislation, communication and funding of both sectors. There is a large untapped potential for new institutional and operational models to provide joint health services to remote populations; this is particularly relevant with regard to ongoing health sector reforms and the human resource crisis. Further, there is a potential for innovative, cost-effective approaches to the control of zoonoses. Pan-African networks would be the best justification for setting up a global fund for zoonoses, similar to and/or linked to the Global Fund to fight HIV/AIDS, Tuberculosis and Malaria. .

[Ethiop.J.Health Dev. 2008;22(Special Issue):105-108]

Introduction

Human and veterinary medicine still appear as well separated sectors and entities in most countries. Veterinarians are generally not allowed by law to treat humans and physicians only rarely treat animals. However, there are many overlapping issues, mostly in the realms of public health and in the control of diseases transmissible between animals and humans (zoonoses). In such cases cooperation between both sectors becomes crucial, e.g. ranging from informing each other on the emergence of new diseases to long term perspectives on integrated control.

The cooperation between two well structured entities is not very easily achieved as by the example human Rift Valley fever outbreaks in Mauritania that where mistakenly identified as Yellow fever. The correct diagnosis only occurred after contacts with the livestock services, who had observed abortions in livestock due to Rift Valley fever (1). In sub-Saharan Africa, clinicians attribute most fever to malaria, even though an estimated 50-80% of fevers result from other causes (2). In a case study on fever related diseases in Mali, physicians paid attention to potential zoonotic diseases only after veterinarians identified risk factors for transmission(3). A lack of awareness may very likely be due to limited capacity and resources for diagnosis and surveillance of zoonoses, and - equally importantly - the focus of the clinician on the patients and not on their surroundings. Consequently, governments often neglect zoonotic diseases. Here we will explore the concepts of closer cooperation between human and animal health initially coined as “one medicine”, and present examples of its application and future potential emphasising the African context.

From “one medicine” to “one health” a brief historical background

Ancient healers were priests and cared for both humans and animals (4). They gained anatomical and pathological skills from slaughtering sacrificial animals and deciding on their purity for sacrifice (Leviticus 1,3). Human medicine was integrated into the medieval universities, whereas veterinary medicine remained largely in the hands of equerries until the 18th century (5). Claude Bourgelat, the founder of the first veterinary school in Lyon (1762) was heavily criticised when he recommended human clinical training for the veterinary curriculum (6). However, in the 19th century, the advent of cellular pathology encouraged the pioneers of the microbiological revolution to (e.g. Rudolf Virchow cited in (7)) develop a strong interest in linking human and veterinary medicine as a form of comparative medicine based on discovering similar disease processes in humans and animals. In the 20th century, both sciences specialised to such an extent that their association was hardly visible and less often practiced. It was Calvin Schwabes’ thorough rethinking of the concept of “one medicine” in 1976, that fully recognized the close systemic interaction of humans and animals for nutrition, livelihood and health (4). Today, the earliest forms of healing of humans and animals are still widely practised in traditional pastoral societies. It is thus not surprising that the “one medicine” idea is actually of African origin.

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It was conceived and conceptually consolidated during Calvin Schwabe’s work with Dinka Pastoralists (8). It basically means that there is no difference of paradigm between human and veterinary medicine. Both sciences share a common body of knowledge in anatomy, physiology, pathology, and on the origins of diseases in all species (4). Later, international organizations such as the WHO and the Food and Agriculture Organization (FAO) institutionalized it partly as Veterinary Public Health (VPH). More recently “ecosystem health” has emerged, seeing sustainable development expressed as the mutualism of the health of humans, animals and the ecosystems in which they co-exist (9) and extending the concept of “one health” to that of the whole ecosystem including wildlife (10-12). Conservationists have recognized, what is known as the “Manhattan principles” (13), that the health and sustainable maintenance of wildlife in natural reserves is mutually interdependent with the health of communities and the livestock surrounding them (14). Finally, many of the causing agents with bioterrorist potential are zoonoses and hence require mutual animal and public health vigilance for rapid detection (15). The “one medicine” hence evolves towards a “one health” concept which reflects the contemporary thinking on health and ecosystems and their relevance for global health development (16).

What does “one health” really mean
While it is accepted that human and animal health should be much more closely interlinked, the operational strategies still require a substantial re-thinking. To fully exploit synergistic benefits between human and animal health, closer cooperation is required at all levels ranging from international organizations, governments, research and technology, health systems and education.

Governments and international organizations
WHO, FAO and OIE (World Organization for Animal Health) are the focus of discussion. While they cooperate on zoonotic diseases with transboundary importance such as Avian Influenza (AI), their respective roles and responsibilities are still not fully clarified or based on pragmatic considerations of the most effective approaches for surveillance and control. Achieving this closer cooperation would provide a strong signal to national governments and all institutions concerned. For example, following the recent outBreaks of AI and RVF in East Africa, many governments, including Ethiopia, have created ad hoc task forces between the concerned ministries of agriculture, livestock production and health. Such cooperation between sectors should be formalized and its mode of operation and responsibilities clarified to make it effective not only in response to crises but even much more as a tool for risk analysis, prevention and coordinated, integrated control (16). Many other zoonoses like Q-fever (17), Anthrax and rabies (18) would benefit from such cooperations and interlinkages, which should finally also strengthen links within and between African countries (19).

Research, technology and health systems
In many countries zoonotic diseases are not considered as important simply because the diagnostic capacity to detect them hardly exists. For example, bovine tuberculosis in Chad was not considered important until the first tuberculosis laboratory in the country was able to demonstrate it (20). Joint human and animal surveillance and research on zoonoses accelerates time to detection and the identification of reservoirs (15). Under resource constraints diagnostic facilities could easily be shared (21). Governments often consider the control of zoonoses as too expensive. However, combined societal economic assessments show that their control may actually be highly cost-effective if intervention costs are shared between sectors (22,23). Observations of higher vaccination coverage in cattle than in children in nomadic pastoralists in Chad have led to joint livestock and human vaccination campaigns by cooperation between the expanded programme of immunisation (EPI) and the veterinary services in Chad (24). Veterinarians are often the only health person in remote rural areas and would be competent – after some training - to sell a limited set of essential human drugs under conditions where pharmacists and pharmacies are lacking (25). Such cross-sector arrangements are certainly more effective and also more ethical than leaving the rural population at the mercy of illegal drug sellers and drug peddlers. Moreover, novel models of integrated social services exploiting links between education, public health, animal health, and the environment (26) could make veterinary services profitable in areas where they can hardly make a living under the current privatised schemes, and would therefore significantly contribute to improved rural health service coverage. Veterinarians could also be instrumental in organizing joint animal–human vaccination services (25). Accepting these approaches implies rethinking institutional and operational models of joint health services provision, which is of particular relevance in view of the current human resource crisis in the health sector (27). Community based surveillance of animal diseases as proposed by OIE at the N’Djamena conference in February 2006 (28) could be extended to public health to accelerate detection of new outBreaks. Current academic and technical curricula should be revised to provide medical doctors with more knowledge of the ecology of zoonoses, and veterinarians with better knowledge of public health and health systems. In conclusion, the major challenge in achieving these interlinkages lies in effectively combining public health, animal health and ecosystem health under a common umbrella for comprehensive public health action.

Vision for the future
Zoonoses and their control are certainly the most prominent example of the need to combine human and
animal health. The interaction of humans and animals in Africa is much closer and directly visible e.g. by the breakdown of livestock production due to the HIV epidemic (29) or the livelihood consequences of animal diseases (30). Moreover, we should not forget the past disaster of Rinderpest imported to Ethiopia during colonial rule (4). These inextricable links show the need for a thorough rethinking of institutions, legislations, communication and funding of both sectors. There is a large untapped potential for new institutional and operational models to provide health services jointly to remote and/or neglected populations, which is highly relevant to ongoing health sector reform programs and the human resource crisis. Limited laboratory capacity and infrastructure can easily be shared between sectors, and needs no further justification as the pathogens dealt with are the same for humans and animals. The populations concerned in rural and urban areas have specific knowledge about diseases in their surrounding which can be better used for community based surveillance, but also to define priorities for action and the translation of evidence into policy, comparable to the East African REACH consortium with their activities to link research outcomes with political and strategic decision makers (31). These examples certainly enhance the urgent need to improve communication between sectors and will also allow much better use to be made of non-Western knowledge from “integrated” pastoral societies, with their own pragmatic solutions for problem-solving (4).

In conclusion, there is potential for innovative, cost-effective approaches to national zoonoses control (23), this cooperation between the human and animal health sectors should be extended internationally, as exemplified by the concerted approach to rabies control in South America (19). Pan-African networks for zoonoses control would be the best justification for a global fund for zoonoses similar to and/or linked to the Global Fund to fight HIV/AIDS, Tuberculosis and Malaria.

Acknowledgement

Wellcome Trust and National Centres for Competence in Research North-South (NCCR North-South – mitigating syndromes of global change, Integrated Project 4/Work package 3 “health & wellbeing”) are acknowledged for funding.

Reference


Cross-disciplinary and participatory livestock and human health research for successful control of zoonoses in the developing world

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Abstract
Conventional disciplinary research approach is losing momentum in the face of dynamic health challenges of the 21st century. There is a need for a new, suitable approach, to tackle these emerging and re-emerging human and animal diseases through integrating livestock and human health research in a cross-disciplinary approach for greater impact. This is particularly important for the developing world owing to the closer contact of humans with animals as well as the consumption of raw animal products, worsened by low levels of literacy. Animals are the major source of today’s emerging and re-emerging infectious diseases that threaten both human and animal populations of the world. Among recent examples are SARS (severe acute respiratory syndrome), the Hendra and Nipah virus infections, BSE or mad cow disease (bovine spongiform encephalopathy) and now highly pathogenic avian influenza (HPAI), or bird flu. In addition, bovine tuberculosis and rift valley fever (RVF) are some examples of important re-emerging zoonoses. In recent years, bovine tuberculosis has become increasingly important with the HIV and AIDS pandemic in the developing world. This paper highlights the past and current research portfolio of ILRI and its partners, focusing on diseases that are transmissible between human and animals in the context of developing countries. [Ethiop.J.Health Dev. 2008;22(Special Issue): 109-116]

Introduction
Veterinary public health (VPH) is not integrated into the mainstream of public health services in sub-Saharan African countries. There are no formal mechanisms within government public health services through which veterinary skills and resources can be effectively harnessed to bear upon community health. There is no conscious, overt or substantial effort by public authorities to incorporate VPH services in the overall approach to public health. VPH activities cover mainly the control of the major animal diseases transmissible to man (zoonoses), meat inspection and, to a limited degree, the quality control of milk, fish and their products. These services are carried out by the Veterinary Services of the Ministry of Agriculture in each country (1).

Zoonotic diseases, transmitted between humans and animals, are mostly associated with people who have close direct contact with animals or indirect contact via vector or other transmission vehicles. Farmers, pastoralists, veterinarians, butchers and abattoir workers are people with a high risk of zoonotic infections. Zoonoses have important impacts on public health and the economies of the people who depend on animal agriculture. Taylor et al. (2) reported the number of zoonotic infections at 868 representing 61% of all infectious organisms identified to be pathogenic to humans. Some of these infections have been recognised early in history (e.g. rabies) while others have recently emerged (e.g. SARS, BSE, HPAI). For example, raw milk consumption was recognised and proved as means of transmission for bovine TB only two decades ago, and pasteurisation drastically reduced the disease occurrence in Europe.

Measures taken to control major diseases in Europe include disease surveys, monitoring, and control through concerted efforts of important key players in the food chain (e.g. slaughterhouses and milk processors); these have reduced the prevalence of many zoonotic diseases. In contrast, many zoonotic infections are still common in the sub-Saharan African countries. The occurrence of zoonotic diseases such as bovine TB, rabies, RVF, HPAI, brucellosis, and anthrax in humans could be reduced or eliminated if the diseases are controlled in livestock. Hence, vaccinating livestock or companion animals against these diseases should also be considered as part of public health measures.

Despite the above challenges that could have acted as a focal point for change, there has not been a cross-disciplinary breakthrough in collaboration among relevant professionals and other key stakeholders in targeting zoonoses. This paper summarises research focus areas at ILRI, areas of collaboration, and a way forward for zoonoses in the context of developing countries.

Concept of cross-disciplinary approach – a need for change
Despite huge international and national efforts towards improving health and livelihoods of people, there has not...
been substantial progress towards achieving the Millennium Development Goals (MDGs) set for health. In spite of the increasing challenge from emerging and re-emerging zoonotic infections in the world, collaboration among professionals has not achieved momentum. There is a need for a strategic shift in the types of collaborations – from mono-disciplinary to cross-disciplinary and participatory approaches.

