Bovine tuberculosis in Ethiopian local cattle and wildlife: Epidemiology, economics and ecosystems

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To my parents

Table of contents

1. Acknowledgments

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2. Summary

Ethiopia has recently been focusing on intensive dairy cattle farming in order to supply the growing demand for milk. For this purpose Holstein Frisians have been imported, bred and distributed in farms especially around Addis Ababa. However, Holstein (*Bos taurus*) although giving more milk than traditional zebus (*Bos indicus*) seem to be more susceptible to bovine TB (BTB). Ethiopia ranks worldwide 8th in the number of newly diagnosed TB cases, and the incidence of extra pulmonary TB was shown to be high. Therefore, the following research questions have been raised: What is the prevalence of BTB in cattle? What is the contribution of *M. bovis* in the national TB prevalence? Which strains of *M. bovis* are present in cattle and humans? A consortium funded by the Wellcome Trust (UK) and involving institutions from the UK, Switzerland, Ethiopia, Ireland and Kenya was established in 2005 study BTB in Ethiopia and to address these questions. The overall ultimate goal of the project is to measure the cost of BTB by assessing its impact on the livestock and public health sector and to suggest the most profitable intervention strategies. This thesis contributes to the overall BTB project by providing baseline epidemiological and economical data needed for the development of a SIR animal-human transmission model, and for the estimation of the economical impact of BTB in the livestock sector in Ethiopia.

We assessed field prevalence of BTB using the comparative intradermal test (CIDT) in 6194 cattle in 5 different Woredas (districts) from Amhara, Oromia, and SNNPR regions. Four of our study sites were located in the Ethiopian Highlands and were characterized by extensive mixed crop-livestock farming with predominantly local zebu breeds, whereas the last study site was a pastoralist area in the lowlands of south Omo (SNNPR). We performed a repeated cross-sectional study in cattle in 3 of the Woredas, over 3 years. In comparison to Central Ethiopia, which is characterized by the presence of a higher number of exotic breeds and more intensive farming practice, apparent prevalence of BTB in our study sites was very low (when using the official OIE definition for positive reaction), with a minimum of 0% in the Bale Mountains and a maximum of 1.3% in

Bako-Gazer and South Omo. We discussed the use of different cut-offs for the interpretation of the skin test results.
In addition we interviewed in those study sites 450 farmers whose cattle were tested for BTB in order to assess the risk for skin test positivity in cattle and TB diagnosis in humans. Purchase of cattle and the presence of livestock other than cattle were a significant risk factor for skin positivity in cattle. None of the classical risk factors such as consumption of raw animal products, and close contact with animals were significantly linked to the presence of human TB cases in households.

Since wildlife has been shown to be a potential reservoir for BTB in other countries we started the first BTB survey in Ethiopian wildlife, in close collaboration with the Ethiopian Wildlife Department and professional and recreational hunters. We sampled specimens from 133 animals and performed serology in order to try to validate the rapid test (RT), as well as culture (gold standard) followed by molecular typing of lymph node samples. So far, no *M. bovis* were isolated from the 28 mammal species sampled (but molecular analysis is still pending for a number of samples). However, 23% of the tested animals, including flagship endemic rare species were sero-positive. Since we also sero-tested live animals, culture could not be performed nor diagnosis confirmed in these animals. Nearly half of the culture of samples yielded environmental Mycobacteria, their role has still to be assessed.

In an additional investigation, 684 farmers were included in two independent surveys in our study sites. This investigation highlighted the delicate balance between livestock, cereal cropping and natural resources, the trend towards unsustainable use of natural resources, fuelling a decrease in grazing land, human conflicts, and encroachments on wildlife habitats and the need of a more holistic approach to secure future sustainability of natural resources. Herd structure analysis showed that 52% of the total animals were males, among them ¼ were oxen. Since oxen are intricately associated with agriculture (e.g. ploughing, harvesting, threshing), farmers need to maintain a minimum herd size in order to secure at least 2 draft animals, at all times. A disease such as BTB is therefore likely to have a major impact on draft animals rather than on milk production in rural

areas. Diseased draft animals are very likely to work less in the fields, leading to decreased cropping yields, thus contributing to poverty and famine.
Some studies assessing the economical impact of BTB on the livestock sector were started during this PhD but will go beyond the timeframe of the thesis. Economical analysis of the impact of BTB to the society is not part of this thesis but the approach will be discussed. We assessed the baseline productivity of cattle kept under traditional extensive husbandry practice by following 21 farms (700 animals) over a period of four years and keeping for each farm a herd book on productivity parameters (weight, milk, fertility, entry, exit).In addition a long term study was started in collaboration with other members of the consortium, to assess the impact of *M. bovis* on live animal and carcass weight in six abattoirs.

Finally the data collected during the thesis suggest that the final analysis of BTB (economical impact, transmission model, and intervention strategies) should be performed on two distinct levels: 1) the urban and peri-urban level characterized by intensive dairy farms, high numbers of exotic breeds and their cross breeds, high milk production but also high BTB prevalence and 2) the rural level characterized by extensive farming, local zebu breeds, low BTB prevalence and probable impact of the disease on draft power rather than on milk.

3. Zusammenfassung

Äthiopien hat sich seit einiger Zeit auf intensive Milchkuhhaltung konzentriert, um der steigenden Nachfrage nach Milch gerecht zu werden. Es wurden Holsteinrinder importiert, gezüchtet und an Bauernhöfe verteilt, vor allem in der Umgebung von Addis Ababa. Die Hosteinkühe (*Bos taurus*) geben zwar mehr Milch als traditionellen Zebu Rassen (*Bos indicus*), scheinen aber empfänglicher für Rindertuberkulose zu sein. Äthiopien kommt weltweit an achter Stelle bezüglich neu diagnostizierten Tuberkulosefällen (TB) und die Inzidenz von extrapulmonärer TB ist sehr hoch. Aus diesem Grund wurden folgende wissenschaftliche Fragen gestellt: wie hoch ist die Prävalenz von Rindertuberkulose beim Rindvieh? Welcher Anteil hat und welche Rolle spiel *Mycobacterium bovis* in der Nationalen TB Prävalenz? Welche *M. bovis* Stämme existieren beim Rindvieh und beim Mensch? Ein Konsortium von Institutionen aus der Schweiz, UK, Äthiopien, Irland und Kenia, finanziert vom Wellcome Trust (UK), wurde 2005 aufgestellt mit dem Ziel die Rindertuberkulose in Äthiopien zu untersuchen und obige Forschungsfragen zu beantworten. Das Projekt hat das Ziel, die Kosten, die durch die Rindertuberkulose verursacht werden zu erfassen, indem man die Bedeutung der Krankheit sowohl im Nutztiersektor als auch im Gesundheitswesen berechnet, um schliesslich kostengünstige Interventionsstrategien vorschlagen zu können. Diese Dissertation trägt zum gesamten Projekt bei, indem sie epidemiologische und wirtschaftliche Daten generiert, die nötig sind um nachfolgend ein Tier-Menschkrankheits-Übertragungsmodel zu entwickeln, und um die wirtschaftliche Auswirkung von Rindertuberkulose im Nutztiersektor in Äthiopien zu beurteilen.

Die Prävalenz von Rindertuberkulose wurde bei 6194 Rindern mit dem komparativen intradermalen Tuberkulintest (CIT) getestet. Die Rinder stammten aus 5 verschiedenen Woredas (Distrikte) in der Amhara, Oromia und Southern Nations, Nationalities, and People's Region (SNNPR) Regionen. Vier unserer Studienorte befanden sich im Äthiopischen Hochland, wo Bauern sowohl extensive Rindviehhaltung als auch Ackerbau betrieben und wo vor allem lokale Zebu Rassen gehalten wurden. Unser letzter

Studien Ort befand sich im südlichen Omo (SNNPR), ein Gebiet, das vor allem durch pastorale Hamer und Karo Völker bewohnt war.
In 3 der Woredas wurde eine Querschnittstudie mit dem CIT über 3 Jahre durchgeführt. Verglichen mit Zentraläthiopien, wo sich eine hohe Anzahl an exotischen Rindvieh Rassen befand, die oft intensiv gehalten werden, haben wir in unserer Studie eine sehr niedrige apparente Rindertuberkulose Prävalenz gefunden, mit einem Minimum von 0% in den Bale Bergen und einem Maximum von 1.3% in Bako-Gazer und Süd Omo (Benutzung der offiziellen OIE Definition für Positivität). Die Interpretation der Resultate bei verschiedenen cut-offs wird auch diskutiert.

Zusätzlich wurden 450 Bauern interviewet, deren Tiere für Rindertuberkulose getestet worden sind, um die Risikofaktoren zu erfassen, die zu einem positiven CIT beim Rind und zu TB Diagnose beim Mensch führen. Der Zukauf von Rindern und die Präsenz von anderen Nutztieren waren signifikante Risikofaktoren für einen positiven CIT beim Rindvieh. Es wurde kein statischer Zusammenhang gefunden zwischen den möglichen Risikofaktoren wie beispielsweise das Einnehmen von rohen tierischen Produkten und dem Vorkommen von TB bei den Leuten.
Auch in anderen Ländern ist beschrieben worden, dass Wildtiere als Reservoir für Rindertuberkulose agieren. Wir haben die erste TB Studie in Äthiopischen Wildtieren durchgeführt in enger Zusammenarbeit mit dem Äthiopischen Wildtier Departement, sowie beruflichen und Hobbyjägern. Es wurden Proben von 133 Wildtieren gesammelt. Wir haben den serologischen „Rapid Test“ validiert und Bakterienkulturen von Lymphknoten (Goldstandard) gemacht, welche dann weiter mit Molekularmethoden untersucht worden sind. Bis jetzt wurden aus den 28 Säugetier Proben keine *M. bovis* isoliert, aber die Analysen sind noch am laufen. Hingegen, waren 23% der serologisch getesteten Tiere positiv, davon auch seltene endemische Tierarten. Da auch lebende Tiere serologisch getestet worden sind, konnte für Diese keine Kulturen angesetzt werden, und somit die endgültige Diagnose nicht gestellt werden. Wir isolierten Umweltmykobakterien in fast der Hälfte der Kulturen, und deren Rolle ist noch weitgehend unbekannt.