Problems are usually multidimensional and interlinked and the search for solutions calls for application of the combined methodologies as well as mobilisation of new ideas of expertise and application of theoretical frameworks which transcend traditional professional boundaries (3). Cross-disciplinarity is a cover term for different types of collaboration. By definition a discipline is a complex phenomenon with social as well as cognitive aspects, “a community, a network of communication, a particular set of values and beliefs, a domain, a mode of enquiry, and a conceptual structure” (4). Rosenfield (1932, 1951) cited in Aargaard-Hansen et al. (3) distinguished three different levels of cross-disciplinary collaboration:

A. Multidisciplinary – researchers work in parallel or sequentially from disciplinary-specific base to address common problem

B. Interdisciplinary – researchers work jointly but still from disciplinary-specific base to address common problem

C. Trans-disciplinary – researchers work jointly using shared conceptual framework drawing together disciplinary-specific theories, concepts, and approaches to address common problem. Cross-disciplinary approach can provide more useful answers to the pertinent problem because it applies more holistic view. Moreover, collaboration between different disciplines increases the possibility of raising new and innovative research questions and provision of cross-fertilisation in terms of methodologies and theories and direct academic benefit per se.

Therefore, it is time for change in addressing zoonoses research to generate viable control options through cross-disciplinary collaboration for all professionals and stakeholders.

Zoonoses research by the ILRI

ILRI conducts research to protect and enhance the physical human capital of the poor by developing strategies to reduce health risks and improve nutritional benefits associated with livestock keeping. Other projects focused on the use of water and feed for livestock also consider human health impacts. ILRI is working to improve understanding of the links between livestock keeping and the health and nutrition of poor people, particularly those engaging in smallholder livestock production and marketing. Activities underway include field studies, literature reviews and explorations of the ways in which livestock keeping might benefit the care of people with HIV/AIDS.

Poor people in developing countries have a high risk of exposure to zoonoses. ILRI is helping to bridge the artificial divide between animal and human health, and helping to bring out more clearly the links between agriculture and health. With over three-quarters of human infections having a zoonotic origin, the need to examine the epidemiological relationships between pathogens and their animal and human hosts is paramount. Poor households that keep livestock often live in close proximity to their animals, with animals and people alike living in poor sanitary conditions (5). The several species of livestock kept by the poor benefit from little if any veterinary care, and medical facilities for the people themselves are also scarce. Poor keepers and non-keepers of livestock alike typically consume livestock products that have not been subject to inspection or improved processing and storage. Furthermore, many developing countries lack the information, awareness and control strategies needed to control zoonotic diseases, often because conventional disease control and food safety strategies are ill-suited or too expensive for smallholder production and marketing systems. Typically, little investment has been made to develop appropriate control strategies and there is a lack of coordination between the relevant veterinary and medical sectors. Through a joint research programme with the Swiss Tropical Institute, ILRI research is addressing the strategic methodological challenge of integrating veterinary-medical assessments of the impacts of the zoonotic disease burden on livelihoods of the poor.

I. Bovine tuberculosis

Due to the global importance of tuberculosis as one of the most prevalent infectious diseases and leading cause of death and because the infection caused by bovine tubercle bacillus, Mycobacterium bovis, is clinically indistinguishable from tuberculosis caused by Mycobacterium tuberculosis, ILRI has recognized the disease as one of the most important zoonosis for collaborative research. In this connection ILRI has been undertaking research in bovine TB both directly and indirectly in collaboration with partners, examples are highlighted below:

1. The role of ILRI in contributing to novel vaccination strategies for control of bovine tuberculosis in cattle and understanding the immunological basis of breed differences in susceptibility to the disease:

The first context in which ILRI became involved in bovine tuberculosis research was in evaluation of heterologous prime-boost vaccination regimes based on priming with plasmid DNA or avian poxvirus antigen constructs and boosting with the same recombinant antigen expressed in replication attenuated poxviruses in
cattle. Such strategies had previously been demonstrated to induce immunity, based on CD4 and CD8 T cells, against several diseases in both rodents and primates. The ILRI study demonstrated that conceptually similar prime-boost vaccination strategies using the p85a antigen of *M. tuberculosis*, which is conserved with the *M. bovis* homologue, were highly effective in inducing antigen-specific gamma interferon secreting CD4 and CD8 T cells, detected using a bovine ELISPOT assay, in Bos indicus cattle (6). T cell responses induced by priming with either plasmid DNA or fowlpox p85a recombinant antigen expression constructs were enhanced by boosting with modified vaccinia virus Ankara (MVA) administered intradermally. Intradermal priming was markedly more effective than intramuscular delivery of the priming dose for MVA boosting in cattle. Fowlpox or plasmid DNA priming were both effective and using either fowlpox or DNA priming there was a significant bias toward induction of CD4, rather than CD8 T cell responses. These data illustrated the general applicability of prime-boost vaccination strategies for induction of antigen-specific T-cell responses and suggested that the method may be useful for development of veterinary vaccines. Due to the lack of a BSL3 containment facility at ILRI it was not possible to perform challenge experiments with virulent *M. bovis* to test whether the T cell responses induced might be protective.

A growing body of evidence suggests that Zebu cattle (Bos indicus) are more resistant to *Mycobacterium bovis* infection than exotic (Bos taurus) cattle. As long ago as 1940, Carmichael concluded that the incidence of bovine TB in Uganda, was dramatically lower in Zebu (Bos indicus) cattle 0.1–0.7%, compared to Ankole (Bos taurus) 12.5–41.4% (7). More recently, a comparison of slaughterhouse examinations in India indicated that Bos indicus breeds were less affected by bovine TB than pure European breeds like Jersey, Holstein-Friesians and Brown-Swiss (8). Skin testing results compiled in Ethiopia further support the hypothesis that cattle breeds differ in their relative susceptibility to bovine TB. Tadelle (9) found that in central Ethiopia local Bos indicus breeds had much lower prevalence rates (5.6%) than exotic breeds (mainly Holsteins, 86.4%); crosses showed 13.9% prevalence. Similar differences in prevalence rates and severity of pathology have also recently been demonstrated between Holsteins and Zebus north-east of Addis Ababa, by Ameni and co-workers (10).

In view of this evidence for differential susceptibility of breeds to bovine TB derived from field studies, ILRI is currently performing a comparative analysis of the immune responses and pathology of experimentally infected cattle of East African Boran (Bos indicus) and Friesian (Bos taurus) breeds. This represents one module within a Wellcome Trust-funded Animal Health in the Developing World project, involving an international consortium which seeks to make a systematic survey of the prevalence, genotypes and economic impact of bovine TB in both livestock and humans, focused on Ethiopia. It is envisaged that the results may be synergistic with the definition of genomic regions associated with differences in bovine TB prevalence identified using single nucleotide polymorphisms that differentiate Bos indicus and Bos taurus. These data may ultimately provide insights into the molecular basis of the resistance/susceptibility phenotype in cattle.

2. **Comparison between comparative tuberculin and gamma-interferon tests for the diagnosis of bovine tuberculosis in Ethiopia** (11)

Comparative cervical tuberculin (CCT) test as standard test for the detection of bovine tuberculosis (12) has been in use since the last two decades and measuring gamma-interferon release in a whole-blood culture system has been developed (13) as a new diagnostic method for bovine tuberculosis. Diana and Carole (14) found that single caudal-fold skin test as more sensitive than the commercial IFN-γ test. However, the sensitivities and specificities of the IFN-γ and CCT tests have not been compared before. A study aimed at determining and comparing the sensitivities and specificities of the comparative cervical tuberculin (CCT) and gamma-interferon (IFN-γ) tests for the diagnosis of bovine tuberculosis was conducted on 30 Zebu oxen in Ethiopia. The results of the tests were compared with the presence of acid-fast bacilli found by bacteriological culturing and histopathological examinations. The results indicated that the sensitivity of CCT was 90.9% and its specificity was 100%. Those of the commercial IFN-γ test were determined to be 95.5% and 87.7%, respectively. No significant differences were found between the sensitivities or the specificities of the two tests. It was therefore concluded that the choice between the two tests depends on their cost and simplicity and on livestock management and time factors rather than on their respective diagnostic value.

3. **Kinetics of interferon-γ (IFN-γ) release in the peripheral blood of calves vaccinated with BCG**

Buddle *et al.* (15), studying on the efficacy of BCG, have shown that a low dose of BCG will protect calves from experimental infection with *M. bovis*. One of the mechanisms by which BCG protected calves from experimental infection is by inducing the release of IFN-γ. We conducted a study (16) on 13 Friesian-Zebu crossbred calves, the ages of which lie between 6 and 18 months, to investigate the kinetics of IFN-γ release in the peripheral blood following Bacille Calmette Guerin (BCG) vaccination. After being screened for bovine

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tuberculosis (BTB), the calves were vaccinated with 1ml inoculums containing $6 \times 10^6$ CFU of BCG. The level of IFN-$\gamma$ in the peripheral blood was measured two times before vaccination and seven times after vaccination, using a sandwich ELISA. The kinetics of IFN-$\gamma$ post vaccination presented itself in three phases: rising, falling and steady phases. The concentration of IFN-$\gamma$, before and after vaccination, both in stimulated and non-stimulated samples, was statistically significant ($P<0.01$). Strong positive correlation ($r=0.86$) was recorded between the levels of IFN-$\gamma$ release in avian PPD- and bovine PPD-stimulated samples. Of the total 13 calves, 11 ($84.6\%$) reacted positively to tuberculin inoculation 15 weeks post vaccination. We concluded that the IFN-$\gamma$ rises immediately after BCG vaccination, reaching its peak two weeks post vaccination, and then declines gradually in the following weeks. The strong positive reaction of calves to tuberculin inoculation 15 weeks post vaccination showed the capability of BCG in causing the release of IFN-$\gamma$ in the peripheral blood, indicating its role in protection against infection with $M$. bovis in calves.

4. Cross-sectional studies of bovine tuberculosis in selected dairy farms and smallholder farms in Ethiopia

Zoonotic tuberculosis is prevalent in animals of many developing countries where surveillance and control activities are often inadequate or unavailable; Ethiopia is one of these countries where many epidemiologic and public health aspects of the infection remain largely unknown. We conducted a cross-sectional study (17) to generate baseline information on the prevalence of bovine TB and related risk factors in selected dairy farms in Ethiopia using 1171 dairy cattle in 12 randomly selected state-owned, private or research dairy farms. Comparative intra-dermal tuberculin (CIT) test and bacteriologic study through milk culturing were employed. An overall individual animal prevalence of 46.8% and a herd prevalence of 91.7% were recorded. There were significant differences in individual prevalence between farms and breeds (pure Holstein and their crosses with Zebu). It was also found out that herd size and management (sanitation levels) affect the prevalence of bovine tuberculosis. Particularly, breed and management affected the prevalence of bovine TB as confounding variables. $M$. bovis was isolated in the milk of 4 cows out of the 13 reactor cows. The widespread occurrence of bovine TB in the study farms and isolation of $M$. bovis from the milk of reactor cows signified its economic importance and potential risk to public health. Generalization and improved use of milk pasteurization within all dairy sub-sectors is recommended, and this would affect the competitiveness of the dairy sector in Ethiopia.

With the rationale that the introduction of exotic and crossbred cattle into Ethiopia has created favourable condition for the spread of bovine TB, leaving cattle, cattle owners, and consumers of raw cattle products at risk for infection from $M$. bovis, we considered the Wuchale-Jida district, which is one of the 12 districts in the North Shewa Zone (Ethiopia) where dairying is commonly practiced using small herd size. The extent of bovine TB in the district in both cattle and humans, however, was not known. We conducted a cross-sectional study (10) on 94 households and 763 (575 crossbred and 188 indigenous) cattle to determine the prevalence of bovine TB and assess its public health implications in these smallholder farms. Cluster sampling, CCT test, a questionnaire, and mycobacteriology were used. Based on the CCT test, herd prevalence was 42.6% and individual animal prevalence was 7.9%. The individual animal prevalence was significantly affected by herd size, age and body condition of the animal. Among the interviewed households, 24.5% had experienced at least one human tuberculosis case in the family. Of these families, 43.5% had reactor cattle. Nevertheless, no statistically significant association was observed between reactor cattle and human tuberculosis cases in households. The habit of milk and meat consumption was affected by occupation and location of household residence. Although the level of education influenced the habit of milk consumption, it did not impact the habit of meat consumption. Less than half (38.3%) of the respondents knew about bovine TB, and only 30.8% of the respondents were conscious of its transmission from cattle to humans. Secondary data analysis from Muka-Turri human clinic indicated that 85.6% of the human tuberculosis cases were from rural parts of the district. Although the bovine TB prevalence seems low, its potential risk to public health was important based on food consumption, poor sanitary measures, and the lack of understanding about its zoonosis. Presently there are two projects on bovine TB at ILRI (one on-going and one planned):

- Bovine tuberculosis in the developing world: WP5 (the on-going Wellcome Trust funded project)
- Bovine tuberculosis in Ethiopia: molecular epidemiology and recombinant vaccine development using DNA and attenuated poxviruses constructs (a planned PhD research proposal for Fufa Dawo)

II. Sleeping Sickness (African Trypanosomosis) and Cysticercosis

ILRI has been working on sleeping sickness (18-25) and cysticercosis (26-28), which are among the major 

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neglected zoonoses. Building on its long research history in trypanosomosis research, ILRI has collaborated with local partners, the University of Edinburgh, and FAO in understanding better the potential role of veterinary interventions to control Sleeping sickness in Uganda. The use of mouse model in the design of control of trypanosomosis and malaria (29-32) is significant. Cysticercosis is a highly complex disease affecting both people and pigs. ILRI has been participating in a Cysticercosis Working Group of Eastern and Southern Africa (CWGESA), which promotes effective communication, collaboration and coordination of integrated research and control activities aimed at combating cysticercosis.

III. Rift Valley fever (RVF)
Rift Valley fever (RVF) is an acute viral disease, affecting mainly livestock but also humans. The virus is transmitted to humans through mosquito bites or by exposure to blood and bodily fluids. Drinking raw, unpasteurized milk from infected animals can also transmit RVF. Routine vaccination of livestock in Africa has been prohibitively expensive, leading to endemicity of RVF in most African countries. Reports in September 2000 first documented RVF occurring outside of Africa in the Kingdom of Saudi Arabia and Yemen. Prior to this outbreak, the potential for RVF spread into the Arabian Peninsula had already been exemplified by a 1977 Egyptian epidemic. This appearance of RVF outside the African Continent might be related to importation of infected animals from Africa.

IV. Avian influenza or bird flu
World concern over the devastation the pandemic has wrought on the poultry business and the deaths, and risk of death, of people whose livelihoods depend on poultry in some affected countries is overshadowed by the risk that this lethal avian influenza virus will mutate and cross over to humans, where it would be transmitted from person to person and could cause a human pandemic. The danger is real: flu pandemics in the 20th century killed 20 million people in 1918, 2 million people in 1957 and 1 million people in 1968.

In Africa, national task forces are being assembled in countries threatened by the introduction of avian influenza through wild bird migrations. South Africa has strong technical capacity and recent experience in successfully eradicating a type of pathogenic avian influenza not caused by H5N1 from its ostrich population. The African Union’s Inter-African Bureau of Animal Resources is the coordinating body for combating trans-boundary diseases in Africa. As the implementing agency of the Pan-African Rinderpest Campaign and the Pan-African Control of Epizootics program, AU-IBAR has supported national programs in epidemic-surveillance and provided technical support to national veterinary services.

The Consultative Group on International Agricultural Research (CGIAR) and its partners can support developing countries in their efforts to prevent and control avian influenza in two main ways.

(1) Provide empirical research results and approaches to determine the following:
- The sector-wide impacts of avian influenza and the predicted distributional impacts of options for its control. Studies by the ILRI and its partners of the impacts of disease and disease control options on poor people in Asia and Africa provide relevant approaches and methodologies. ILRI’s operating project on animal health and food safety for trade is a partner in a proposal recently submitted for such a study in Southeast Asia. The International Food Policy Research Institute (IFPRI) has investigated the impacts of human health issues such as HIV-AIDS on the poor and analysed risks to the poor related to food safety and biosecurity issues.
- Alternative institutional and market chain arrangements, norms and standards for poultry products, the feasibility of implementing different control plans, and alternative veterinary service delivery options to meet the needs of poor poultry producers and consumers. This research should be linked to ILRI, IFPRI and FAO’s Pro-Poor Livestock Policy Initiative (PPLPI) projects on livestock markets in Southeast Asia.
- The benefits and risks of employing alternative methods of preventing and controlling avian influenza outbreaks for poor people and the world at large to guide equitable decision-making on the choice of disease control methods and the incentives needed to ensure compliance with them. The CGIAR has a comparative advantage in conducting studies that assess the impacts, risks and trade-offs of disease control on different groups of poor people.
- The impacts of avian influenza control options on developing countries in terms of their national, regional and international markets and trade in poultry and poultry products as well as other livestock products. This would require research by ILRI and IFPRI to integrate field-level information to enhance the precision of more aggregate macro-economic models, the latter of which are already in existence.

(2) Provide immediate support to help strengthen the capacity of national and regional organizations in developing countries (33), particularly in Africa, in epidemi-surveillance, risk assessment, field study design, analysis of options for new approaches to disease control.
veterinary service delivery, and decision-support methods and tools. This support can be provided to AU-IBAR and national task forces by ILRI along with FAO, CIRAD, South Africa’s Onderstepoort Veterinary Institute, and others. The CGIAR is unlikely to engage in technical research on vaccines and diagnostics for avian influenza, given the comparative advantage of alternative suppliers world-wide. The Group has important roles to play nonetheless. Beyond making immediate responses to help control today’s outBreaks of avian influenza, the CGIAR is well-positioned to play a strategic role in accelerating the capacity of developing countries to understand emerging diseases and to design appropriate strategies to deal with them effectively.

One of the important aspects of the work on livestock vaccines involves host functional genomics as it relates to livestock diseases that can be transmitted to humans. A project investigating resistance to trypanosomosis in cattle is shedding light on some of the basic questions of disease resistance, which may have implications for human medical treatment. ILRI researchers first identified several regions of the cattle genome in which genes contributing to resistance or susceptibility must lie. They then identified genes within a part of the bovine genome that affects anaemia, a characteristic of the disease. Remarkably, significant differences between cattle breeds that are susceptible and resistant to the disease were found in one of the candidate genes. Such a result makes it possible that the gene in question is responsible for the difference in susceptibility to anaemia in the two breeds. This is now being further investigated. More recent results of this trypanosomosis genomics research appear to have implications for medical research on cholesterol.

VI. Livestock, water quality, and human health
ILRI has recently initiated limited research on water-mediated impacts on human health and on INRM approaches to reducing health risks. Most of this research falls within ILRI’s collaboration with the CGIAR Challenge Program on Water and Food and the CGIAR Comprehensive Assessment of Water Management and Agriculture. Key issues include the transmission of water-borne pathogens such as coliform bacteria, cryptosporidium, and Fasciola that result from animal manure contaminating domestic water supplies and where simple remedial interventions are feasible. One recent MSc thesis demonstrated, for example, that expansion of irrigation into dryland areas may increase the prevalence of sheep Fasciolosis. Livestock keeping may also affect the ecology of water-dependent disease vectors such as mosquitoes. In collaboration with the CGIAR System Wide initiative on Malaria and Agriculture (SIMA), an effort is being made to assess the potential for using cattle management options that can reduce malaria transmission.

VII. Livestock feed quality and human health
Aflatoxin in milk – a possible hazard to human health: ILRI in collaboration with ICRISAT is investigating aflatoxin contamination of fodder (mainly crop residues) as a source of aflatoxin content in milk. In selected sites in Andhra Pradesh, India, close to 50% of the milk samples contained non-permissible levels of aflatoxin. At the same time, only one of the collected fodder samples (groundnut cake) contained non-permissible levels of aflatoxin. Aflatoxin in milk can clearly present a health hazard to the consumer. There appears to be a mismatch between non-permissible levels of aflatoxin in fodder (30μg/kg) and milk (0.5μg/kg) which needs further investigation.

VIII. Food safety associated with livestock and livestock products
This research program has focused on identifying the public health risks associated with the marketing of unpasteurized milk (34-37), with an emphasis on developing policies and technologies for improved quality and safety without jeopardizing market access for the poor. An outcome of this work has been changes in government policies towards more acceptance of raw milk marketing in several East African countries, based on the identified low risks and high dependence of resources poor people on these markets. This work is being expanded, in cooperation with IFPRI, to examine the marketing of other livestock and livestock products, particularly in South Asia. Studies provide policy-relevant analyses of the risks and economic benefits to poor farmers, market agents, and resource-poor consumers (38). ILRI in collaboration with Cornell University (USA) is also analysing the risks faced by the poor of contracting zoonotic and food-borne diseases and modelling the dynamics of these complex, under-reported diseases.

Demand for better quality and safe food is increasing among urban consumers, especially among affluent ones. This poses threats to the market opportunities of smallholder producers who often are unable to access technology, inputs and services to produce high quality products demanded by the market chains serving high-end consumers. ILRI research is trying to understand the nature of quality and safety attributes demanded by consumers, their willingness to pay for such attributes and how smallholders may respond to these through participation in market chains.

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Towards effective control of bovine tuberculosis in Africa: A case for public engagement in science

Jakob Zinsstag

The present paper summarizes a presentation given at the first stakeholder meeting on bovine tuberculosis held in Addis Ababa in June 2007. Reflections refer to animal health as a whole but are particularly applicable to the case of bovine tuberculosis in Africa.

Innovation in research requires open spaces to evolve. Numerous examples show us that it is often the unexpected detail, observed by a prepared mind that leads to discoveries and innovative approaches to problem solving (1). This is not disputed and remains a principle for applied research and for development oriented projects. On the other hand we face a huge gap between increased research capacity in Africa and persistent pressing problems of poverty, disease and hunger (2). There appears to be an evident disconnection between research and development action. At least some research should reflect the actual needs of society and produce outcomes that can be applied fast. This is increasingly recognized and existing initiatives require to be assessed for their effectiveness.

One such example is the REACH policy initiative between Kenya, Uganda and Tanzania (www.idrc.ca/geh/ev-101251-201-1-DO_TOPIC.html) aiming at the development of an evidence brokerage for health policy in East Africa. The REACH initiative recognizes the current failure on the one hand of research to reach the level of policy making and the development of effective interventions, and on the other hand of policy problems which are not taken up by research. Its approach is to create a cyclical process involving scientists and policy makers, connecting policy and practice questions via research and development, advocacy for evidence, new research agendas, policy-relevant research funds to research action. At the same time research studies are synthesized to summarize evidence and package it for policy development by appropriate advocacy. In turn new policies may undergo another iteration of scrutiny, producing new sets of policy relevant research questions. This process however requires active involvement and brokerage, negotiating actual priorities.

Another comparable approach has evolved in the framework of the National Competence Centre for Research North-South (NCCR North-South) on research for health services to nomadic pastoralists in Sahelian countries of Africa. Thereby a trans-disciplinary process (connecting science and society) established continuous dialogue between researchers, the concerned population and government authorities, which validated iterative cycles of research and pilot interventions leading to the formulation of a new integrated policy for nomadic pastoralists in Chad (3).

What now is the potential of the above examples to work towards effective control of bovine tuberculosis in Africa? Bovine tuberculosis is still largely unknown or under-diagnosed in many African countries. Its cost to livestock production and its impact on public health are not known, or limited to only a few countries (4). Control strategies are mostly limited to the inspection of carcasses at slaughterhouses. Effective compulsory “test and slaughter” strategies are virtually non-existent in most countries of the continent. Governments are not able to compensate for slaughtered animals, which is a central condition for the successful elimination of bovine tuberculosis in many industrial countries. Diagnosis of tuberculosis relying essentially on sputum microscopy cannot identify human Mycobacterium bovis infection. Since national governments do not have sufficient capacity and means, effective action against bovine tuberculosis needs participation and ownership by all the public and private stakeholders involved.

A participative stakeholder process involving livestock holders, veterinary and public health authorities and decision makers started in 2007 with initial funding from the Wellcome Trust Livestock of Life initiative. It aims to strengthen efforts of capacity building and policy development to assess and control BTB in Africa as North-South and South-South networks. Currently over 25 countries have participated in two workshops in Bamako and Arusha with the specific objectives: (a) to identify and collate all available data on M. bovis in both human and animal populations in each country, (b) determine on-going diagnostic capacity and protocols in each country, together with national policies and strategies relating to the treatment, prevention and control of disease in human and animal populations, (c) develop capacity in diagnosis and epidemiology and control policy, (d) initiate surveillance of BTB with the aim of establishing the burden to the livestock production and human health in order to assess the cost of disease to society, (e) raise awareness of M. bovis as a potential zoonotic component of the human tuberculosis epidemic and the consequences for disease control and prevention, (f) establish a policy dialogue between African countries with a view to identifying locally adapted control policy
options and (g) determine future research and evaluation needs to identify the most cost-effective locally adapted interventions. Participating countries engage in identifying funding for research and policy development.

The documented public health component of bovine tuberculosis in Nigeria, Tanzania and Uganda justifies efforts to make it part of current global programs and initiatives on tuberculosis control [5,6]. Recognition of these facts ought to result in applications by affected countries to the Global Fund to Fight AIDS, TB and malaria (GFATM) [7]. Furthermore international bodies like OIE, FAO and WHO should foster support to Africa, establishing standards for zoonoses surveillance and control. This is also part of the WHO International Health Regulations that came into force in mid-2007 and that require all countries to do a better job of disease surveillance for diseases that can spread between countries (www.int/edwha/pdf_files/WH58-REC1/english/Resolutions.pdf). In conclusion, bovine tuberculosis in Africa cannot be controlled without extensive public engagement in animal health research. This calls for a rethinking of research and control efforts and its economic consequences as bovine tuberculoses still goes unrecorded in most cases.