In einer weiteren Studie wurden 684 Bauern aus unseren Studiengebieten in zwei unabhängigen Befragungsrunden eingeschlossen. Diese Studie zeigte das sensible Zusammenspiel zwischen Viehhaltung, Ackerbau und natürlichen Ressourcen. Es zeigte die unvermeidbare Intensivierung an der Schnittstelle Mensch-Vieh-Wildtier, dass zu unnachhaltiger Nutzung von natürlichen Ressourcen führt, zu weiterer Verminderung von Weiden, zu Konflikten und zu Übergriffen auf Wildtierhabitate. Eine Herdenstrukturanalyse hat aufgezeigt, dass 52% der Herden aus männlichen Tieren bestanden davon ein Viertel Ochsen. Ochsen sind eng mit der Landwirtschaft verbunden (Pflügen, Treschen, Ernten, Transport). Die Bauern müssen eine Mindestzahl an Rindern halten um ständig mindestens 2 Ochsen zu haben. Eine Krankheit wie die Rindertuberkulose kann demzufolge schwere Folgen auf die tierische Zugkraft haben in ländlichen Gebieten. Kranke Tiere vermögen nicht mehr die Felder zu bearbeiten, was zu einer verminderten Ernte, Armut und Hungersnot führen kann.

Zwei andere Studien haben die wirtschaftliche Auswirkung von Rindertuberkulose auf das Nutzvieh studiert. Diese Studien sind noch nicht abgeschlossen wegen der benötigten langen Datensammelzeit, werden aber während dem Jahr nach der Dissertation vollständig abgeschlossen. Der Ansatz und Methode für wirtschaftliche Analysen werden beschrieben. Wir haben die Grundlage Produktivität von Rindvieh die unter traditionellem extensivem Systeme gehalten werden untersucht, indem wir 21 Bauernhöfe mit 700 Tieren über 4 Jahren verfolgten. Auf jedem Hof wurde ein Herdenbuch betrieben, in dem Produktivitätsparameter aufgeschrieben wurden (Gewicht, Fertilität, Milch, Neuerwerbungen und Abgänge). Zusätzlich wurde eine Langzeitstudie in 6 Schlachthöfen, in Zusammenarbeit mit einer anderen Gruppe des Konsortiums gestartet, um die Auswirkung von *M. bovis* auf das Schlacht- und Lebendgewicht zu untersuchen.

Abschliessend können die Schlussfolgerungen der Dissertation folgendermassen zusammengefasst werden. Die endgültigen Analysen über Rindertuberkulose in Äthiopien (wirtschaftliche Auswirkung, Übertragungsmodel und Interventionsstrategien) müssen auf zwei Ebenen untersucht werden: 1) Stadt und Umgebung mit einer intensivierten Milchviehhaltung mit hohen Anzahlen von exotischen Rassen und deren Kreuzungen. Diese Tiere haben eine höhere Rindertuberkuloseprävalenz als traditionelle Zeburassen , 2) ländliche Gebiete, wo man fast ausschliesslich traditionelle Zebu Rassen

extensiv gehalten werden, und niedrige Rindertuberkuloseprävalenzen aufweisen, aber die Auswirkung der Krankheit auf die tierische Arbeitskraft grosse Auswirkungen haben kann.

4. Résumé

L'Ethiopie s'est récemment embarquée dans un programme d'élevage intensif de bovins afin de répondre aux demandes grandissantes des villes en lait. Du bétail de race Holstein a été importé, élevé puis distribué dans les fermes notamment dans les environs d'Addis Abeba. Les vaches Holstein (*Bos taurus*), bien que fournissant plus de lait que les zébus traditionnels (*Bos indicus*) semblent par contre être plus susceptibles à la tuberculose bovine. L'Ethiopie termine au 8ème rang mondial dans le classement des cas de TB nouvellement diagnostiqués et l'incidence de la tuberculose extra-pulmonaire y est élevée. Cette situation a soulevé de nombreuses questions, notamment quelle est la prévalence de la tuberculose bovine dans le bétail? Quel rôle joue *M. bovis* dans la prévalence nationale de la tuberculose? Quelles souches de *M. bovis* trouve-t-on chez le bovin et chez l'homme? Un consortium financé par le Wellcome Trust (UK) a été mis sur pied en 2005 pour répondre à ces questions et pour étudier la tuberculose bovine en Ethiopie. Celui-ci comprend des institutions du Royaume-Uni, de la Suisse, de l'Ethiopie, de l'Irlande et du Kenya. Le but final du projet est de calculer le coût de la tuberculose bovine pour la société éthiopienne par l'évaluation de son impact sur le bétail ainsi que sur le service de santé public, afin de pouvoir proposer par la suite les stratégies d'intervention les plus profitables. Cette thèse contribue au projet car elle fournit des données épidémiologiques et économiques de base, nécessaires ultérieurement d'une part au développement d'un model de transmission entre les animaux et les hommes et d'autre part à l'estimation de l'impact économique de la tuberculose bovine sur l'élevage en Ethiopie.

Nous avons fait une étude transversale de tuberculinisation intradermale comparative, afin d'évaluer la prévalence de la tuberculose bovine chez 6194 bovins provenant de 5 Worédas (districts) différents se trouvant dans les régions Amhara, Oromia et Southern Nations, Nationalities, and People's Region (SNNPR). Quatre de ces régions étaient situées dans les Highlands Ethiopiens, caractérisés par une détention extensive du bétail comprenant principalement des races bovines locales) et d'une agriculture céréalière. La dernière était une région pastoraliste dans le sud de l'Omo (SNNPR). Nous avons répété l'étude sur 3 ans dans 3 de ces 5 régions. Nous y avons observé une prévalence faible

avec un minimum de 0% dans les montagnes Balé et un maximum de 1.3% à Bako-Gazer et Omo (en utilisant la définition du cut-off officiel de l'OIE), contrairement aux résultats trouvés en Ethiopie centrale, où l'on trouve un plus grand nombre de races exotiques (Holstein) tenues de manière plus intensive. Nous avons également discuté de l'interprétation du résultat du test intradermal en utilisant différent cut-off.
Nous avons interviewé dans ces sites 450 paysans dont les animaux ont été testés. Ceci afin d'évaluer les facteurs de risques expliquant une positivité du test intradermal chez le bovin et/ou une tuberculose confirmée chez les hommes. L'achat de bétail ainsi que la présence d'animaux d'élevage autres que le bétail ont été des facteurs statistiquement important menant à un test intradermal positif. La présence de cas de tuberculose humaine n'a pu être lié à aucun des facteurs à risque traditionnels connus, comme par exemple l'ingestion de produits animaliers crus ou encore un contact proche avec les animaux.

Le fait que les animaux sauvages puissent être un réservoir pour la tuberculose bovine, nous a incité à lancer la première étude sur la faune sauvage en Ethiopie, en collaboration avec l'"Ethiopian Wildlife Department" et des chasseurs. Nous avons échantillonné 133 animaux, fait une sérologie afin de valider le test rapide et avons également fait une culture cellulaire (standard or) suivi par un typage moléculaire des échantillons des ganglions. Aucune souche *M. bovis* n'a été isolé dans les échantillons provenant des 28 espèces de mammifères testés à ce jour (analyse moléculaire en cours). 23% des animaux testés sérologiquement ont été positifs, incluant également des espèces endémiques rares. Vu que nous avons aussi testé sérologiquement des animaux vivants, le diagnostique n'a pas toujours pu être confirmé par culture chez ces animaux. Des mycobactéries environnementales ont été isolées dans presque la moitié des cultures. Leur rôle doit encore être élucidé.

Dans une étude supplémentaire, 684 paysans ont été inclus dans deux rondes indépendantes d'interviews. Cette étude a montré l'équilibre délicat existant entre l'élevage, l'agriculture et les ressources naturelles ainsi qu'une tendance vers une utilisation non pérenne des ressources naturelles menant à une diminution des pâturages, des conflits ainsi qu'à une utilisation des habitats à faune sauvage. Une approche plus

globale est nécessaire, afin de sécuriser la pérennité des ressources naturelles. Le pourcentage de mâles dans le cheptel est élevé (52%), dont ¼ sont des boeufs. Vu que ces derniers sont étroitement liés à l'agriculture (labourage, moisson, battage), les paysans doivent garder un certain nombre d'animaux afin de d'assurer en permanence un minimum de 2 boeufs. Une maladie comme la tuberculose aurait, dans ces milieux ruraux, un plus grand impact sur ces animaux à traction que sur la production laitière. Ces animaux ne seraient plus capables de travailler dans les champs comme des animaux en bonne santé, ce qui diminuerait la production céréalière contribuant ainsi à la pauvreté et aux famines.

Certaines études visant à évaluer l'impact économique de la tuberculose ont commencé pendant ce PhD mais ne sont pas encore terminées. L'analyse économique de l'impact de la tuberculose ne fait pas partie de cette thèse mais l'approche analytique y est discutée. Nous avons évalué la productivité de base du bétail en détention extensive traditionnelle en faisant des visites bimensuelles dans 21 fermes (700 animaux) sur une période de 4 ans. Pour chaque ferme et animal, les paramètres de productivité ont été notés dans un "herd book". De plus, une étude à long terme a débuté en collaboration avec un autre groupe du consortium afin d'évaluer l'impact de *M. bovis* sur le poids des animaux vivants et le poids des carcasses dans 6 abattoirs.

En conclusion, les données collectées pendant cette thèse permettent de suggérer que l'analyse finale de la tuberculose bovine (impact économique, model de transmission et interventions) devrait se faire à deux niveaux différents: 1) le niveau urbain et périurbain caractérisé par une détention plus intensive de races exotiques, une production laitière plus élevée mais aussi une plus haute prévalence de la maladie et 2) le niveau rural caractérisé par une détention extensive de zébus locaux, une prévalence basse de tuberculose bovine et un impact probable de la maladie sur les animaux à tractions plutôt que sur la production laitière.