Reference
Mycobacterial genomics and its application to disease control

Stephen V. Gordon

Abstract
The publication of the Mycobacterium tuberculosis genome sequence in 1998 heralded a new phase in mycobacterial research. The subsequent decade has seen an enormous increase in our understanding of the basic biology, virulence mechanisms, drug targets and evolution of the M. tuberculosis complex, an acceleration in knowledge that would not have been possible without the genome. This review summarises some key findings from mycobacterial genomics and highlights how this knowledge can be applied to disease control in the developing world. [Ethiop. J. Health Dev. 2008;22(Special Issue):119-122]

Introduction
From anywhere in the world with an internet connection, researchers can now access the genome sequences of all the major mycobacterial pathogens, such as Mycobacterium tuberculosis, Mycobacterium leprae, Mycobacterium bovis and Mycobacterium ulcerans, as well as the vaccine strains M. bovis BCG and Mycobacterium microti OV254. Indeed, through the Wellcome Trust Sanger Institute, The Institute for Genome Research, and the Broad Institute we currently have genome sequences for six different strains of M. tuberculosis (H37Rv, CDC1551, 210, C, F11, Haarlem), with many more to follow through projects underway at the Broad. The “TubercuList” M. tuberculosis H37Rv genome database regularly receives more than 70,000 “hits” per month from all over the world (S. Cole, personal communication), showing how the genome has become a cornerstone of mycobacterial research; we now all work in a post-genomic landscape.

How has the availability of this mass of genome data changed our understanding of the tubercle bacilli? How are we to apply this information to improve disease control? This review will briefly summarise key findings from the genome sequences of M. tuberculosis, M. bovis and M. bovis BCG, and highlight how genome findings are being applied in the fight against disease.

Evolution
The emergence of M. tuberculosis has often been viewed as a classic zoonotic infection, with the generalist animal pathogen M. bovis crossing the species barrier into man at the time of cattle domestication. Initial work on defining genetic diversity across the M. tuberculosis complex using single nucleotide polymorphisms (SNPs) suggested that M. tuberculosis arose ~15,000 years ago (1), a similar date to that proposed for cattle domestication (2), seemingly providing further support for the zoonosis model. However, subsequent attempts to date the clonal expansion of M. tuberculosis using SNP data gave a date of ~35,000 years ago (3). Indeed, using SNP data to define a molecular clock may not be appropriate for M. tuberculosis, given its small effective population size and distinctive DNA repair systems.

An alternative method to explore the evolution of the M. tuberculosis complex employed comparative genomics. With the M. tuberculosis genome as a starting point, the genomes of all members of the M. tuberculosis complex were scanned for deletion events (4). This defined a set of 12 deletions that could then be mapped across a reference collection that encompassed the diversity of the M. tuberculosis complex (5). This comparative analysis allowed a novel evolutionary scenario to be proposed, with M. tuberculosis closer to the common ancestor of the complex than M. bovis (Figure 1). This scenario appears to overturn conventional evolutionary thinking, suggesting that tuberculosis is in fact a reverse zoonosis given by man to domesticated animals. However, the host association of the common ancestor of the complex is unknown, and valid cases can be made for a human or animal associated ancestor.

Recent work from strains causing tuberculosis in East Africa has added a further twist to the evolution of the M. tuberculosis complex (6). These strains, classed as “smooth” tubercle bacilli due to their characteristic colony appearance, were characterised using multilocus sequencing and revealed to have an unexpected degree of sequence variation. However, most surprising was evidence for horizontal gene transfer among this population of strains, which goes against current data suggesting a purely clonal population structure for the M. tuberculosis complex. Based on these data the authors proposed that these in fact were isolates of the progenitor of the M. tuberculosis complex, Mycobacterium

1. http://genolist.pasteur.fr/Tuberculist
2. http://genolist.pasteur.fr/Leproma
5. http://genolist.pasteur.fr/BCGLList
7. www.sanger.ac.uk
8. www.tigr.org
9. www.broad.mit.edu

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prototuberculosis (6). However, this latter suggestion has been challenged since recombination within the studied population would inflate the estimation of diversity of this group (7). Clearly, a greater sampling of strains causing tuberculosis on a worldwide level, and in particular from Africa, is needed to allow a better understanding of how smooth tubercle bacilli fit into the evolution of the *M. tuberculosis* complex.

Using a combination of genome deletion events and SNPs it is possible to define a phylogeny for the members of the *M. tuberculosis* complex, with *M. tuberculosis* sitting closer to the common ancestor than *M. bovis*. The deletion events RD9 and RD4 serve as unequivocal markers of species; hence, strains deleted for RD9 and RD4 are defined as *M. bovis*. The mmpL6 SNP is at codon 561, where AAC is replaced by AAG; hence, *M. bovis* presents the AAG codon.

![Figure 1: An evolutionary scenario for the *M. tuberculosis* complex.](image)

**Physiology**

Analysis of the genome sequence of *M. tuberculosis* revealed a remarkable capacity for both catabolism and anabolism of lipids, reflecting complex synthetic requirements for the cell wall and the capacity to metabolise a wide variety of lipid substrates (8). The radiance of lipid metabolic genes is found across all members of the *M. tuberculosis* complex; however, comparative analysis of genes encoding key enzymes of central metabolism has uncovered the metabolic basis for notable phenotypic differences between the tubercle bacilli. For example, *M. bovis* requires pyruvate to be added to media where glycerol is the sole carbon source, while *M. tuberculosis* has no such requirement; the metabolic basis for this was unknown, but the answer presumably lay in the glycerol utilisation pathway. Once the *M. bovis* genome had been sequenced, it was relatively facile to compare all genes between *M. bovis* and *M. tuberculosis* that encoded proteins involved in glycerol utilisation. This revealed a nonsynonymous point mutation in the gene encoding pyruvate kinase which charged a conserved glutamic acid residue to aspartic acid (9). Enzyme assays showed that *M. bovis* lacked pyruvate kinase activity, while complementation with the *M. tuberculosis* allele restored pyruvate kinase activity and the ability to grow on glycerol. Furthermore, the complemented strains of *M. bovis* switched from the typical “dysgonic” appearance of *M. bovis* colonies on glycerol-based media to abundant, “eugonic” colonies as seen with *M. tuberculosis*. As pyruvate kinase is one of the few irreversible steps in glycolysis, the lack of a functional pyruvate kinase in *M. bovis* blocks glycolytic intermediates from feeding into oxidative metabolism. Hence, *in vivo*, *M. bovis* must rely on amino acids or fatty-acids as a carbon source for energy metabolism; *in vitro*, media for the isolation of *M. bovis* must be supplemented with pyruvate.

**Drug Targets**

The current length of tuberculosis chemotherapy and paucity of frontline drugs are driving the emergence of MDR (multi-drug resistant) and XDR (extensively drug resistant) strains. There is clearly an immediate need for novel drug targets to be identified in *M. tuberculosis*. Genomics and allied functional genomic techniques have helped galvanize drug discovery initiatives. A key starting point was to identify which of the ~4000 genes are essential for the bacillus, as proteins essential for bacterial viability are considered the most promising targets for rational drug design. Using a combination of saturation transposon mutagenesis and microarray-based identification of insertion sites, Sassetti, Boyd and Rubin defined a minimal *in vitro* gene set for *M. tuberculosis* (10); 570 of these genes were also found to be essential by a similar experimental approach adopted by Bishai and colleagues (11). To prioritize these genes as targets for drug development further genomic criteria can be used, including the presence of druggable protein domains that bind potent compounds, situation at a unique position in a metabolic chain or “chokepoint”, or phylogenetic restriction to avoid homologues in host or host flora.
In addition to identifying potential targets for drugs, genomics has greatly assisted in identifying the targets of drugs with known antibacterial activity. Previously this had to be done by laborious and time consuming genetic screens but the ease of full genome sequencing means whole genome comparisons can be used to locate resistance-conferring mutations. For example the molecular target for the diaryquinoline R207910 was identified by comparing the genomes of resistant *M. tuberculosis* and *M. smegmatis* strains selected *in vitro* with the wild-type genome. Drug resistance conferring mutations localised in both species to the *atpE* genes, that encodes part of the ATP synthase, which was subsequently confirmed as the site of drug action (12).

PA-824 is a nitroimidazole prodrug whose activation requires reduction via an F420-dependent glucose-6-phosphate dehydrogenase; however, this activity is not sufficient to confer sensitivity. To identify further factors involved in sensitivity to PA-824, Barry and colleagues isolated PA-824 resistant strains with wild-type F420-dependent glucose-6-phosphate dehydrogenase activity, and resequenced their genomes using high density oligonucleotide arrays (13). Resistant isolates were shown to harbour mutations in *Rv3300c*, encoding a nitroimidazo-oxazine-specific nitroreductase, further elucidating the action of PA824. This approach of rapid full genome comparisons also represents a powerful new tool for characterizing the phenotypic consequences of single point mutations in other settings, such as the microevolution of *M. tuberculosis* within hosts or during transmission cycles.

**BCG: a family of duplicating daughters**

BCG is not a single vaccine, but rather a family of daughter strains derived from an original stock sent out by the Institute Pasteur to laboratories around the world (14). Accumulating evidence suggests that these daughter strains posses significant differences in terms of genome content, immunostimulatory activity, and protective efficacy. Indeed, despite being the most widely used vaccine in the world, the molecular basis for the attenuation of BCG has never been fully described, although the loss of the RD1 locus was a key attenuating event (15). The recent completion of the genome sequence of *M. bovis* BCG Pasteur, and comparative analyses across BCG daughter strains, exposed all the all genetic differences between BCG Pasteur and the virulent *M. bovis* and *M. tuberculosis*, and provided further evidence for strain specific genome content that may impact on vaccine efficacy (16).

A key finding from the BCG sequencing project was the presence of duplication events in the BCG genome. These duplications are the first to be described in a mycobacterial genome, and show variable configurations across the daughter strains. For example, DU1 is a duplication around the origin of chromosomal replication, *oriC*, that is only found in BCG Pasteur. On the other hand, DU2 defines a duplication that is present in all BCG strains, but in 4 different arrangements. Hence, after the original DU2 duplication event, internal regions were spliced out and duplicated a number of times, suggesting a selective pressure was driving the gene duplication events. Clues to what the in vitro selective pressure was are revealed in the core region of DU2 which is duplicated across the strains but that did not subsequently undergo deletion. This core region comprises three complete coding sequences (CDS), namely *glpD2* (encoding a putative glycerol 3-P dehydrogenase), *phoY1* (encoding a potential repressor of the high affinity phosphate uptake system), and *Rv3300c* (encoding a protein of unknown function). This duplication would serve to increase the production of glycerol 3-P dehydrogenase; glycerol was the carbon source used by Calmette and Guérin during the derivation of BCG from *M. bovis*, so it is possible that increased copy number of *glpD2* conferred a selective advantage for growth on glycerol. However, glycerol 3-P dehydrogenase is not a known metabolic bottleneck for glycerol growth; one might instead expect to have seen *glpK*, encoding glycerol kinase and a known rate limiting enzyme in glycerol utilisation, undergoing amplification. A second possibility is that increased production of the potential repressor of high affinity phosphate uptake, *phoY1*, was selected. BCG is known to have lesions in the high affinity phosphate uptake system compared to *M. bovis* and *M. tuberculosis*, suggesting that switching this system off may have been advantageous to the bacillus.

**Application to disease control**

In a collaborative project between the Armauer Hansen Research Institute (Ethiopia), Imperial College (UK), Trinity College (Ireland), the Swiss Tropical Institute (Switzerland), the Institute for Livestock Research (Kenya), and VLA (UK), we aim to measure the cost of bovine TB to Ethiopian society by assessing its impact on livestock productivity and human health. A necessary step in this project is to ensure that we can correctly culture and discriminate the members of the *M. tuberculosis* complex from both human and animal clinical samples. Findings from the mycobacterial genome projects feed directly into this endeavor. For example, from the *M. bovis* genome project we know that *M. bovis* can not use carbohydrates as a carbon source for energy production; pyruvate must be added to the media to allow *M. bovis* to grow (9). Hence, knowledge from the genome informs our choice of media for the improved isolation of *M. bovis* from clinical samples. Similarly, from comparative genomics we now know that deletion events provide unequivocal markers of species (5, 17). From the schematic in Figure 1, we can see that isolates that are deleted for RD9 and RD4 are *M. bovis*, while strains that have the RD9 and RD4 loci intact are *M. tuberculosis*. The presence or absence of these
deletions can be determined using a simple PCR reaction; this is a significant improvement over biochemical tests (such as nitrate reduction or niacin production) which can prove misleading. Hence, genetic tests based on deletions provide confidence that the isolates are correctly identified as *M. bovis* or *M. tuberculosis*. Access to a specific, portable PCR for strain discrimination is an obvious benefit when one is trying to determine the burden of *M. bovis* infection in the human population.

**Conclusions**

Genomics has fundamentally altered our understanding of the members of the *M. tuberculosis* complex. The advances outlined above are also providing tools that can now be applied to disease control, with simple PCR-based strain identification offering a case in point. A key requirement is to ensure that mycobacterial genomics continues to drive efforts in disease control, and that novel findings are translated to the clinical and field setting where their application can have the greatest impact.

**Acknowledgements**

This work was funded by the Wellcome Trust and the UK Department for Environment, Food and Rural Affairs. The author wishes to acknowledge the guidance and support of Glyn Hewinson, Noel Smith, Roland Brosch, Thierry Garnier, and Stewart Cole.