5. Summary in Amharic

ማጠቃለያ

ከቅርብ ጊዜ ወዲህ፣ ኢትዮጵያ ውያን የወተት ፍላጎት ለማሟላት፣ የወተት ከብቶች የተጠናከረ ርባታና ልማት ላይ ያተኮረች ትገኛለች። ለዚህም ተግባር ንዲውሉ ሆልስቲይን ፍሪሲያን ላሞች ወደሀገር ገብተው ለማብርና፣ በተለይም በአዲስ አበባ ዙሪያ ተሰራጭተዋል። ነገር ግን ሆልስቲይን (ቦስ ቶረስ) ከነባሮቹ ዜቡ (ቦስ ኢንዲከስ) የበለጠ ወተት ቢሰጡም ለከብት ቲቢ ግን የበለጠ ተጠቂ ናቸው። ኢትዮጵያ ባለም የቲቢ ህመም አዲስ የሚገኝባቸው ሰምች ቁጥር 8ኛ ደረጃ ላይ ስትሆን ከሳንባ ውጪ የሚገኝ ቲቢ ቁጥርም በከፍተኛ መጠን ይቲይባቲል። ከዚህ በመነሳት የሚከተሉት የምርምር ጥያቄዎች ተነስተዋል፣የከብት ቲቢ ስርጭት መጠን ምን ያህል ነው? በአገሪቷ ውስጥ ካለው ቲቢ ከከብት የሚተላለፍ ቲቢ ድርሻ ምን ያህል ነው? በሰውና ከብት ውስጥ የሚገኙት የማይኮባክቲርያም ቦቪስ ዝርያዎች ምን ዓይነት ናቸው? ንዚህን ጥያቄዎች ለመመለስ በ2005 ዓ.ም ን.ኤ.አ የዩናይትድ ኪንግደም፣ ስውዘርላንድ፣ ኢትዮጵያ፣ አየርላንድ ና ኬንያ የምርምር ተቋሞች በዋልካም ትረስት (ዩናይትድ ኪንግደም) የሚደገፍ አንድ ቡድን (ኮንሰርሽየም) አቋቁሙ። የዚህ ፕሮጀክት አጠቃላይ ግብ የከብት ቲቢ በንስሳትና በህብረተሰቡ ጤና ላይ የሚያደርሰውን ኪሳራ መጠን ለመገምገምና ይህን ለማዳን መደረግ የሚችሉ አትራፊ ስትራቴጅያዊ ተግባሮችን ለይቶ ተመራጭ የመፍትሄ ሳሰቦችን ለማቅረብ ነው። ይህ ቴሲስ መሠረቲዊ የኤፒዳሞሎጂና የኢኮኖሚ መረጃዎችን መነሻ ያደረገ የከብትና ሰው መሃል የበሽቲ መተላለፍ ኤስ አይ አር ሞዴል ተመርኩዞ በንስሳት ሀብት ዘርፍ ላይ የከብት ቲቢ የሚያደርሰውን የኢኮኖሚ ጉዳት መጠን ማምት በማውጣት ለአጠቃላይ የከብት ቲቢ ፕሮጀክት የበኩሉን አስተዋጽኦ ያደርጋል።

በ5 የተለያዩ የአማራ ፣ ኦሮሞና ደቡብ ክልል ወረዳዎች የሚገኙ 6194 ከብቶች ላይ የቆዳ መርፌ ምርምራ (ኮምፓራቲቭ ኢንትራደርማል ቴስት) በማድረግ የከብት ቲቢን የስርጭት መጠን መርምረናል ፣ አራቱ ያጠናናቸው ቦቲዎች በኢትዮጵያ ደጋማ ክልሎች የሚገኙ የከብቶች ርሻ ቅልቅል ሰፊ የአዝመራ ቦቲዎችና የዜቡ ዝርያ ከብቶች የሚገኙባቸው ሲሆኑ አምስተኛው ቦቲ በደቡብ ኦሞ (ደቡብ ክልል) የሚገኝ ቆላማ የአርብቶ አደር ቦቲ ነው።

በ3ቱ ወረዳዎች ተመላልሰን 3 ክሮስ ሴክሽናል ጥናት ለ3 ዓመት አካሄደናል። በዛ ያለ የውጭ ዝርያ ካለበትና የከብት ልማት ባሰፊው ከሚካሄድበት ከመሃል ኢትዮጵያ በተለየ በነዚህ ጥናት ባደረግንባቸው ቦታዎች (በኦ አይ ኢ የቆዳ መርፌ ምርመራ አወሳሰን መሠረት ያለው የከብት ቲቢ ስርጭት መጠን በጣም ዝቅተኛ ሆኖ አግኝተነዋል። ዝቅተኛው መጠንም በባሌ ተራራዎች 0% ሲሆን ከፍተኛው በባኮ ጋዘርና በደቡብ አሞ ^ስክ 1.3% ብቻ ነበር። በቆዳ መርፌው ምርምራ ውጤት አነባብ ላይ የተለያዩ የበሽታውን መኖርና አለመኖር መለያ ወሳኞች በበሽታው ስርጭት መጠን ላይ የሚያሳድሩትን የአተረጓጎም ሁኔታዎች ተንትነናል። በተጨማሪም ፣ በጥናቱ ቦታ የከብቶች ባለንብረት የሆኑ 450 ገበሬዎች ጋር ሰው ላይ ስለሚከሰተው ቲቢና በከብቶች የቆዳ ምርምራ ስለሚገኘው የከብት ቲቢ መነሻ ምክንያት ላይ ውይይት አድርገናል። አዲስ ከብት ገዝቶ መጨመርና ከከብት ጋር ሌሎች የቤት ^ንስሳት መኖር ለመርፌ ቆዳ ምርመራው የከብት ቲቢ መገኘት ዋነኛ ተጠርጣሪ ምክንያት መሆናቸውን አውቀናል። ዱሮ ከሚታወቁት ተጠርጣሪ ምክንቶች መሃል ለምሳሌ ጥሬ የ^ንስሳት ውጤቶች መመገብ ና ከ^ንስሳት ጋር በቅርብ መኖር ለሰው ቲቢ መገኘት ተጠርጣሪ ሆነው አልተገኙም።

የዱር አራዊት ለከብት ቲቢ መነሻ መሆናቸው በሌሎች አገሮች ስለተረጋገጠ ፣ በኢትዮጵያም ከኢትዮጵያ የዱር ^ንስሳት ክፍልና ከባለሙያና የመዝናኛት አዳኞች ጋር በመተባበር የመጀመሪያውን የዱር አራዊት የከብት ቲቢ ስርጭት ጥናት አካሄድን። ከ113 ንስሳት ደም ወስደን ራፒድ ቴስትን ለመፈተሽ ፣ የ^ጢ ናሙና የተውሳክ ማሳደግ ጥናት (ካልቸርና) የስሮሎጂ ምርመራና የ ^ጢ ናሙና ሞለኪዩላር ምርመራ አካሄድን። ስካለሁን ከ28 የአጥቢ ^ንስሳት ዘር (ማማል ስፒሽዬስ) ዓይነቶች መሀል አንድም ኤም ቦቪስ አልተገኘም። (የሞለኪዩሰር ምርመራው ግን ^ንደቀጠለ ነው።) ነገር ግን ፣ በሰሮሎጂ ምርመራ ለመጥፋት የተቃረቡ ብርቅዬ ^ንስሳት ዝርያዎችን ጨምሮ በ23% የተመረመሩ አራዊት ውስጥ የበሽታው ምልክት ተገኝቶአል። ደም የተወሰደላቸው አራዊት በህይወት ያሉም ጭምር ስለነበሩ የሥጋ ምርመራ በማድረግ ተውሳክ አሳድጎ ማረጋገጥ አልተቻልም። ካልቸር ከተደረጉት ውስጥ ማማሽ ያህሉ

የአካባቢ ማይኮባክቴሪያ አሳድገዋል። ይህም ምን ማለት ^ንደሆነ ገና መረጋገጥ ይኖርበ<ል። በመቀጠል፣ በሁለት የተለያዩ ቦ<ምች 684 ገበራምችን ያካተቱ ሁለት ጥናቶች ተካሂደዋል። ከዚህ የተገኘው ውጤት የገለፀው ነገር ቢኖር በክበት ^ርባ<፣ በአዝርዕት ^ርሻና በተፈጥሮ ሀብት መሀከል ያለውን ስስ ሚዛን ና የተፈጥሮ ሃብት መመናመን የሚያስከትለውን የማጦሽ መራት ማነስ፣ የለው ማጭት፣ የዱር ^ንስሳት ክልል ላይ ^የደረስ ያለውን ጥበት ^ና የ^ዝህ ሁሉ ችግሮች መፍትሄ ^ና ለወደፊቱ የአካባቢ ልማት ለዘለቂ<ው በአንድ ወጥ ^ቅድ መመራት ያለበት መሆኑን ነው።

የክበት አያያዝ ጥናት ^ንዳሳየው ክባለንብረቶች ክብቶች መሃል 52% ወንዶች ሲሆኑ 1/4 ኛው በራምች ናቸው። በራምች ከ^ርሻ ጋር ጅማ የተጣመሩ በመሆናቸው ^ርሻ፣ ምርት ና ^ህል መውቃት) አርሶ አደሮች በማናቸውም ጊዜ ቢያንስ አንድ ጥማድ ^ንዲኖራቸው ያስፈልጋል። ^ንደቲቢ አይነቱ ሀመም ከወተት ምርት ይልቁን በገጠር የ^ርሻ አቅም ላይ የበለጠ ጉዳት ያመጣል። የ<መሙ የ^ርሻ በራምች ማሳ ውስጥ ጠንክረው ለመሥራት ስለማይችሉ ለ^ርሻ ምርት ማነስና ለድህነትና ረሃብ የበኩላቸውን አስተዋጽኦ ያደርጋሉ።

በዚህ የፒ ኤች ዲ ሥራ የተጀመሩ የክበት ቲቢ በክበት ልማት ዘርፍ ላይ የሚያስድረውን የኢኮኖሚ ተጽ^ኖ የሚመለክቱ አንዳንድ የምርምር ሥራዎች ከዚህ ቴሲስ በኋላም ይቀጥላሉ። የክበት ቲቢ በህበረተሰቡ ላይ የሚያሳድረው የኢኮኖሚ ዝርዝር ንፅፅር የዚህ ቴሲስ አካል ባይሆንም የጥናቱን አቅጣጫ ^ናብራራለን።

በልማዳዊ የክበት አያያዝ ዘዴዎች ሥር የተያዙ 21 ^ርሻዎች (700 ^ንስሳት) ላይ ለ^ያንዳንዱ ባለንብረት የምርት መጠን (ክበደት፣ወተት፣ ^ርባ<፣ ግዝር፣ ሽያጭ) በመመዘገብ ለአራት ዓመት ክትትል ^ያደረግን ነው። ከምርምር በድኑ ሌሎች አባላት ጋር በመተባበር በ6 ቴራምች የ<ረዱ ክብቶችን ክብደት በመውለድ ቲቢ በዕርድ ^ንስሳት ላይ የሚያደርሰውን ክሳት ለማጥናት የረጅም ጊዜ ምርምር ^የተካሄደ ነው።

ለማጠቃለል ፣ ለዚህ ቴሲስ በተደረገው ጥናት የተሰበሰቡ መረጃዎች የሚጠቁሙት የከብት ቲቢ ጉዳይ (የኢኮኖሚ ጉዳት፣ የስርጭት ሞዴል ና የመፍትሄ ^ስትራቴጂ) በሁለት ደረጃዎች መ<ቃየት ^ንዳለበት ነው፣

1. በከተማና ከተማ ዙሪያ የተሰባሰበ የወተት ከብት ልማት ፣የውጪ ንፁህ ዝርያና ክልስ ከብቶች በብዛት መኖር፣ ከፍተኛ የወተት ምርት ^ና ነገር ግን ከፍተኛ የከብት ቲቢ ስርጭት

2. በገጠር - የተበ<ተነ የከብት ልማት፣ የሀገር ውስጥ የከብት ዝርያዎች፣ ዝቅተኛ የከብት ቲቢ ስርጭትና ምናልባትም ከወተት ጎብት ይልቅ በ^ርሻ አቅም ላይ ጉዳት የሚያደርስ ችግር ናቸው።

6. Abbreviations

AFB	Acid Fast Bacilli
AHRI	Armauer Hansen Research Institute
AIDS	Acquired Immune Deficiency Syndrome
ALERT	All Africa Leprosy, Tuberculosis and Rehabilitation Training Centre
BCG	Bacillus Calmette-Guèrin
BTB	Bovine Tuberculosis
BVDV	Bovine Virus Diarrhoea Virus
CDC	Center for Disease Control
CIDT	Comparative intradermal test
CMI	Cell Mediated Immunity
DA	Development agent
DOTS	Direct Observed Treatment Strategy
DTH	Delayed Typed Hypersensitivity
DNA	Deoxyribonucleic Acid
EPTB	Extra pulmonary tuberculosis
FAO	Food and Agriculture Organization
FIV	Feline Immunodeficiency Virus
GEE	Generalized Estimating Equation
GDP	Gross Domestic Product
HIV	Human Immunodeficiency Virus
IDP	Internally displaced people
ILRI	International Livestock Research Institute
IUCN	International Union for Conservation of Nature
MTC	Mycobacteria Tuberculosis Complex
NGO	Non Governmental Organization
NTM	Non Tuberculous Mycobacteria
OIE	Office international des Epizooties
PCR	Polymerase Chain Reaction

PPD	Purified Protein Derivate
rRNA	Ribosomal Ribonucleic Acid
RT	Rapid Test
SIV	Simian Immunodefiency Virus
SNNPR	Southern Nations, Nationalities, and People's Region
STI	Swiss Tropical Institute
TB	Tuberculosis
TBLN	Tuberculosis lymphadenitis
TLU	Tropical Livestock Unit
TVET	Technical and Vocational Education Training
VLA	Veterinary laboratories Agency
WCS	Wildlife Conservation Society
WHO	World health organization
WTO	World Trade Organization
ZN	Ziehl Neelson

7. Introduction

7.1. Bovine tuberculosis

7.1.1. Aetiology

Bovine TB is an infectious disease caused by *Mycobacterium bovis*, a member of the *Mycobacterium tuberculosis* complex (MTC), a closely related group of Mycobacteria including *M. tuberculosis, M. africanum, M. bovis, M. bovis* bacilli Calmette-Guérin (BCG), *M. canetti* and *M. caprae comb. nov*. (Fig 1) [121, 4, 84]. MTC bacteria are 99.9% similar in regard of their DNA, with identical 16S rRNA sequences [17]. However, there are distinct differences in phenotype, host ranges and pathogenicity [7]. *M. bovis* is a very resistant pathogen; it has been reported to survive in cow faeces for more than 5 months and in soil for up to 2 years depending on weather conditions [81, 58, 132, 31]. In developing countries manure fertilization of arable land is a common practice, which might represent a potential source of infection for animals and humans through contaminated pastures and vegetables [7].

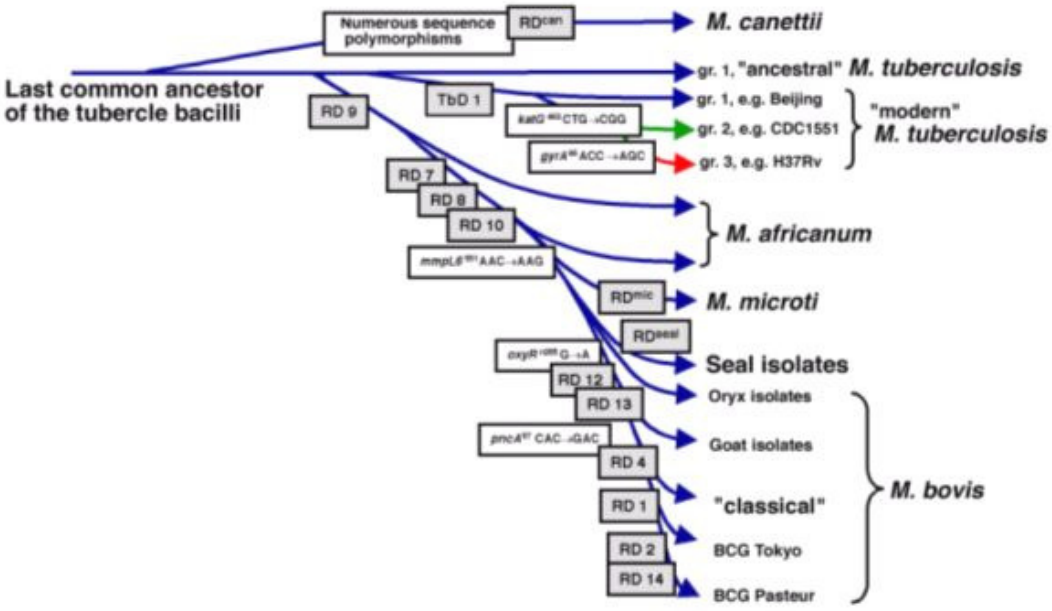

Fig. 1. Evolutionary scheme of the members of the M. tuberculosis complex
(Source: Brosh et al; Natl Acad Sci. 2002, 99:3684-9)

7.1.2. Host species

Cattle are considered to be the main hosts of *M. bovis*. However, most mammalian species are susceptible to various degrees to BTB and the disease has been reported in human beings, domesticated animals and various free-ranging and captive wildlife species [39]. The list of reports is long (appendix 4). The pathogen was isolated from, among others, ovidae [74], suidae [130, 99, 86], camelidae [120], bovidae [9, 66, 42, 106], caprinae [54], cervidae [118, 91, 59, 72, 90], mustelidae [41, 21], rodents [37, 75], primates [108, 65], marsupials [41], lagomorphs [27], perrissodactyls [111], procyonidae [98], canidae [55, 19], felidae [69, 16], ursidae [19] and elephant [71].

7.1.3. Transmission of *M. bovis*

Main transmission pathways

The respiratory and the alimentary routes are the main transmission pathways of BTB. Less described modes of disease transmission are vertical and genital transmission [88]. Percutaneous transmission was described in kudu and large predators resulting in granulomatous skin or muscle infections [104].

Animal to animal transmission

Infectious animals shed *M. bovis* in a number of ways: in feces, milk, discharging lesions, saliva and urine [100, 89, 60]. Animal age and behavior, environment, climate and farming practice can modulate the infection [101, 100]. Aerosol exposure is considered to be the most important route of infection of cattle. Neill et al (1991) stated that the inhalation of one single *M. bovis* bacillus in an aerosol droplet would be enough to infect an animal. More recently, Dean et al (2005) found experimentally that 1 CFU (6-10 bacilli) could induce tuberculosis immuno- pathology. Close contact between animals (e.g. intensive farming practice, water points, salt licks, market places, transports, auctions) contributes therefore to the effective spread of *M. bovis*. Ingestion of contaminated products (e.g. carcasses/prey, pastures and water) is considered as a secondary way to spread the disease in cattle [77], however it is an important pathway in

wildlife [104]. Congenital infections, vertical and genital transmission are very rarely observed in cattle [88].

Animal to human transmission

M. bovis is a zoonotic pathogen. Humans get infected either by inhalation of dust-particles and bacteria-containing aerosols shed by infected animals (close contact between humans and animals) or by ingestion of contaminated animal products (e.g. raw milk, raw milk product, and raw meat) [30, 35, 53, 124, 110, 50, 49]. Ingestion of raw wildlife products or aerosol exposure with infected wildlife have also been described as a source for *M. bovis* infection in humans [70, 125, 129].

Other transmission

Human patients with genitourinary BTB can be a source of infection for cattle when they urinate in cowsheds or on pastures [52]. However, this transmission route has been rarely described in the literature. *M. bovis* seems to be less virulent in humans than *M. tuberculosis* [122] and transmission between humans is rare but has been reported in immunosuppressed HIV patients [15, 121, 51, 50].

7.1.4. Clinical features and pathology

Forms of BTB

Pulmonary TB due to *M. bovis* is the most prevalent form of BTB in cattle and is the result of inhalation of bacteria containing aerosols shed by infected animals. In contrast, pulmonary TB due to *M. bovis* is rare in man and more commonly seen in developed countries as an occupational hazard in abattoir and farm workers [30]. Lesion distribution and pathology show predominant involvement of the upper and lower respiratory tract and associated lymph nodes [88, 127]. On the other hand, extra pulmonary BTB is the result of ingestion of tuberculous contaminated products. This is the most common form in man resulting from the ingestion of contaminated milk and milk products. Extra pulmonary TB involves predominantly cervical lymph nodes [63, 54] but also intestines,

liver, spleen, kidneys, pleura and peritoneum together with their associated lymph nodes [88].

Pathogenesis and immunology

Mycobacterial infection triggers a Th1-induced cell-mediated immune response (CMI) which leads to release of cytokines such as tumour necrosis factor-α, Interleukin- 12 (IL-12) and Interferon gamma (IFN-γ). This pathway is essential to activate macrophages [97]. Depending on the balance of cytokines involved, three outcomes are possible: 1) macrophages kill and eliminate the bacteria 2) the bacteria lies dormant (latency), 3) the bacteria can not be contained by the immune system and the disease develops to active TB [126].
Containment of the bacteria results in the formation of nonvascular nodular granulomas known as "tubercles". Lesions show typically a centre of caseous necrosis with some degree of calcification surrounded by a cell wall of epitheloid cells, lymphocytes and neutrophils [46]; Unlike in man, these primary lesions are rarely contained by the immune system in cattle and bacilli spread by lymphatic and haematogenous routes, resulting in tubercles in other organs [119, 88].