**References**

Abstract
The Wellcome Trust is one of the world’s largest independent research-funding charities, with a mission to foster and promote research with the aim of improving human and animal health. Based in London, UK, the Trust funds a wide range of research, from basic science to the history of medicine, and supports more than 3,000 researchers in 54 different countries. The Trust’s total annual charitable expenditure is over £500 million; in 2006 it spent £73 million on health research and capacity building in developing and restructuring countries. Although the Wellcome Trust is more generally known for funding research into human health, it views animal health as important in its own right and, recognising the importance of livestock diseases, in 2003 launched a global five-year initiative termed ‘Animal Health in the Developing World – Livestock for Life’. This initiative is supporting a spectrum of activities that focus on livestock diseases in developing countries and their impact on human health and wellbeing. [Ethiop.J.Health Dev. 2008;22(Special Issue):123-125]

Introduction
The Wellcome Trust was created in 1936, under the will of Sir Henry Wellcome (1853–1936), and is now one of the world’s largest and most diverse independent biomedical charities. Our mission statement, which is based on the will of Sir Henry Wellcome, is to foster and promote research with the aim of improving human and animal health. Its broad definition allows us to undertake a wide range of activities and to respond flexibly to both medical needs and scientific opportunities.

Each year, we spend about £500 million to support biomedical and health research in the UK and internationally. Our research portfolio ranges from fundamental biological studies through to clinical and public health research, and support is also provided for public engagement with science, for technology transfer to encourage commercial application of research to meet medical needs, and for research and capacity building in biomedical ethics and the history of medicine.

International funding
Each year, around 10–15 per cent of the Trust’s budget is spent on research outside the UK – in 2006 we spent £73 million on health research and capacity building in developing and restructuring countries. To build on the success of our biomedical health research programmes in public health and tropical medicine, in 2005 we agreed to increase funding for researchers in developing and restructuring countries who work in these fields, and we have recently announced a new international strategy (http://www.wellcome.ac.uk/globalhealth). The core aims of this strategy are:

- Broadening the base for scientific endeavor by investing in excellent scientists who show they have the greatest promise to advance knowledge, and ensuring they have the resources that they need to carry out their work.
- Increasing our support to areas of science that have the most potential for increasing health benefits for people and livestock in the developing world.
- Supporting international networks and partnerships that are focused on health research in developing and restructuring countries, and that will facilitate the sharing of ideas and resources, and ultimately accelerate scientific progress.

We believe that building sustainable research capacity starts with developing people, as it is talented researchers who drive the formation of productive programmes and networks that ultimately accelerate scientific progress. Examples of where this has been successful are our four Major Overseas Programmes in Kenya, Malawi, South-east Asia and South Africa. Each programme is embedded within a local institute, and provides a base for high-quality research into important local health issues and aims to put research into practice.

In Ethiopia, the Trust has funded a variety of research projects over the past 40 years. For example, awards have been made to Dr Abdulkadir for a ‘Clinical and epidemiological study of malnutrition related diabetes mellitus’, to Professor Prince on the ‘Relative impact of major depression upon clinical course, disablement and service use in rural Ethiopia’, to Dr Larenson for the ‘Pathogens of Canidae in multi-species host systems: reservoirs, transmission and effective control’, and to Professor Morton on ‘Engaging with the corporate sector for pastoral development in Ethiopia’.

Funding animal health research
Although the Wellcome Trust is more generally known for funding research into human health, since its inception the Trust has funded research in animal health. Today, such studies are funded in two main categories:
Recognising the importance of livestock diseases and their impact upon human health, in 2003 we launched a fixed-term initiative called ‘Animal Health in the Developing World – Livestock for Life’. This initiative, which emphasized practicalities, actual needs and real solutions, aimed to fund research that:

- improves the understanding of the epidemiology of disease to predict and improve control of outbreaks
- stimulates efforts to understand biology of transmission
- exploits genomics for new disease control measures
- produces livestock with enhanced resistance to disease
- raises awareness and understanding of animal health issues to maximize health gain from existing strategies
- strengthens links between local communities, livestock keepers, practitioners, researchers, scientists, policy makers and other stakeholders to make a significant impact on animal health.

**Figure 1:** Overview of fellowships available to researchers based in developing countries

**Box 1:** Web links

Twelve awards in biomedical science were made in December 2004. Most of the awards were to large international consortia, with collaborating researchers from 28 different countries, and included a grant for the investigation of bovine tuberculosis in Ethiopia, led by Professor Douglas Young (Imperial College London, UK). Other awards included studies into the epidemiology, genomics, immune responses, prevention and diagnosis of livestock diseases, and covered animal diseases such as bovine tuberculosis, African swine fever, babesiosis, peste des petits ruminants, theileriosis.
and cysticercosis. Details of the awards can be found at www.wellcome.ac.uk/doc_wtx025304.html.

For the Livestock for Life scheme, 19 smaller projects were funded. Many went to collaborative public engagement programmes, with award holders representing 15 different countries across Africa, Latin America and Southern Asia. Programmes that were funded aimed to enhance dialogue between different stakeholders, strengthen education and training, or involve various parties in policy and advocacy issues pertinent to the health of livestock. One such project is led by Professor Jakob Zinsstag (Swiss Tropical Institute, Switzerland) on ‘Effective management of bovine tuberculosis in Africa: towards adapted control policy, in Africa’. Full details of all the awards can be found at www.wellcome.ac.uk/doc_WTX035581.html.

Although some of the projects started quite recently, advances in animal health and the translation of research into policy and practice have already begun. Examples include the development of protective vaccines against diseases such as cysticercosis and schistosomiasis, which are presently being tested in the field, and epidemiological studies that are shedding light on the impact of livestock diseases such as trypanosomiasis.

**Funding opportunities**

Although there are no plans for a new ‘Animal Health in the Developing World – Livestock for Life’ initiative, researchers can apply to the Trust for support through our standard funding schemes. Funding opportunities are available for UK- and Republic of Ireland-based researchers, and for developing country scientists who wish to carry out research in their home country. Full details of how to apply for support are available on www.wellcome.ac.uk/funding. In general, researchers from a developing country who wish to work in a developing country, and are based at an eligible institute, can apply for a fellowship in public health and tropical medicine (Figure 1), while those who wish to work in collaboration with researchers in the UK or Republic of Ireland can apply for a collaborative project or programme grant.

We have recently changed our eligibility rules so that applicants who have previously been successful in obtaining Wellcome Trust funding a principal applicant can apply directly to use without the requirement for a UK partner. This includes eligibility to request support for multi-user items of equipment, under our equipment grant scheme, as well as applying for a Strategic Award, which is our new flexible form of support, to facilitate under other existing schemes.

In summary, it is the Trust's policy to focus on the best people and the best teams who are asking innovative questions. Through long-term funding, we aim to make a difference in human and animal health issues of global importance.

**References**

1. The Wellcome Trust: www.welcome.ac.uk
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4. Wellcome Trust funding: www.wellcome.ac.uk/funding.
The role of bovine tuberculosis as zoonotic disease in Ethiopia

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Abstract
Bovine tuberculosis (BTB) is endemic in Ethiopia although the epidemiology and zoonotic importance of the disease is not well known. In order to assess the magnitude of BTB in animals and its zoonotic impact in humans a comprehensive surveillance study has to be conducted in all the geographical zones of the country. Tackling a disease like BTB which affects both livestock and humans requires joint activities between the ministries of Agriculture and rural Health. The close working relationship developed between the Ministry of Agriculture and Rural Development (MoARD) and the Ministry of Health (MoH), such as the National Coordination Committee created for the avian human influenza and Rift Valley fever threat can be used for BTB as well. Through this development an effective response can be mounted against BTB and other serious livestock diseases that have a significant public health component. A joint effort of the two ministries as well as national and international research institutes is required more than ever to handle old and newly emerging zoonotic diseases. [Ethiop.J.Health Dev. 2008;22(Special Issue):125-127]

Introduction
Zoonoses are defined as those diseases and infections naturally transmitted between people and vertebrate animals. In most cases, animals play an essential role in maintaining the infection in nature and contribute in varying degrees to the distribution and actual transmission of infection in human and animal populations. These diseases have a variety of transmission mechanisms that may be direct such as in rabies and anthrax, or indirect, via vectors, food, water and the environment, as in the case of bovine tuberculosis and cysticercosis. Many, such as brucellosis, also have multiple routes of infection. With the constant and inevitable interaction of man and animals, zoonotic diseases remain a genuine threat to health and survival for people, their livestock, companion animals and wildlife.

The significance of zoonotic diseases is expanding and their health and socioeconomic impacts are increasingly being experienced by many countries, particularly the developing ones. In countries such as Ethiopia, the establishment and implementation of adequate measures for livestock and consumer health protection against zoonoses, especially those that are new and emerging, has proven to be very difficult. Thus zoonotic diseases continue to further burden public health systems as well as to undermine efforts to boost livestock production and exports.

Bovine tuberculosis
Bovine tuberculosis is a significant zoonosis that can spread to humans through aerosols and by ingestion of raw milk. In developed countries, eradication efforts have significantly reduced the prevalence of this disease, but reservoirs in wildlife make complete eradication difficult. Bovine tuberculosis is still common in developing countries, and economic losses can occur in cattle and buffalo from deaths, chronic disease, and trade restrictions. Infections may also be a serious threat to endangered species.

In Ethiopia bovine tuberculosis is endemic, although the epidemiology and zoonotic importance of the disease is not well known due to lack of nation-wide investigation. However, the disease has frequently been reported in Ethiopia from small scale studies, though the magnitude of the impact of BTB on animal health is largely unknown. Since the disease is known to affect a large host range (cattle, goat, sheep, camels, humans, and wildlife) it makes its assessment difficult.

Local and miliary pathological lesions are seen in abattoirs during meat inspection, and the disease has been described in cattle and in wildlife. Up to 50% of dairy farms in Addis Ababa were shown to be positive. M. bovis was also isolated from humans. A study conducted on 788 cattle subjected to a Single Intra-dermal Comparative Cervical Tuberculin (SICCT) showed a prevalence of 29% of which 188 (23.8%) were positive to bovine PPD, and 46 (5.8%) were doubtful reactors. There was a significant difference in prevalence between farms ranging from 4.2% to 90.8%, the highest being commercial dairy farms. Analysis of risk factors revealed that prevalence of BTB was significantly associated with management and breeds (1,2,3,4).

A total of 265 human sputum, bovine milk and tissue samples were cultured and 102 samples were positive for bacterial growth on primary culture. However, only 81 were positive for acid fast bacilli on subculture and subjected to niacin test: 36 (44.4%) were niacin positive indicating M. tuberculosis, and 45 (55.6%) negative indicating M. bovis or atypical mycobacterium. Niacin
positive organisms were isolated from sputum and milk samples, suggesting the possibility that raw milk and its products act as a vehicle for the transmission of *M. tuberculosis* (1) from cattle to humans. Isolation of niacin negative organisms from human sputum is an indication of zoonotic importance of BTB.

**Routes of transmission**

Agents transmissible between livestock and humans (zoonotic agents) may have an important impact on the health status of livestock keepers because they live in close contact to their animals. A number of zoonoses are among the oldest known diseases and many are entrenched, particularly in rural agricultural communities. Increasingly they are also found in urban areas where people keep livestock and live in close contact with their animals.

Tuberculosis can be transmitted either by the respiratory route or ingestion. In cattle, aerosol spread is more common. Infectious bacteria can be shed in the respiratory secretions, feces, milk, and in some individuals in the urine, vaginal secretions, or semen. Not all infected animals transmit the disease; asymptomatic and anergic carriers occur. *M. bovis* can survive for several months in the environment, particularly in cold, dark, and moist conditions.

In Ethiopia, where pasteurization of milk is very limited the most likely route of transmission is through consumption of raw milk, but few studies have demonstrated the shedding of mycobacterium organisms through milk (5). Tuberculosis in humans is also increasing at an alarming rate and affects mainly the active working age group (15 – 45 years). This may have a negative influence on the national economy. More than 30% of TB patients have extra-pulmonary tuberculosis (6) and the majority of them were directly or indirectly in contact with cattle. This suggests the possible association that may exist between extra-pulmonary TB and *M. bovis*.

**Types of study to be conducted**

In order to assess the magnitude of BTB in animals and its zoonotic impact in humans a comprehensive surveillance study has to be conducted in all the geographical zones of the country. National studies should be carried out to investigate geographical areas affected, especially in pastoral areas, field prevalence and economical impact of the disease. Baseline epidemiological information is needed for assessing the most cost effective intervention for Ethiopia. Education and working capacity has to be strengthened and existing national structures should be used. Tackling a disease like BTB which affects both livestock and humans requires joint activities between the Ministry of Agriculture and the Ministry of Health. Only then can a control strategy can be developed based on knowledge based policy decisions.
Conclusions & recommendations

The close working relationship developed between the Ministry of Agriculture and Rural Development (MoARD) and the Ministry of Health (MoH), such as the National Coordination Committee created for the avian human influenza and Rift Valley fever threat can be used for BTB as well. Through this development an effective response can be mounted against BTB and other serious livestock diseases that have a significant public health component. Agreement can be reached on a joint framework to conduct a comprehensive surveillance program to assess the magnitude of human/bovine tuberculosis and come up with sound control strategies suitable for Ethiopia. The experience gained in developing preparedness and response plan for the threatened avian human influenza pandemic has helped to explore the opportunities for sharing resources between the two ministries, where appropriate, and to avoid unnecessary duplication. Therefore a joint effort of the two ministries as well as national and international research institutes is required more than ever to handle old and newly emerging zoonotic diseases.