The initial cell-mediated immune response (CMI) response is followed later in time by a humoral antibody response, which is caused by a shift of Th1 to Th2 cell activation [44]. A state of anergy may occur in advanced stages of the disease and a CMI response is no more detected. Initial pathological changes are associated with the onset of CMI response [30] (Fig.2.). CMI response can be affected by the animal's nutritional state (e.g. deficiency in energy, protein and micronutrients) [101], by stress [45, 18] or concurrent diseases, which lead to a reduction of the host resistance, caused for instance by the classical immunodeficiency viruses such as HIV in man [135], Bovine Virus Diarrhoea Virus (BVDV) in calves [83], feline immunodeficiency virus (FIV) in cats, Simian immunodeficiency virus (SIV) in primates [104].

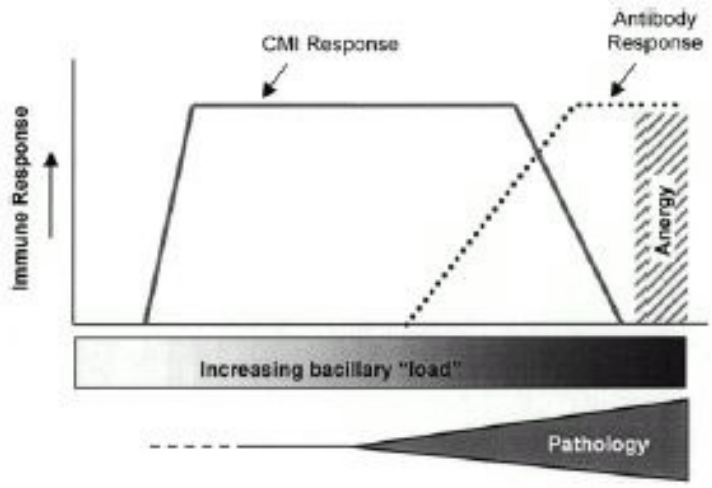

Fig.2. Temporal spectrum of immune responses in cattle following infection of *M. bovis* (Source: Pollock and Neill. The Vet. J. 2002. 163:115-127)

7.1.5. The tuberculin skin test

The above mentioned CMI is used in the diagnosis of tuberculosis, when performing the skin test. The test is based on the fact that infection with Mycobacteria produces a delayed type hypersensitivity (DTH) skin reaction mediated by Th1. T cells sensitized prior by an infection are recruited in the skin after a PPD injection, where they release lymphokines, inducing at the site of injection induration and edema through local vasodilation within 48-72 hours [126, 82].

7.1.6. Clinical signs

In humans, TB due to *M. bovis* is indistinguishable from that due to *M. tuberculosis* in terms of clinical signs, radiological and pathological features [7]. BTB is a chronic debilitating disease. Often, the disease remains asymptomatic until advanced stages with disseminated lesions [77]. Pulmonary TB may result in cough, dyspnoea and respiratory distress. Extra pulmonary TB may lead to various clinical signs, depending on which organs are affected. Enlarged lymph nodes may obstruct air passages, the alimentary tract or blood vessels. Cervical lymphadenitis (scrofula) is typically found in milk-borne TB infection in humans and is characterized by visually enlarged lymph nodes of the head

and neck, which can sometimes rupture and drain (Fig 3) [35, 54]. In developing countries, tuberculous lymphadenitis is one of the most frequent causes of lymphadenopathy and the most common form of extra pulmonary TB [68]. In terminal stages of BTB, extreme emaciation and weakness may occur (Fig 4, 5).
Some wildlife species show very typical signs such as the greater kudus (*Tragelaphus strepsiceros*), which develop characteristic swollen head and neck lymph nodes with draining sinus tracts visible from a distance as well as blindness (Fig 6) [10, 104]. Carnivores often show limb swellings and lameness [104].

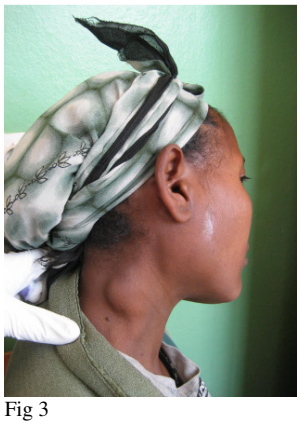

Fig 3

Fig 4

Fig 6

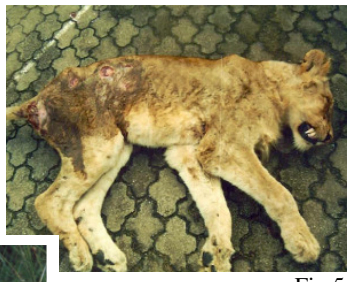

Fig 5

Fig. 3-6. Clinical signs of bovine tuberculosis

Fig.3. Cervical lympadenitis in an Ethiopian patient (Photo: R. Tschopp)

Fig.4. Emaciated Holstein cow with BTB, Ethiopia (Photo: R. Tschopp)

Fig.5. Emaciated lion (*Panthera leo*) that died of BTB, RSA (Photo: D. Cooper)

Fig.6. Swollen neck lymph node in a kudu (*Tragelaphus strepsiceros*), RSA (Photo: D. Cooper)

7.2. Epidemiology of BTB

7.2.1. Overview

Tuberculosis is responsible for the death of more people each year than any other infectious disease [47]. The World Health Organization (WHO) reported 9.2 million new cases and 1.7 million deaths in 2006 [128], with Sub-Saharan Africa having the highest annual risk of infection with tuberculosis. WHO estimates that 70% of humans co-infected with TB and HIV live in Sub-Saharan Africa [30].

Global prevalence of human TB due to *M. bovis* has been estimated at 3.1% of all human TB cases accounting for 2.1% of pulmonary and 9.4% of extra pulmonary TB cases [30]. A recent UK study showed that *M. bovis* accounted for 0.5-1.5% of all culture confirmed TB cases [38].

Although the epidemiology of *M. bovis* is well documented in developed countries and control and elimination strategies implemented for many decades in those nations by a policy based on systematic slaughter of infected animals, meat inspection in abattoirs and milk pasteurization, 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* [30]. Political factors also often account for the failure to control and eradicate BTB: political instability, wars, both resulting in displacement of large human and animal populations; insufficient collaboration with bordering countries (lack of quarantine, smuggling of live animals) and lack of veterinary expertise [7]. Only seven nations in Africa consider BTB as a notifiable disease and therefore apply control measures [30]. The isolation of *M. bovis* and thus the differentiation between infections caused by *M. tuberculosis* and *M. bovis* in diagnostic laboratories is poor to non-existent and the epidemiology of BTB in most part of Sub-Saharan Africa is still largely unknown.

7.2.2. BTB prevalence in Sub-Saharan Africa

Nearly 2 million TB cases occur each year in Sub-Saharan Africa, fuelled by the HIV epidemic. The role played by cattle-derived *M. bovis* in this increasing epidemic is unknown [33]. BTB is prevalent in 33 (80%) of 43 African member countries of the OIE [34]. However, none of the national reports submitted to the OIE and the WHO by African member states mention the importance of BTB in human cases [7], and there is very little systematic data on the extent of BTB either as a veterinary or as a human health problem in the developing world [30]. The proportion of *M. bovis* infections causing human TB is not well known and very likely to be underreported, since only a few laboratories are capable of differentiating *M. bovis* from *M. tuberculosis* and other members of the MTC group [28]. Approximately 85% of the cattle and 82% of the human population in Africa live in areas where BTB is either only partly controlled or not controlled at all, and large communities are exposed to direct contact with animals and consume unpasteurized milk and milk products [85, 30]. Only very few reports mention isolation of *M. bovis* from both humans and animals in the same area [105, 29, 26]. Thus the assessment of risk factors of disease transmission among animals and between animals and humans, as well as the potential role of *M. bovis* as a zoonosis, is still largely unknown in sub-Saharan Africa.

BTB prevalence in man

Pulmonary and extra pulmonary BTB in humans was confirmed in several African countries [30]. In Egypt, *M. bovis* was found in 0.4-5.4% of sputum of positive TB patients [48]. In Nigeria, Idigbe et al (1987) found *M. bovis* in 4% of patients with lower respiratory tract symptoms. *M. bovis* seems to play a minor role in human TB in Burundi, despite the high prevalence of both HIV in humans and BTB in livestock [105]. Rasolofo et al (1999) observed in Madagascar *M. bovis* in 1.25% of pulmonary and 1.30% of extra pulmonary TB patients. In Tanzania, Daborn et al (1997) isolated *M. bovis* in 4 lymph nodes out of 19 lymph node biopsies from suspected extra pulmonary TB lesions. Recent studies in Tanzania showed a prevalence of 10.8% of *M. bovis* from adenitis biopsies [78]. In Uganda, Oloya et al (2007) isolated *M. bovis* from 3 out of 43 biopsies (7%).

BTB prevalence in African livestock

M. bovis was isolated in numerous African countries and there seem to be an endemic state of bovine tuberculosis in some of these nations. In Burundi, Rigouts et al (1996) isolated *M. bovis* in 38% of clinically suspected bovines. A large scale skin test study in Uganda showed a BTB prevalence of 6% in cattle [11]. Furthermore, 19 out of 61 abattoir lesions (31%) were confirmed as being *M. bovis* [93]. Diagbouga et al (1999) described prevalence of BTB in slaughtered cattle in Burkina Fasso to vary between 0.2-13%. Vekemans et al (1999) found 13% positive reactors to intradermal PPD injection in the area of Bobo Dioulasso.

Schelling et al. (2000) found a 17% prevalence of BTB in Chadian livestock using the PPD tuberculin test. In Ghana, an individual animal skin test prevalence of 13.8% was described by Bonsu et al (2000). Omer et al., (2001) found, in a cross-sectional study of BTB in dairy farms in Asmara (Eritrea), that 14.5% of the PPD tested animals reacted positive. In Tanzania, low prevalence was found in cattle: Jiwa et al (1997) reported a 0.2% prevalence of BTB in the Lake Victoria area of Tanzania. Cleaveland et al (2007) reported individual cattle prevalence using skin test of 0.9%. However, *M. bovis* was also isolated in 2 out of 805 (0.2%) milk samples in the Southern Highlands of Tanzania [62]. In Sudan, 39.9% of abattoir lesions were confirmed as *M. bovis* [112].

M. bovis was also isolated in livestock other than cattle. BTB was reported in sheep in Sudan [114], and camels (*Camelus dromedarus*) in Mauritania [22].

However, accurate livestock prevalence of BTB is still unknown in many African countries.

BTB prevalence in African wildlife

Wildlife maintenance hosts have been described in many parts of the world and are known to hamper costly national control and eradication programs in developed countries since they represent a persistent source of infection for livestock. These maintenance hosts are for example as already described earlier, the brushtail possum (*Trichosurus vulpus*) in New Zealand, the wild boar (*Sus scrofus*) in Spain, the white-tailed deer (*Odocoileus virginianus*) in the US, and the European badger (*Meles meles*) in the UK.

In Africa, BTB has been described in many wildlife species and the number of reports is increasing [104]. However, most exhaustive studies are from southern Africa, whereas existence of BTB in wildlife is still lacking in most African countries.

The African buffalo (*Syncerus caffer*) is generally recognized as being the classical maintenance host [130, 64, 79]. However, other species such as the Kafue Lechwe (*Kobus Leche*) or the greater Kudu (*Tragelaphus strepsiceros*) are also potential reservoirs for the disease, whereas most other wildlife species are thought to be spill-over or dead-end hosts.
Already in 1978, Clancey reported 33% of wild lechwe (*Kobus leche*) to be infected with *M. bovis*. Twenty years later, Zieger et al (1998) also isolated *M. bovis* in Lechwe in Zambia. Woodford (1982) confirmed *M. bovis* infection in warthog (*Phacochoerus aethiopicus*) in the Ruwenzoni National Park in Uganda. Tarara et al (1985) and Sapolsky & Else (1987) reported *M. bovis* in baboons (*Papio spp*.) in Kenya. BTB in baboons was also described by Keet et al (1996), in Krueger National Park, RSA. Recent studies in Tanzania isolated *M. bovis* from 11.1% of migratory wildebeest carcasses (*Connochaetes taurinus*), and 11.1% of topi (*Damaliscus lunatus*). In addition, 4% of lions tested (*Panthera leo*) were sero-positive for BTB, as well as 6% of buffaloes tested [25].

However, most reports of BTB in wildlife are coming from South Africa, where reports of BTB were first made in 1928 in greater kudu in the Eastern Cape Province [104]. Extensive studies have been done in the last decades, for example in Krueger National Park and Hluhluwe-Umfolozi National Park, where buffaloes play a major role in maintaining BTB in the ecosystem. They are therefore a major threat for other wildlife species, not only herbivores grazing on contaminated pastures but also carnivores consuming infected prey. BTB has been diagnosed in lions (*Panthera leo*), whose main prey are buffaloes; this has led to a massive decline in the lion population in Krueger. Leopards (*Panthera pardus*), cheetahs (*Acinonyx jubatus*) and hyenas (*Crocuta crocuta*) have also been described as being infected with *M. bovis* [79, 80].

From a conservation point of view, BTB may pose a serious threat to endangered wildlife species. On the other hand, BTB infected wildlife may represent a source of infection for predators, for livestock and for the whole environment. The role played by the different wildlife species in the epidemiology of BTB at the human-livestock-wildlife interface is still largely unknown.

7.3. Current situation in Ethiopia

7.3.1. Country overview

Ethiopia, land locked in the Horn of Africa, is the third most populated country in Sub-Saharan Africa with a population of 78 million in 2008 for a territory of 1,127,127 km2 and an annual growth rate of 2.2 % [23, 32]. The country has a mosaic of people with over 80 different ethnic groups, the major one being Oromo (32%), followed by Amhara and Tigraway (36%). Culture, tradition and religion differ from region to region. Muslims (33%) and Ethiopian orthodox (50%) are the two main religions found in Ethiopia [23]. Ethiopia has good resource potential for development (different ecological zones, agriculture, biodiversity, water resources, minerals etc.). However, the country is faced with complex poverty, which is broad, deep and structural; it is one of the poorest countries in the world with 50% of its population in 2004 living below the poverty line (World Bank). Economy is based on agriculture, which accounts for 45% of GDP, 90% of exports and 85% of total employment [20]. The agriculture sector suffers from recurrent droughts and poor traditional cultivation practices resulting in massive land degradation [116, 12] and famine [56]. Furthermore, the country has been undermined by civil war, a war with Eritrea (1998-2000) a war with Somalia (2007) and clashes along the borders, resulting in hundreds of thousands of refugees from Eritrea, Sudan and Somalia, as well as 200 000 internally displaced persons (IDPs) [23].

7.3.2. Poverty reduction

In some countries, economic growth is a primary national policy goal. However, the core objective of the Ethiopian government is poverty reduction rather than economic growth (World Bank). Government strategy focused in the last decade on crop intensification

[20]. In addition, an intensive cattle breeding program was started around the capital city of Addis Ababa, which increased the number of productive exotic breeds (e.g. Holstein), and consequently milk yields, in order to satisfy the rapidly increasing urban milk demand.

Publications have highlighted that exotic breeds (*Bos taurus*), though producing more milk, are also more susceptible to bovine tuberculosis than zebu cattle (*Bos indicus*) [1, 3]. Therefore, the strategy to increase milk yields by increasing the Holstein population may lead to increased problems with bovine tuberculosis (BTB) in Ethiopia.

7.3.3. BTB in humans

In common with other African countries, TB case notifications in Ethiopia have increased from 97 per 100 000 of the population in 1997 to 151/100 000 in 2006 [128]. This increase can be attributed both to improved case findings under DOTS and to the spread of HIV/AIDS (adult HIV/AIDS prevalence is estimated to be between 2.8% and 6.7%) [128]. A very high prevalence of extra pulmonary TB is found in Ethiopia: 35% of the cases notified under the Ethiopian DOTS program in 2002 had extra pulmonary TB, exceeding all other forms of TB (WHO).The reason for this unusually high prevalence is unknown and HIV may contribute to it. However, Kidane et al. (2002) found that among 35 PCR positive cases of TBLN from Southern Ethiopia, 29 (82.9%) were caused by *M. tuberculosis* and 6 (17.1%) by *M. bovis,* and described TBLA in 90% of HIV positive and in 86% HIV negative patients thus suggesting that factors other than HIV might be more likely to be responsible for the high prevalence of TB Lymphadenitis (TBLA) in Ethiopia.

The country has, with 43 million cattle the biggest herd in Africa [32]. The habit of consuming raw milk and milk products, and raw meat (e.g. *kitfo*) is a very common feature in Ethiopian society. Ameni et al (2003) showed that in Central Ethiopia, only 3% of the interviewed people boiled milk, and only 1% consumed cooked meat. Therefore, it was speculated that bovine tuberculosis caused by *M. bovis* might be the reason for these high EPTB rates.

7.3.4. BTB in Ethiopian 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%). Ameni et al (2003) found an individual animal prevalence of 7.9% using CIDT in the Wuchale district (Central Ethiopia). A recent large scale study involving 5424 cattle carried out in Central Ethiopia showed that the overall prevalence in cattle was 13.5%, with higher prevalence found in Holstein (22.2%) compared to local zebus (11.6%) [3]. In high density herds maintained under intensive farming conditions, BTB prevalence was found as high as 50% of Holstein cattle at the Holetta National Insemination Centre.

M. bovis was also isolated during abattoir surveys. Asseged et al (2004) found 1.5% of 1350 examined cattle in Addis Ababa abattoir displaying tuberculous lesions; *M. bovis* was isolated from 50% of these lesions. *M. bovis* was also isolated in an abattoir survey from Hossana (Central Ethiopia) where 4.5% of the animals showed tuberculous lesions [117]. These studies also showed that the efficiency of routine abattoir inspection was very low. In Asseged's study only 55% of the tuberculous cattle could be detected by meat inspection, whereas Teklul noticed that only 29.4% of the carcasses with lesions could be detected during routine meat inspection.

BTB is endemic in cattle in Ethiopia; the disease has been reported from different field and abattoir studies [5, 2, 3]. However, the prevalence of the disease is not well established in livestock and most studies focused mainly on Central Ethiopia, whereas large areas in the country remain un-investigated.
Furthermore, no studies have so far been carried out to investigate BTB prevalence in wildlife species at the livestock-wildlife interface, and thus the possible existence of a wildlife reservoir for the disease.

7.4. Economic and social impact of BTB

A described above, BTB represents a serious health threat for livestock, wildlife and humans. But the disease is also an important economical and financial burden to society, linked with economic losses: loss of productivity of infected animals (e.g. reduced milk yields and meat production, reduced fertility), animal market restrictions, control and eradication programs, human health costs, loss from tourism sector etc. [13, 76].
In Argentina, the annual loss due to BTB is approximately US$63 million [30]. The socio-economic impact of BTB to the agriculture and health sector in Turkey has been estimated between 15 and 59 million US$ 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 [75].

In Africa, the economic losses associated with livestock infected with BTB have not been examined sufficiently, or have not been studied at all [7]. For the public health sector, WHO estimates total TB control costs in Ethiopia of US$14.2 million per year and US$129 per patient [128].

7.5. Rationale and research framework

In order to embark in a future national BTB control program, the epidemiology of the disease has to be assessed in terms of prevalence in animals and humans, and in terms of risk factors for disease transmission. Elaboration of a transmission model is therefore a prerequisite for any further economical assessment of the most profitable intervention in Ethiopia. Not taking into consideration a potential wildlife reservoir can lead to difficulties in realizing the economic benefits of control strategies, and ideally should be included in transmission models. Tests-and slaughter programs, such as conducted in industrialized countries, are likely not to be feasible in developing countries for technical

and economical reasons. Alternative approaches would be increased disease surveillance in abattoirs, and tracing of BTB animals back to the herds of origin with subsequent sequestration. Vaccination of cattle is another option to reduce disease transmission [30], however, the commonly used BCG vaccine proved to have a suboptimal efficacy [96, 73].

The introduction of a control and eradication program for many zoonoses is based on analysis of the profitability of control efforts (cost-benefit analysis of interventions). Interventions on the animal side are very likely to affect the disease in humans, in diseases with dual impact on animal and public health [107], and control can therefore lead to dual benefit. Economic analysis of possible interventions to control BTB should include the impact on human health costs and the impact on livestock production [134]. Baseline data on bovine TB in Ethiopia are lacking, with a few reports existing on prevalence in cattle in Central Ethiopia, however, no data of cattle prevalence exist from most other regions. The contribution of *M. bovis* to total human TB is unknown. The different TB strains involved have not been investigated; data on risk factors of disease transmission are sparse, studies on prevalence in wildlife are non-existent, and the social impact of the disease largely unknown.

The Wellcome Trust program to study BTB in developing countries therefore started a consortium in Ethiopia, involving different complementary work packages (WP) led by the following institutions : Imperial College (London, UK), Veterinary Laboratories Agency (VLA, Weybridge, UK, WP3/5/7), Trinity College (Dublin, Ireland, WP4), Armauer Hansen Research Institue (AHRI/ALERT, Addis Abeba, Ethiopia, WP1/2), Swiss Tropical Institute (STI, Basel, Switzerland, WP6), and the International Livestock Research Institute (ILRI, Nairobi, Kenya, WP7). The consortium plans to address the above epidemiological, immunological and social questions within the framework of an economical evaluation of the impact of the disease.