Reference

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

Abraham Aseffa

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 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 field 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 society. The study will move beyond the veterinary and human-health perspective and include social, environmental, and economic costs in a comprehensive cost-benefit analysis framework.
controls to determine risk factors. Analysis of the health will be conducted on bovine TB cases and matched 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.

Acknowledgements

The Bovine TB Project team includes Douglas Young (Principal Investigator), Glyn Hewinson, Howard Engers, Dan Bradley, Jakob Zinsstag, Steven Gordon, Richard Bishop, Abraham Aseffa, Martin Vordermeier, Getu Melese, Gobena Ameni, Rea Tschopp, Stefan Berg, Yonas Hirutu, Endalamaw Gadisa, Lawrence Yamuah, Rebuma Firdeessa. Brian Robertson and Aaron J Rae are acknowledged for various contributions. Meseret Habtamu, Yusuf Sani and Girma Berhanu at AHRI give technical support. Collaborating partners in Ethiopia are the National Artificial Insemination Centre, MoARD, AL-Institute of Pathobiology, AAU, Oromiya, Amhara, SNNPR Regional Health Bureaus and Animal Health Offices and the abattoirs and Hospitals in Gondar, Woldeya, Butajira, Jinka and Ghimbi. The support of the Federal Ministries of Agriculture and Rural Development and Health is acknowledged. The project is funded by the Wellcome Trust.

References


A comparative study on the epidemiology and immuno-pathology of bovine tuberculosis in *Bos indicus* and *Bos taurus* cattle in Ethiopia

Gobena Ameni¹,², Abraham Aseffa², Howard Engers², Douglas Young³, Stephen Gordon⁴, Glyn Hewinson⁴ and Martin Vordermeier⁴

**Abstract**

Bovine tuberculosis is a disease of dual effect, having public health and economic implications. The present study was conducted on its epidemiology and immuno-pathology in Holstein and Zebu breeds of cattle. Skin test, post mortem examination and pathology scoring, bacteriology, whole blood gamma interferon assay, ELISPOT assay, and lateral flow assay were used. An overall prevalence of 13.5% (n=5,424) was recorded; both prevalence (χ² =61.8; P<0.001) and severity of pathology (mean pathology scores ± SEM: 6.84±0.79 vs. 5.21±0.30; P=0.018, Mann-Whitney test) were significantly higher in Holstein than in Zebu. Similarly, IFN-γ responses to avian PPD (0.49±0.10 vs. 0.39±0.07), bovine PPD (0.63±0.11 vs. 0.43±0.07), or the ESAT6-CFP10 protein cocktail (0.43±0.01 vs. 0.30±0.05) were significantly higher (for all antigens: p<0.02) in Holstein than in Zebu cattle. However, both Holstein and Zebu exhibited similar T cell and antibody responses to different mycobacterial antigens i.e. no repertoire difference was observed between the two breeds. Thus, the present study showed increased susceptibility of Holsteins to bovine TB as compared to Zebu, similarity between Holsteins and Zebus in their antigen responses, and a positive correlation between IFN-γ responses and severity of pathology of bovine TB. [Ethiop.J.Health Dev. 2008;22(Special Issue):132-134]

**Introduction**

Despite the ability of several countries to eradicate bovine tuberculosis successfully, many countries continue to encounter *M. bovis* infection in their cattle population (1,2). Reasons for the failure of these countries to control/eradicate the disease include the presence of a feral reservoir of *M. bovis* in developed world, and the inability of developing countries to apply the test and slaughter control method. Other alternative control methods such as the use of vaccination, improvement of cattle husbandry and/ or breeding of relatively resistant cattle breeds may be particularly applicable in developing countries. It was stated that housing predisposes cattle to tuberculosis so that the disease is more common and serious in these forms of husbandry (3). Zebu type cattle are thought to be more resistant to tuberculosis than European cattle and the effects of the disease on these cattle are much less severe as stated earlier (4). One of the difficulties in generating more data on such observations was the problem of getting the two breeds under identical cattle husbandry. Zebu is limited to Africa and Asia while European breeds are mainly found in Europe and other developed countries. Therefore, the objectives of this study were assess immune responses to mycobacterial antigens and investigate the epidemiology and immuno-pathology of bovine TB in *Bos indicus* and *Bos taurus* cattle in central Ethiopia.

**Materials and methods**

**Study animals and sampling:** The study was conducted in dairy rearing areas of Holeta and Selalle, central Ethiopia. The sites were selected because of the concentration of Holstein or/and crosses alongside native Zebus (mainly of the Arsi breed). The target population is smallholders that keep at least one Holstein/cross. There are 30,000 Holsteins/crosses kept by smallholders in the study area. Similarly, about equal number of zebus are kept in the target herds. For epidemiological studies, 10% (n=2846) of the Holsteins/crosses were sampled and about 9% of the Zebus (n=2578) were sampled. Thus, a total of 5424 cattle were sampled for the epidemiological study. For the investigation of immune response and pathology, 123 (50 Holsteins and 73 Zebus) skin test reactors were recruited from the above sampled population on the basis of the level of skin indurations and willingness of the farmers to sell their animals. Furthermore, 30 reactor Holsteins were recruited from one intensive farm.

Mycobacterial antigens: ESAT-6 family, heat-shocked proteins of mycobacteria, and secreted antigens/lipoproteins (5).

Comparative intradermal tuberculin test: Two sites on the right side of the mid-neck, 12 cm apart, were shaved and the skin thicknesses were measured. One site was injected with an aliquot of 0.1ml containing 2500 IU/ml

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bovine purified protein derivative (PPD) (Veterinary Laboratories Agency, Addl Estone, Surrey KT15 3NB, U.K.). Similarly 0.1ml of 2500 IU/ml avian PPD (Veterinary Laboratories Agency, Addl Estone, Surrey KT15 3NB, U.K.) were used according to the Office International des Epizooties (6).

Whole blood gamma interferon assay: Whole blood cultures stimulated with mycobacterial antigens were incubated at 37°C in a humid 5% CO2 atmosphere, for 48 hours and supernatants harvested (7) and frozen. Levels of IFN-γ in the supernatants were measured with ELISA using the bovine IFN-γ (Bovigam) test kit (Commonwealth Serum Laboratories, Victoria, Australia) as per the manufacturer’s instructions.

Enzyme-linked immunospot assay: Peripheral blood mononuclear cell (PBMC) was isolated from 16 Zebus and 14 Holsteins and the ELISPOT was done according to (5).

Multiple antigen print immunoassay: MAPIA was performed as described previously (8,9) on sera of 16 Zebus and 14 Holsteins and the ELISPOT was done according to (5).

Post mortem examination and pathology scoring: The severity of the gross lesions was scored by applying the semi-quantitative procedure developed by Vordermeier et al. (10), with minor modifications to facilitate performance under field conditions (11).

Bacteriology: Suspicious tissues were further processed for isolation of mycobacteria as described by the OIE (6).

Statistical analysis: Logistic regression analysis was used to assess the association between prevalence and animal risk factors using STATA statistical software, (STATA Corporation, 4905 Lakeway Drive, College Station, Texas 77845 USA). The Mann-Whitney test was used to compare pathology scores and IFN-γ responses between cattle types.

Results
Prevalence: The overall prevalence of bovine tuberculosis was 13.5% (n=5,424) in central highlands of Ethiopia. The prevalence was significantly higher in Holstein than either in zebus (22.2% vs. 11.6%, \( \chi^2 = 61.8; P <0.001 \)) or in zebu-Holstein crosses (22.2% vs. 11.9%, \( \chi^2 = 50.7; P <0.001 \)).

Effect of risk factors on prevalence: Holsteins were more than twice as likely to present as tuberculin positive than Zebu cattle (OR=2.32; CI=1.89, 2.85). Similarly, animals aged between 5 and 9 years were at higher risk of infection (OR=2.37; CI=1.80, 3.12) with TB compared to those aged 2 years or below.

T cell response to mycobacterial antigens in zebu and Holsteins: Higher T cell count (response) was observed against heat-shock protein 65 (Hsp65) both in Holsteins and Zebus. Nevertheless, no significant difference (P<0.05) was observed in T cell count (response) between Holsteins and Zebu in their recognition of mycobacterial antigens.

Antibody response to mycobacterial antigens in Holsteins and zebu: Both Holstein and Zebu cattle exhibited similar antibody responses to different mycobacterial antigens. Although generally weak responses were observed in both breeds, stronger antibody responses were recorded to M. bovis culture filtrate (MBCF) and 16kDa alphacrystallin/MPB83 fusion proteins (16/83).

Breed and pathology: The severity of pathology in Holsteins [mean pathology scores \( \pm \) SEM: 6.84\( \pm \)0.79; median scores (range): 6.0 (2-42)] was significantly higher (P=0.018, Mann-Whitney test) than the severity of pathology in Zebu cattle [mean scores \( \pm \) SEM 5.21\( \pm \)0.30; median (range): 5.0 (1-17)]. The mesenteric lymph nodes were the most severely affected (mean pathology scores=SEM, 1.95\( \pm \)0.08) followed by the retropharyngeal (0.80\( \pm \)0.05) and the caudal mediastinal (0.8\( \pm \)0.06) lymph nodes.

Bacteriology: Fifty-six percent (81/145) of the animals with gross TB lesions were culture positive. Culture positivity of suspicious lesions did not differ (\( \chi^2 =0.13, P=0.72; EPI6 \)) between Holstein (54%, n=50) and Zebu (51%, n=73) breeds under identical field husbandry.

Discussion
Moderate prevalence was recorded by this study. Previously, a similar prevalence (14.2%, n=416) (12) was reported in southern Ethiopia, where cattle farming is similar to Holeta and Selalle. However, a lower prevalence (4.1%; n=460) was reported in Zebu cattle under traditional management in the Boji district of western Ethiopia (13) while a significantly higher prevalence (46.8%; n=1,171) was reported in 12 intensive dairy farms which keep crossbreed and Holstein cattle (14). The prevalence was significantly higher in

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Holsteins than in either cross-breeds or in Zebus. Thus, the prevalence of bovine TB is affected by cattle husbandry and cattle breed (11). Furthermore, the severity of pathology was significantly higher in Holstein than in Arsi Zebus. Historical reports also indicated that Bos taurus (the group to which Holsteins belong) are more susceptible to bovine TB as compared to Zebu cattle (3,4).

The level of IFN-γ responses to the tested mycobacterial antigens was significantly lower in Zebu compared to Holstein cattle, which would support the correlation of IFN-γ release with the severity of pathology of bovine TB in Holstein (10). It is also noteworthy that the IFN-γ responses observed in Holstein cattle in Ethiopia were considerably lower than those reported for Holsteins in the United Kingdom, Ireland, or New Zealand (15). A likely explanation could be that a higher proportion of Holstein cattle in Ethiopia suffer from advanced disease as the test and slaughter is not applied in Ethiopia. In addition, multiple parasitic infections, which prevail in the study population (personal observation), could also modulate the IFN-γ responses to mycobacterial antigens. A previous study showed that infection with either Fasciola spp. or Strongyulus spp. significantly reduced skin indurations to bovine PPD in M. bovis infected heifers compared to de-wormed M. bovis infected heifers (16).

This study has shown a comparable T cell and antibody responses to mycobacterial antigens in both Holsteins and Zebus. Strong T cell response to Hsp65 was found in both Holstein and Zebu breeds. Similarly, strong antibody response was observed to M. bovis culture filtrate (MBCF) and 16/83 in both breeds

The present study showed susceptibility of Holsteins to bovine TB as compared to Zebus, similarity between Holsteins and Zebus their antigen recognition repertoires and a positive correlation between IFN-γ responses and severity of pathology of bovine TB.

Acknowledgements
The Wellcome Trust is acknowledged for its financial support to run this project.

References

Approach to assess the economic impact of bovine tuberculosis in Ethiopia

Rea Tschopp1,3, Melese Getu2, Abraham Aseffa3, Jakob Zinsstag1

Abstract
Bovine TB is prevalent in Ethiopian cattle and represents a serious zoonotic risk. However, extensive epidemiological data in the human and livestock sector are lacking. Create a dynamic transmission model of disease between animal and human, as a prerequisite for economic analysis of the most profitable intervention to control BTB in Ethiopia. Study on-going (2005-2010), epidemiological (prevalence, risk factors) and cost (human and livestock) data are collected in eight sites over a period of four years and fed into a compartmental trans-sectoral framework that simulates disease transmission. Different intervention scenarios will then be simulated in the model. The most profitable intervention to control BTB in Ethiopia has to be assessed as well as the cost sharing scheme between the public health and agricultural sectors. It has been postulated that a test and slaughter policy would have a negative economic impact in Ethiopia. Alternatives need to be assessed.