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8. Goals and objectives

8.1. Goal

To collect large-scale and long-term epidemiological field data on BTB in cattle kept under traditional husbandry systems and in wildlife in Ethiopia. The data will be used ultimately to develop a transmission model between animals and humans, to estimate the economical impact of the disease to the Ethiopian society and to assess the most profitable intervention strategies for the country. The latter will be achieved beyond the framework of this PhD. This work thus contributes to the overall Wellcome Trust project on BTB.

8.2. Objectives

- Assess the field prevalence of BTB in cattle kept under traditional husbandry system in Ethiopia using the comparative intradermal skin test (CIDT).

- Assess BTB prevalence in Ethiopian wildlife.

- Assess possible risk factors of disease transmission between animals and between animals and humans.

- Assess the baseline productivity of Ethiopian cattle.

- Assess the herd structure of cattle in Ethiopia.

- Assess the impact of BTB on animal live and carcass weight in abattoirs.

9. Study sites

Study sites

The field work involved during this PhD was carried out in different regions of Ethiopia. Photos illustrating the different study sites and work performed are shown in appendix 1. The study sites covered various ecosystems, with different vegetation and climate, and with altitude ranging from 400 m (Hamer) to 4200 m (Bale Mountains). All sites were part of the Ethiopian Highlands or Middle Lands, characterized by sedentary mixed livestock-crop farming systems, with the exception of the Hamer site, located in the lowlands of Southern Omo and which is inhabited by semi-nomadic pastoralists, in our study site by Karo and Hamer tribes.

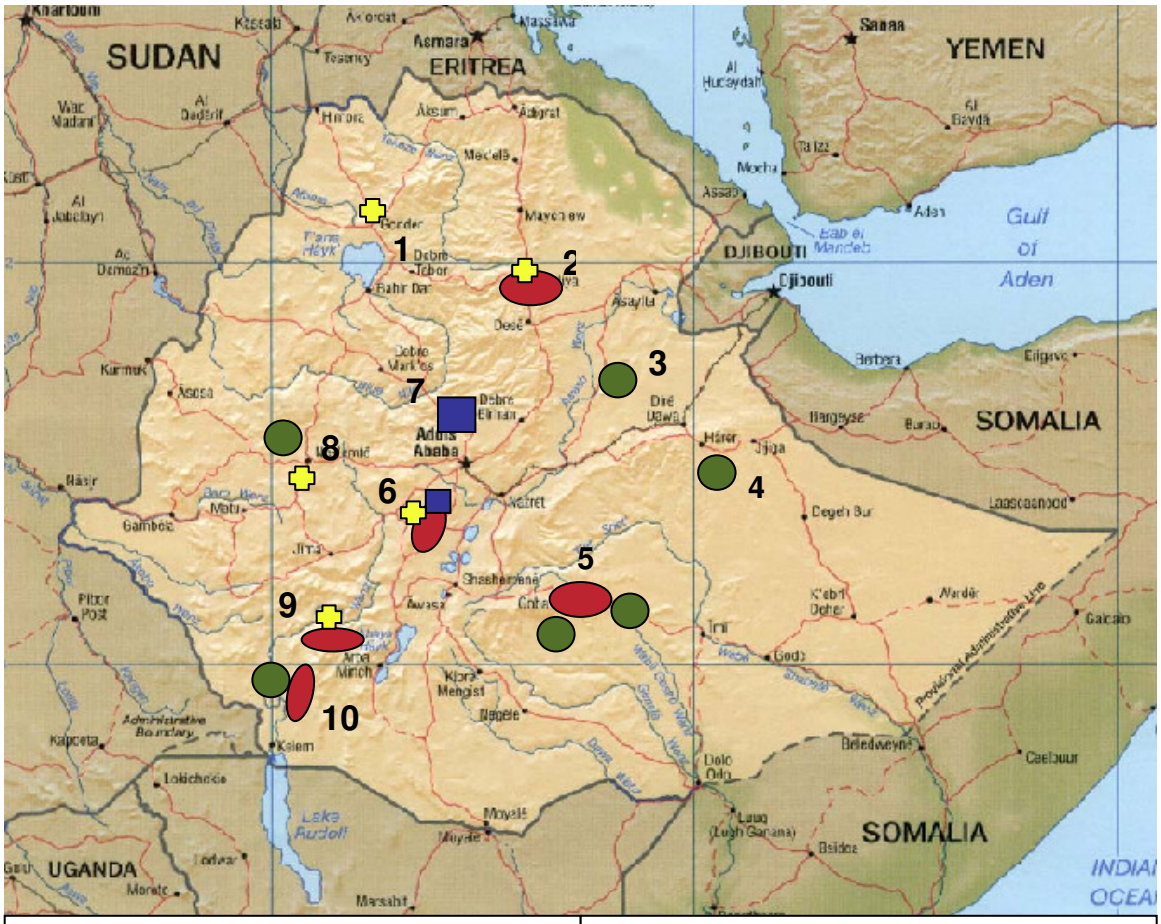
Cross-sectional PPD surveys in cattle were carried out in Meskan Mareko (Rift Valley, SNNPR), Woldia (Amhara), Bako-Gazer (SNNPR), Hamer, South Omo (SNNPR), and the Bale Mountains (Oromia). Study sites in the Bale Mountains regrouped 4 Woredas: Dinsho, Robe, Goba and Goro.
Measurements on live animal and carcass weight were performed in the municipal abattoirs of Gondar (Amhara), Woldia (Amhara), Ghimbi (Oromia), Addis Abeba, Butajira (SNNPR), and Jinka – Bako Gazer (SNNPR). In these abattoirs, lymph node samples were collected as well for the molecular typing study of work package 1.

The productivity survey in cattle was carried out in 20 farms in Sellale (Shoa, Oromia) and 1 farm in Butajira (Rift Valley, SNNPR).
Collection of market prices was done in the same sites as the cross-sectional surveys of PPD in cattle and abattoir surveys.

Wildlife samples were collected in South Omo (SNNPR), Bale Mountains (Oromia), Babille elephant sanctuary (Oromia), Afar, and Wellega (Oromia).

All farmer interviews were done in the sites where PPD in cattle was carried out, see above

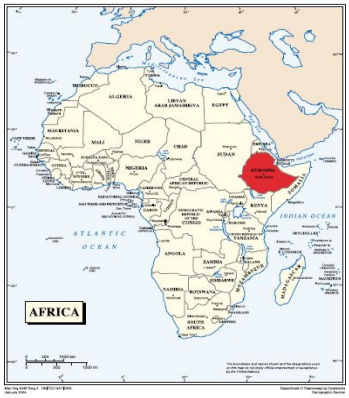
Fig 1: Map of Ethiopia showing all study sites of this PhD



Abattoir and human BTB studies	Cattle productivity studies
Cattle PPD field testing	Wildlife studies

Legend of the different study sites:

1. Gondar (Amhara)
2. Woldia (Amhara)
3. Afar
4. Babille (Oromia)
5. Bale Mountains (Oromia)
6. Butajira (Gurage-SNNPR)
7. Sellale (Shoa-Oromia)
8. Ghimbi/Welega (Oromia)
9. Bako-Gazer (SNNPR)
10. South Omo (Hamer-SNNPR)



10. Repeated cross-sectional skin testing for bovine tuberculosis in cattle in traditional husbandry system in Ethiopia

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Abstract

Representative repeated cross-sectional skin testing for BTB was conducted over a period of three years in a total of 5377 cattle in three randomly selected Woredas in Ethiopia (Meskan, Woldia and Bako-Gazer), that were never previously tested for BTB. The animals included were 99% local zebus kept in traditional husbandry systems. The comparative intradermal testing and two diagnostic thresholds were used to define positive test results, one according to the OIE recommended cut-off of >4 mm and the other with a cut-off of >2 mm. Data analysis was performed using a logistic regression model with a random effect at the village level. Applying the OIE definition, the overall representative apparent prevalence of BTB skin test positive local zebus was 0.9% (CI 95% 0.6-1.3 %). Using a cut-off > 2 mm the overall representative prevalence increased to 4% (CI 95% 2.4-4.8%). Due to low apparent prevalence, true prevalence could only be calculated in Meskan (4.5%) and Bako-Gazer (2.4%) for >2 mm cut-off. With the exception of Meskan, prevalence by Woreda was not statistically significantly changing over the years. *M. avium* reactors were found in all study sites but with significant geographical variation. Overall, bulls and oxen were more at risk for being a positive reactor (OR 1.6, CI 95%: 1.1-2.3; OR 2, CI 95%: 1.4-2.6), as well as animals in good body condition (OR: 2, CI 95%: 1.5-2.9). Similar results were found at Woreda level with the exception of Woldia, where none of the analyzed variables were associated significantly with a positive test result.
In conclusion, this study shows a very low apparent BTB prevalence in cattle in rural traditional husbandry systems, but with regional variations.

Key words: Ethiopia, bovine tuberculosis, comparative intradermal tuberculin test, repeated cross-sectional study, zebu breeds

Introduction

Bovine tuberculosis (BTB) is a chronic debilitating disease caused by *Mycobacterium bovis*. The causative agent belongs to the Mycobacterium tuberculosis complex (MTC), which comprises the phylogenetically closely related *M. tuberculosis*, *M. africanum*, *M. microtti*, *M. bovis* bacilli Calmette-Guérin (BCG), and *M. canetti* (Mostowy and others 2005).

Eradicated or at least controlled in most parts of the developed world, BTB remains prevalent in many sub-Saharan countries. The disease causes not only a potential zoonotic threat through the consumption of contaminated raw animal products and/or close contact with infected animals, but is also an economic burden to the livestock sector and thus to the society, although these have not been quantified yet in Africa (Zinsstag and others 2006). The exact epidemiology of BTB in Africa is still largely unknown and national control strategies in livestock are rare or inexistent.

In developed countries, tuberculin skin testing is universally recognized for BTB diagnosis in live cattle and is the basis for national test-and-slaughter programs (Ayele and others 2004). The use of a cut-off point for the comparative intradermal tuberculin test (CIDT) of >4 mm, as recommended by the Office International des Epizooties (OIE), is used worldwide as standard skin test result interpretation. However, this standard cut-off is more and more questioned since it might not be adapted to the African context, where cattle breeds, prevailing prevalence, epidemiology of disease and diverging official goals of disease control differ from industrialized countries. In a recent study in Central Ethiopia, Ameni and others (2008) suggested the use of a > 2 mm cut-off meaning a difference of skin thickness between bovine and avium reaction greater than 2 mm as being more adapted for skin testing cattle in Central Ethiopia since it increased sensitivity of the test without affecting its specificity. Similarly, a recent study in Chad showed a better performance of the single intradermal comparative cervical test for a cut-off value of >2 mm (Bongo and others 2009).