Introduction
Tuberculosis is distributed worldwide and is one of the most important public health concerns, especially in sub-Saharan Africa. The disease is responsible for the death of more people each year than any other infectious disease: nearly 8 million new cases and 2 million deaths are reported annually (1). Nearly 2 million TB cases occur each year in sub-Saharan Africa alone, and the role played by cattle pathogen *M. bovis* in the rising epidemic of tuberculosis, fostered by HIV in Africa, is largely unknown (2).

Cattle are considered to be the main hosts of *M. bovis*. However, the disease has also been reported in many other species, including human beings, domesticated animals and wildlife (3).

The epidemiology of *M. bovis* is well documented in many countries and control and elimination strategies have long been implemented in the developed world by a policy based on systematic slaughter of infected animals, meat inspection in abattoirs and milk pasteurization. However, BTB is still widely distributed and largely uncontrolled in developing countries, which are unable to support the costs of test-and-slaughter policies and where BTB is often neglected and viewed as secondary to the huge problem posed by the more readily transmissible human disease caused by *M. tuberculosis* (4).

Very little systematic data on the extent of BTB either as a veterinary or as a human health problem are available in Ethiopia. BTB is endemic in cattle in Ethiopia; the disease has been reported from different regions (5, 6). However, the prevalence of the disease is not well established on a national level and large pastoralist communities in the country have been omitted. Over 80% of the Ethiopian population is rural and live in close contact with cattle in areas where BTB is not controlled at all. These communities are exposed to direct contact with their animals and consume unpasteurized milk and milk products as well as raw meat. In addition of being a zoonotic threat, BTB is also an economical and financial burden to society but its cost has rarely been assessed (10) and is largely unknown for Africa.

The aims of this study are to compile large scale and long term epidemiological field data on BTB to create a dynamic animal-human transmission model, which is a prerequisite to simulate intervention strategies to control the disease in Ethiopia. In addition, the impact of BTB is assessed in terms of public and private costs in both the livestock and human health sectors. Field data collection is still ongoing. We present here the approach to estimate the cost of BTB to society and potential benefits of interventions.

Method
A cattle-human compartmental transmission model will be developed to simulate the transmission of BTB between animals (wildlife & cattle) and humans (fig 1). Differential equations are formulated for each compartment and parameters estimated with field data. The parameters consist of demographic data (birth and death rates) and disease transmission data (contact rate, risk factors). BTB transmission can then be simulated as well as the effect of different intervention strategies.

Field data are collected over a period of four years from eight different geographical sites in Ethiopia: the Northern highlands (Gondar, Woldia), the Rift Valley (Butajira), the West (Gimbi), the South (Jinka/Hamer), the South-East (Bale Mountains) and Sellale. The following data are collected: field prevalence of BTB in cattle (intradermal PPD testing), abattoir prevalence of

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BTB, prevalence of BTB in humans, productivity parameters in cattle, cost of animal and animal products (regional, seasonal and annual variation), cost of TB in humans, risk factors of disease transmission and socio-anthropological parameters.

**Figure 1: Adapted model framework for joint human-animal BTB transmission in Ethiopia**

Demographic data (birth and mortality rate) in both humans and cattle are obtained from national statistics. In addition, cattle demographic data are collected from a four year productivity study, which follows 700 cattle in 21 farms, as well as from a herd structure analysis carried out in the sites where cattle PPD is performed.

The burden of disease will be assessed for the livestock sector using BTB prevalence found in the field and in the abattoirs as well as from the impact on their productivity. The burden for the public health sector will be assessed in terms of prevalence of disease in humans, cost of the disease and DALY. Data on cost of the disease will be collected directly in hospitals and health centers as well as through a patient based household survey. Data includes out and in-patient costs, therapy costs, loss of income and coping costs.

Benefits of an intervention will be computed for three different sectors:
1. The agricultural sector: the benefit resulting from the avoided losses in animals and animal products.
2. The public health sector: the benefit resulting from the avoided costs to the public health sector.
3. Private households with patients suffering from TB: the benefit resulting from (i) avoiding payment for treatment, (ii) income loss (= opportunity costs), and (iii) coping costs.

The sum of all three benefits will be considered as a benefit for the society as a whole.

**Discussion**

The disease has been shown in many countries to be an economical and financial burden to society due to economic losses: loss of productivity of infected animals (e.g. reduced milk yields and meat production), animal market restrictions, human health costs etc.

In Argentina, the annual loss due to BTB is approximately US$63 million (4). The socio-economic impact of BTB to the agriculture and health sector in Turkey has been estimated at between 15 and 59 million US$ per year (8). Even in some industrialized countries, where BTB has been eradicated by expensive schemes for control, eradication and compensation for farmers, the disease still has a major economic impact, mainly due to the existence of a permanent wildlife reservoir that reduces the efficiency of control strategies. In the UK, where badger and other wildlife such as deer remain an important source of infection for livestock, approximately £100 million is spent annually in efforts to control the disease. In Africa, the economic losses associated with livestock infected with BTB have not been examined sufficiently or have not been studied at all (9). Since agriculture remains the backbone of many...
African economies, there is an urgent need to control BTB (9). However, before introducing any control and eradication program in a country, profitability of control efforts have to be assessed (cost-benefit analysis of interventions).

Many zoonoses can only be eliminated if the disease is controlled in the animal reservoirs (10). A recent study on brucellosis in Mongolia has shown that mass vaccination of animals to reduce human brucellosis was a profitable intervention for the public health and agricultural sector, if the benefits of the livestock sector are added and the costs shared between the public health and the agricultural sector (11). A similar approach will be chosen for the economical analysis of the impact of BTB in Ethiopia. Disease transmission models provide frameworks to simulate change in prevalence and disease transmission with and without interventions such as test and slaughter or vaccination (10). The disease outcome in animals and humans are needed for dynamic socio-economic assessment of different intervention strategies. Economic analysis of an intervention to control BTB should include the impact on human health costs and the impact on livestock production (12).

BTB presents a serious zoonotic threat, since the disease is prevalent in cattle. Tadelle (1988) found that in Eastern Shoa (central Ethiopia) local breeds had much lower prevalence rate (5.6%) than exotic breeds (Holstein, 86.4%) (7). In high density herds maintained under intensive farming conditions, BTB prevalence was found as high as 50% in Holstein cattle at the Holetta National Insemination Centre (personal communication 2007). The disease burden is difficult to assess accurately since the intraderal test prevalence in cattle might not reflect the clinical stage of the disease (e.g. anergy in advanced stage of BTB; false positive and false negative reactions of the test) and might differ from cattle breed to cattle breed (different breed susceptibility of the intradermal test) and might differ from cattle breed to cattle breed (different breed susceptibility). The disease transmission with and without interventions such as test and slaughter or vaccination (10). The disease outcome in animals and humans are needed for dynamic socio-economic assessment of different intervention strategies. Economic analysis of an intervention to control BTB should include the impact on human health costs and the impact on livestock production (12).

Another difficulty faced by the current research is the low rate of \( M. \) \textit{bovis} detection in human lymphadenitis cases. The reason for this low detection rate is still largely unknown (e.g. low prevalence of \( M. \) \textit{bovis} in humans, sampling and/or laboratory technique) but it might affect the assessment of BTB cost to the public health sector. Alternatively, this cost can be assessed using data collected on patients with \( M. \) \textit{tuberculosis} and then extrapolated for the impact of BTB.

Collection of detailed epidemiological data on BTB on a national level in Ethiopia over a large period of time is therefore a prerequisite before starting any control program within the country. The study of BTB requires a trans-sectoral approach since the disease has a complex epidemiology (animal-human-ecosystem) and affects different sectors of a country (public health, livestock, wildlife, ecology, economy and trade, tourism etc.).

The exact epidemiology of BTB is still largely unknown in Ethiopia, which is a country of extreme diversity (e.g. geography, ecosystem, culture and tradition, cattle breeds probably with different susceptibility to disease) and results from other African countries might not be applicable or replicated here.

Finally, from a cost and logistic point of view, it should also be investigated if the control of BTB in Ethiopia could be linked with those of other zoonosis (e.g. Brucellosis) existing in the country.

**Acknowledgments**

We would like to thank the Wellcome Trust (UK) for funding this study and the Armauer Hansen Research Institute (AHRI) for the logistical support.

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Summary of Working Group Discussions

Four working groups discussed key aspects of the zoonotic problem in Ethiopia. The recommendations of each group were discussed further at the plenary meeting. A summary of each of the discussions is presented below.

**Working Group A: Setting bovine TB in the animal health context in Ethiopia: Animal health and husbandry practices**

*Discussion leaders:* Getachew Abebe, Rudovick Kazwala

*Rapporteur:* Rea Tschopp

**Major threats to the health of livestock and wildlife in Ethiopia**

Ethiopian livestock faces many infectious diseases, most of them being listed by the OIE (Organisation Internationale des Epizooties). The list of diseases is very exhaustive. To name some of them: Foot and Mouth disease (FMD), Chronic Bovine Pleurpneumonia (CBPP), Trypanosomiasis, Brucellosis, Anthrax etc. They are summarized into four categories depending on their degree of threat (livestock health/public health) and economic importance on a national and/or international level. Ethiopia has a monthly reporting system according to OIE formats; reports that are delivered to the federal Ministry of animal development. Diseases often do not occur uniformly throughout the country but are more often associated with different agro-ecological zones, e.g. pastoral areas of the lowlands, highlands.

However, diseases are not the only threat to the health of livestock and wildlife. Poor husbandry practice, inadequate (qualitative and quantitative) nutrition due to overgrazing, recurrent droughts and lack of feed supplementation, lack of proper breeding system, absence of animal research, poor to inexistent veterinary service, poor market access and legal framework are all major threats to livestock health.

Wildlife in Ethiopia faces other challenges and has other priorities regarding its threat to health than livestock. The steady population growth leads to an increased need of grazing land for their cattle (over 80% of the Ethiopian population is rural and linked to livestock). Deforestation and encroaching on habitat leads to habitat loss for wildlife, and forces wildlife to move to other areas. Besides diseases (e.g. rabies, anthrax), also climatic factors are a threat to the health of wildlife (e.g. drought, flooding) as well as lack of legal actions against poachers and, last but not least, the threat of genetic loss (e.g. interbreeding wildlife/domestic animals as described for the Ethiopian wolf).

| Table 1: Ranking by the participants of the 5 majors threats to livestock and wildlife by decreased importance |
|---|---|---|
| Ranking of threats | Livestock | wildlife |
| 1 | Diseases | Human population growth |
| 2 | Feed/nutrition | Deforestation/overgrazing |
| 3 | Husbandry practices | Poaching |
| 4 | Breeds/breeding policy | Diseases |
| 5 | Animal health | Interbreeding |

**Impact of bovine TB on animal health in Ethiopia**

Bovine TB belongs to List B of the OIE and has been frequently reported in Ethiopia from small scale studies. However, the magnitude of the impact of BTB on animal health in Ethiopia is largely unknown. Since the disease is known to affect a large host range (cattle, goat, sheep, camel, humans, wildlife) it makes its assessment difficult. Pathological lesions are seen in abattoirs during meat inspection, and the disease has been described in cattle and in wildlife. Up to 50% of dairy farms in Addis Ababa were shown to be positive. *M. bovis* was also isolated from humans. BTB is present but what is its impact? From the nature of the disease we can assume that it will have an impact on livestock production (weight, milk yield, fertility). BTB is a chronic debilitating disease, therefore affecting directly animal health and indirectly leading to loss of power traction, loss of carcasses and abattoir profits. The presence of BTB in diary farms suggests that BTB might have an important impact on the dairy product market and development of a dairy milk industry (cross breeding program), not to forget its zoonotic importance.

**Comparison of the pattern of BTB with that described in other Sub-Saharan countries**

There is a crucial lack of information regarding BTB in Ethiopia. Some studies have been conducted but mostly focusing on the highlands (dairy farms located in Shoa region). It is therefore difficult to describe a geographical
association of BTB with particular geographical areas. Detailed information is missing and a lot of areas especially the pastoral lowland areas are not covered. The situation of BTB on a nationwide level is largely unknown.

Existing Ethiopian publications show similarities with other African countries: in cattle, the individual prevalence of BTB has been shown to be low whereas herd prevalence is high. BTB is more often found in intensive dairy farming in urban and peri-urban setting (association of BTB with particular farming practice). There is an association with wildlife at the interfaces.

One major issue seems to be the laboratory isolation of *M. bovis* in humans, which is consistent with the problem encountered in other African countries. Only in Tanzania and Uganda (and Ethiopia) so far, has *M. bovis* been successfully isolated from humans. Considering the prevalence described in cattle and the existence of risk factors of disease transmission to humans, the prevalence in humans should be expected much higher. The question arises whether there is a problem of detection in the laboratories.

**Cost effective control of BTB in the context of developing countries**

Collection of detailed epidemiological data on BTB in Ethiopia is a prerequisite before starting any large scale control program. In developed countries, BTB has been eradicated by means of costly test and slaughter programs. This approach is unthinkable in developing countries, who should opt for different, country adapted control strategies. An option would be to integrate BTB control with the control of other zoonotic diseases in order to cut costs.

From an animal health perspective, it is most probably not cost effective to launch a control/eradication program at this stage (e.g. cattle vaccination, test and slaughter, farmer compensation). However, measures can be readily taken from a human health perspective in the absence of data on animal BTB: pasteurization of milk, strengthening of meat inspection, public health education and strengthening awareness about the transmission of BTB (handling of animals, consumption of raw animal products), BCG vaccination of people.

**Building capacity**

A huge capacity building is needed in Ethiopia (technology, human resources, education etc.). There is not enough research done on BTB and baseline information and knowledge on BTB are lacking (national prevalence, impact on productivity, geographical areas affected etc.).

The country lacks institutional capacity (research facilities), diagnostic capacity, surveillance capacity, and networking in the advocacy. Abattoir surveys and meat inspection should be strengthened. A priority is the education of communities, training of professionals and increase extension services. Existing national structures should be used and linkage between institutions dealing with BTB promoted. Also the animal service should become a single entity and not split under many divisions, which make it difficult to solve a problem.

**Conclusions & recommendations**

Bovine TB is prevalent and is probably one of the major zoonotic diseases in Ethiopia, however the magnitude of its impact is largely unknown. Further studies on a national level should be carried out to investigate geographical areas affected, especially in pastoral areas, field prevalence and economical impact of the disease. Baseline epidemiological information is needed for assessing the most cost effective intervention for Ethiopia. Education, and working capacity has to be strengthened and existing national structures should be used. Tackling a disease like BTB affecting both livestock and humans requires joint activities between the Ministry of Health and Ministry of Agriculture. Only then can a control strategy be developed.

If Ethiopia wants to take part in the international market, it has to make use of its own resources, its man power and great numbers of cattle herds. The husbandry system has to be assessed from a sustainability perspective (ecological sustainability) as well as from an economic perspective (economical profitability). The value and importance of indigenous breeds (part of the Ethiopian heritage) has also to be taken into consideration when compared with imported exotic breeds to increase productivity.

Human TB caused by *M. tuberculosis* is declared as emergency by WHO, unlike TB caused by *M. bovis*. If more information is gathered for BTB and its zoonotic implication proved by hard field data then there would be a possibility to approach the Global Fund for BTB funding.
Working Group B – Zoonoses: the role of bovine TB as a zoonotic disease in Ethiopia and identification of other important zoonoses

Discussion leaders: Tafesse Mesfin, Glyn Hewinson
Rapporteur: Martin Vordermeier

Summary of outcome of discussion - The following were highlighted:

• Main problem: lack of data or data capture
• Importance of interfacing human and veterinary medicine:
• Need for a champion inter-ministerial department to take ‘ownership’ of zoonoses including BTB which will generate logistical framework etc.
• Overriding priority to assess importance of BTB (and other zoonoses) is to establish dimension of problem: = research priority/project
• Specifically research should address:
  o Epidemiology of BTB in cattle across country (focus also on milk, meat, plus TT-ST prevalence)
  o Use these data to concentrate surveillance/epidemiology in humans to assess BTB in humans, also link to behavioural practices
  o Also human TB in cattle epidemiological studies needed
  o Need for epidemiology/surveillance also for other zoonoses

• Aim to allow knowledge-based policy decisions
• Centralised Data Capture : Statement Made - Much Info Is Out There, But Is Not Accessible Or Centrally Recorded (Eg Master Theses, Or Data From Regional Offices, Abattoir Records)
• Role of abattoir/meat inspection in surveillance and also to control zoonoses (not just BTB): standards, QC, properly resourced
• Simple, cheap diagnostic tool and centralised surveillance labs
  o Transmission routes/pasteurisation
  o Likely routes: milk, meat, aerosol
• Pasteurisation: very limited (only two plants nationwide). Lack of public awareness as well.
• Need survey to assess % shedders into milk
• Other zoonoses (top 5 only):
  o Rabies
  o Brucellosis
  o Toxoplasmosis
  o Salmonella
  o Anthrax
  o BTB (mainly for surveillance)

• A simple diagnostic test for diagnosis and surveillance would be useful: like a lateral flow system
Working Group C – Community Issues and policy: how to link research to policy more effectively in the context of health.

Discussion Leaders: Eshetu Lemma, Dagninet Yimenu
Rappoteur: Caroline Aylott

Working group C contained representatives from social science, biomedical science, medicine, veterinary medicine and from the Ministry of Agriculture and Rural Development.

It is important to note, however, that the working group did not contain representatives from the farming community or members of the general public. The group was therefore unable to represent the community as a whole.

- **Does bovine TB have a preferential impact on particular social groups?**
  The group considered people that have direct contact with cattle are affected:
  - Farmers – intensive & extensive.
  - Pastoralists and communities that live with their animals (who have a lot to lose through morbidity and loss of productivity).
  - Farm workers.
  - Abattoir workers.
  - Livestock traders and people associated with the sale of meat and dairy products.
  - Women and children – who are associated with milking and transporting of milk.
  - Immunosuppressed people were seen to more at risk as well as sick people.

- **Do farmers recognize bovine TB as a disease?**
  Here we had to distinguish the difference between recognition of disease and bovine TB.
  Farmers recognize signs and symptoms of disease, such as swelling of the neck, however, this is not associated with symptoms of bovine TB as this disease is not known.
  The fact the disease is chronic is thought to contribute to this.
  There is no awareness of how bovine TB is transmitted.
  It was noted that TB patients are generally discriminated against.

- **Does bovine TB have a name in particular languages?**
  Bovine TB as a disease is generally not recognized, see above.

- **Are different farming practices associated with differences in the risk of cattle to human transmission?**
  The following are thought to be associated with risk:
  - Intensive and semi-intensive versus extensive.
  - Traditional farming where people live with their animals.
  - Farming exotic breeds.

- **Does the extra milk yield from exotic cattle outweigh any risks associated with enhanced susceptibility to bovine TB?**
  This question caused some discussion and was thought to be an extremely complex issue.
  Even though farmers are not aware of TB per se, they are aware that exotic breeds yield more milk and make a better workforce. They are also aware, however, that exotic breeds require more feed, need greater care and medication. This indigenous knowledge is passed on and farmers make calculated risks.
  As a group we were unable to decide if extra milk yield outweighs any risks associated with enhanced susceptibility to bovine TB. We were also unable to speak from a farmer’s point of view.

- **Would vaccination be viewed as a socially acceptable intervention for bovine TB?**
  Yes
  Farmers love their cattle and would want them to be protected.
  Cost is a huge issue though and complicates this issue. An example was provided of rift valley fever vaccine, which used to be provided free of charge. With the introduction of partial cost recovery uptake has decreased.
  There is also an issue with farmers failing to recognise bovine TB and the fact it is a chronic illness. In many cases, farmers have to take their cattle many miles to be vaccinated and they are less inclined to do this if their animals are not thought to be diseased.
• Would establishment of elite TB-free herds in Ethiopia provide an important boost to international trade? This point also caused much discussion. From a trade/economic point of view the answer is yes, however, the ethics of providing a super herd to sell abroad needs to be discussed.

• Would you consider a community-based participatory approach in identifying constraints and solutions for bovine TB control important? Yes

• What socio-cultural practices and food habits affect bovine TB transmission that need special attention? Just cattle to human transmission was considered.
  1) Awareness.
  2) Raw milk/meat consumption.
  3) Living conditions.

• What capacity gap is there for ensuring community engagement in bovine TB control? There are gaps in capacity at all levels of communication and engagement. There is a huge need for raising awareness and education. Community engagement needs to consider, who how, where, when and why? Engagement needs to be targeted towards people needs.

• What is the linkage between stakeholders? We need to organise properly formed networks and linkages. To be sustainable, networks should be instigated at the institutional level; it was suggested that the MoH and MoARD should be involved in this. It was recognised that networks should be formed at all levels. The group made a strong recommendation that the MoH and MoARD should come together on zoonosis.
Working Group D - Diagnostic capacity and surveillance of zoonotic diseases in Ethiopia

Discussion leaders: Getachew Tilahun, Ahmed Bedru
Rapporteur: Stefan Berg

The group discussed surveillance and capacities established for zoonotic diseases in 1) humans and 2) animals.

What is the status of diagnostic and surveillance capacity in Ethiopia for zoonotic diseases?
(The workgroup made an inventory of the different institutions responsible for the zoonotic diseases in both animals and humans):

In Ethiopia, there is a task force in place with representatives from Ministry of Health and Ministry of Agriculture. This task force is currently only dealing with a few diseases, but it should be used as a spring point to cover all diseases that are threatening to human and animal health in Ethiopia.

“The status” regarding humans:
The Ethiopian Health and Nutrition Research Institute (EHNRI) is the reference centre for several of the zoonotic diseases but additional institutions, like Armauer Hansen Research Institute (AHRI), are partially complementing EHNRI in its goals. The country has several regional laboratories and hospitals that are dealing with zoonotic diseases, but the level of diagnosis differs, such as in serological diagnosis of patients. Health centres represent the capacities on the local level but they don't have the capacity in diagnosing zoonotic diseases.

“The status” regarding animals:
The National Animal Health and Disease Investigation Centre is responsible for all animal diseases, inclusive zoonotic diseases. The National Veterinary Institute is an additional institution that is responsible for vaccine production, but also diagnostics and surveillance of animal diseases (including those of zoonotic importance). Under these two federal institutions serve 12 laboratories on the regional level. Additional institutions, like AHRI, carry limited capacities regarding diagnostics and surveillance of zoonotic diseases in animals.

The laboratories "set up" for zoonotic diseases:
Institutions exist at national and regional levels for most diseases, but only a few are involved in the majority of the zoonotic diseases in Ethiopia. One such disease is Brucellosis, which is a very serious problem, but there are no facilities for its diagnosis in Ethiopia. Institutions at federal level are better equipped than labs at regional level. There are a very few laboratories where disease organisms (e.g. bovine TB) can be cultured and isolated for strain characterization and for research purposes Such as EHNRI, AHRI and Aklilu Lemma Institute of Pathobiology. The majority of the regional laboratories have only a basic setup (building facilities, staff and basic equipments). Among the regional hospitals there are no hospitals that have a setup for TB diagnostics for culture and sensitivity. In a comparison between laboratories and hospitals, the regional hospitals have less capacity than the regional laboratories in case of diagnostics and culturing of zoonotic disease, and with high turnover of staff. The local health centres have less capacity than the regional hospitals. Some regions do not have a laboratory at all. In Gambella, there is no regional laboratory and the four health centres are not doing TB diagnostics. Patient samples are instead often sent to the hospital. However, the hospital in the Gambella region is not properly set up for doing TB diagnostics. Therefore, quality assured diagnosis of TB is required.

What are the needs for improved diagnostic and surveillance capacity?
Federal institutions: Capacity and capability have to be established in reference laboratories to the extent that the current state-of-the art can be introduced or strengthened. This can be done by reinforcement in equipment and trained staff.

Regional laboratories: The basic level has to be raised so all regional laboratories have the capability for culturing and sensitivity testing of zoonotic diseases. For that purpose, training of staff, complementation of relevant equipment and consumables (test kits etc.) are essential, as is set up of standard operating procedures for diagnostic tests. Set up does, to some extent, exist for diagnosis of zoonotic diseases, but it needs to be properly used. In many laboratories around the country, the situation can be dramatically improved just by providing basic and relatively inexpensive equipment.
and consumables (like microscopes and reagents). Some of the diagnostic tools are very simple, e.g. those for gram-negative staining. Today, many clinicians have low confidence in the diagnostic results produced at the laboratories, due to the poor level of supplies. A higher level of diagnosis would lead to higher frequency of reliable results, an important factor in establishing confident relationships between clinicians and the testing laboratories.

As a minimum improvement at the hospitals, serological test have to be introduced or strengthened. The local health centres would largely benefit from training their staff and make sure that test kits are in place for diagnosis of diseases, at least for those diseases where simple tests are available.

At all levels, there are policy issues and bureaucracy hinders even the basic level of functioning. In addition, lack of manpower, staff turnover, and delays in supplies generate problems.

**How can better collaboration be achieved between human and animal health laboratories and surveillance services? Are there experiences that can be shared?**

It has to be a network and collaboration system in place, initially at the national level and such network should later expand to include also the regional level. For that purpose, an information survey could find where the gaps are. Regarding the zoonotic diseases, it is also very important that networks are established between workers in the veterinary and human health systems - on both regional and local levels.

A public health laboratory system is to be established, where it is to be determined what diseases can be diagnosed and at what level. Zoonoses could also be included in these diagnoses. The minimum acceptable level of service must be established, and the minimal capability of diagnosis should be available across the board. Facilities do exist, but plenty of things are not being exploited. These facilities could be set up to do quality routine diagnostics, but needs more support.

*Organisation and management:* regions should coordinate services, so that capacity is not duplicated and agreements should be reached about requirements. When it comes to surveillances of animal and human diseases, there are set ups for many of those but they have to be substantially improved.

(Compiled by Abraham Aseffa)