BTB has been shown to be endemic in Ethiopian cattle with prevalence ranging between 7.9 % and 34%. However most field and abattoir prevalence studies focused on Central Ethiopia, which is characterized by urban and peri-urban management settings and/or herds with exotic cattle breeds or their cross breeds, which are more productive than traditional zebu breeds (Ameni and others 2003, Asseged and others 2004, Teklul and others 2004, Ameni and others 2007). The Central highlands are also the major area of milk supply for Addis Abeba, a fast growing capital city of 3.1 million people (CSA 2008). This region is subject to more research regarding the burden of bovine TB than other parts of Ethiopia. However, over 80% of the Ethiopian population is rural and directly dependent on livestock for their daily livelihood. In these more remote rural areas, Ethiopian farmers keep mainly zebu breeds, under traditional management system, for crop farming, where animals are used for draft power. BTB prevalence in these areas was up to this study largely unknown.

In this article the authors present a repeated cross sectional study of skin testing in cattle over three years in three rural regions in Ethiopia that were never tested previously for BTB: from the Northern Highlands, across the Rift Valley and down to the Southern part of the country. The primary goal was to assess representative tuberculine positivity prevalence in traditional husbandry system and to follow trends over time in view of a future cost study of disease. The use of the different CIDT cut-offs is discussed as well as the role of *M. avium* in the testing procedure.

Material and methods

Study areas

The authors carried out a repeated cross-sectional study from 2005 to 2008 in two regional zones of Ethiopia (Amhara, and Southern Nations, Nationalities and People Region (SNNPR) between the latitudes of 5.1°N and 11.5°N and the longitudes of 36.1°E and 40.1°E. Within these regions, three Woredas (districts) were randomly selected (see below) according to the further requirements of a larger programme on BTB in Ethiopia (e.g. presence of abattoirs and hospitals in the same areas), namely Woldia (Northern

highlands), Meskan Mareko (Rift Valley), and Bako-Gazer (Southern middle lands). Altitude of the study sites ranged from 1300 m to 3500 m above sea level. The Woldia site had two distinct ecological zones, the lowlands (<2000 m) and the highlands (>2000 m). All farmers were sedentary small holders with mixed livestock-crop farming system and cattle husbandry practice.

Study design

Cattle were selected by a stratified cluster sampling proportional to the size of the cattle population, and considered villages as clusters based on calculated intra-class correlation coefficients. Cattle from individual owners in the study areas were kept communally during the day; therefore herds belonging to individual owners were taken into consideration but animals of all owners were regrouped into one single "village" herd for each village.

Lists of Kebeles (administrative units within the Woredas) and villages within the Kebeles were obtained from each Woreda agricultural office. Kebeles within the Woreda were selected randomly using random numbers generated in Microsoft Excel®; Villages were selected randomly and proportionally to their number within a particular Kebele. Approximately thirty animals were selected per village. Cattle younger than 6 months of age, late stage pregnant cows and clinically sick animals were not included in the study.

The selected cattle from a village were gathered together for testing and reading. If animals were not present on the reading day, house to house visit was conducted. As compensation and incentive for farmer's participation, all tested cattle were dewormed on the reading day with Albendazol boli (Ashialben 2500, Ashish Life Science PVT, Mumbai, India).

The cross-sectional study was repeated three times on a yearly basis in the same villages for all three Woredas, whereby animals were newly selected at each visit. To avoid seasonal impact on test results (e.g. drought, rainy season), cattle were tested in each Woreda in the same month over the years.

The calculation of sample size was done using formulas of Bennet and others (1991) as described in Tschopp and others (2009). We took the intra-class correlation coefficient roh (ρ) as 0.2 and obtained a design effect D of 6.8. Choosing 30 animals (b) per cluster (c), with a disease prevalence of 5% and 17 clusters (total sample size per Woreda = 510 animals) gave us an estimate of the standard error or precision of 0.025. The total sample size n is the product of the number of clusters (c) times the number of samples per cluster (b) [n=b*c], thus equaling 510 animals per Woreda, which totaled a number of required animals of 2040.

Skin testing of cattle

Skin testing was carried out by the same person to avoid technical bias related to testing method. We applied the comparative intradermal tuberculin test (CIDT) in all cattle using both avian and bovine purified protein derivates (PPD) supplied by the Veterinary Laboratories Agency, Weybridge, UK. Intradermal injections of 0.1 ml (2,500 IU/ml) bovine PPD and 0.1 ml (2,500 IU/ml) avian PPD were made in two shaved sites, 12 cm apart from each other in the middle neck region, after having recorded skin thickness with a caliper. Skin thickness was measured again at both injection sites after 72 hours. The reaction at each site was derived as the difference of the skin thickness after 72 hours minus before injection. The data was analyzed using two different diagnostic thresholds to define positive test results: 1) an animal was considered positive if the bovine minus the *M. avium* reaction was greater than 2 mm (Ameni and others 2008, Bongo and others 2009) and 2) an animal was considered positive if the bovine minus the *M. avium* reaction was greater than 4 mm (OIE definition). A village-herd was considered positive if at least one positive reactor was present.

To assess the prevalence of *M. avium*, we defined arbitrarily MAC positive reactors by assessing the skin reaction on the avium site alone as the difference between the initial avium PPD injection and the 72-hour reaction being greater than 4 mm.

Cattle data

In addition to the CIDT, information was collected for each tested animal: sex, breed, age, and body condition score. Animals were categorized into four age groups: calves

younger than one year, juveniles between one and three years, reproductive animals between three and ten years and animals older than ten years. Body condition score of the animals was done using a 1 to 5 scale as described in Msangi and others (1999), but were regrouped into three categories that better reflected the assessment in the field, namely emaciated to thin, normal and musculous to fat. Cattle were differentiated in three different types, traditional zebus, cross-breeds and exotic breeds. Within the traditional zebu breeds, animals were, if possible, further categorized on phenotypical characteristics in three breeds, the Kola/Danakil breed which belongs to the Sanga group and which is found in the North Eastern part of the country, the Ethiopian Boran and a breed found in Bako-Gazer called traditionally Male. Farmers were asked on the testing day in order to avoid recall bias, about the work stamina of their ox and if they had noticed any changes such as weakness, and reluctance to work in the field during the last year.

Statistical analysis

Data were double entered in Access, validated with EpiInfo (version 3.3.2) and analyzed with the software package STATA 10.1 (StataCorp, Texas, USA). A variance component analysis was performed for the different levels of the multicluster sampling (Woreda, Kebele and village) using Generalized Linear Mixed Models with binary outcome and logit link function (GLLAMM add-on). The analysis indicated that most variance was associated with village and Woreda. Village was therefore included as random effect in the logistic regression model.

Data on prevalence and cattle characteristics were analyzed using a logistic regression with random effect on village. Prevalence was calculated as overall prevalence, as prevalence stratified by Woreda and by year as well as an overall prevalence by Woreda combining all 3 years. Apparent prevalence was calculated using both the official >4 mm and a >2 mm cut off. Estimation of the true prevalence and their 95% CI were obtained using the Rogan-Gladen estimator as described by Greiner and Gardner (2000) based on the proportion of test positive cattle (apparent prevalence) and a sensitivity and specificity of the test of 69% and 97%, respectively, as described by Ameni and others (2008). Univariable and multivariable analysis of risk factors inherent to the animal (age,

breed, sex, and body condition) and altitude were performed with the >2 mm cut-off only and using, as mentioned above logistic regression models. The results were expressed in odds ratio, 95% confidence interval for the odds ratio and p-values.

Results

BTB prevalence in cattle

Individual prevalence stratified by Woreda and year are shown in table 1. Overall, a total of 5377 cattle were tested with the CIDT in the 3 Woredas over a period of 3 years. Each Woreda had a unique temporal pattern of prevalence over the years, but prevalence by Woreda was not statistically significantly changing over the years, with the exception of the second year in Meskan, where the peak prevalence of 7.9% (cut off >2 mm) differed significantly from the two other years with lower prevalences (p = 0.13).

The lowest prevalence was found in Woldia (0.3 % and 2.2% when using >4 and >2 mm cut-off respectively). Prevalence with the >2 mm cut-off was 3.3 times higher than with the >4 mm cut-off in Bako-Gazer and 7.3 times higher in Woldia. Prevalence in the lowlands of Woldia was lower (1.7%) than in the highland zone (2.9%) but the results were not statistically significant (OR: 0.6; CI:0.1;3.1; cut-off >2 mm). True prevalence could be estimated only when using a >2 mm cut-off for Meskan and Bako-Gazer which had an overall prevalence of 4.5% (CI: 2.9%-6.1%) and 2.4% (CI: 0.9%-3.9%) respectively. It could not be estimated for Woldia due to low apparent prevalence and low sensitivity of the tuberculin test (65%).

Herd prevalence was, when using the 4 mm cut-off, the lowest in Woldia (6 positive herds out of 22, 27.3%) and the highest in Bako-Gazer with 13 positive herds out of 19 (68.4%). In Meskan, 14 herds out of 21 were positive (66.7%). When using the 2 mm cu-off, herd prevalence reached 100% in Meskan and Bako-Gazer, whereas 20 villages out of 22 were positive (91%) in Woldia.

Risk factors for positive CIDT

Since only three Borans and three Holstein were tested that were negative for the CIDT, these animals were excluded from the univariable and multivariable analysis. The results

of the univariable analysis are shown in table 2. Sex was significant in the univariable analysis including all three Woredas with bulls and oxen showing an OR of 1.6 (OR 95%: 1.1-2.3) and 2 (OR 95%: 1.4-2.6), respectively, when compared to females. Animals with good (musculous to fat) body condition were more at risk for a positive CIDT (OR: 2; OR 95%: 1.5-2.9) than animals with a normal body condition form. Juvenile cattle aged 1-2 years had an OR smaller than 1 for BTB positivity (overall OR: 0.5, OR 95%: 0.3-0.8) when compared to adult breeding cattle (=>3 – 10 years). Oxen accounted for 32.8% (N=1268) of the tested cattle; they were older than 5 years, which is the earliest castration age and 97.4% of the animals were in good body condition.

The analysis of the different breed categories had to be conducted separately for each Woreda since Male cattle were only found in Bako-Gazer and Kola only in Woldia. In Woldia, 36.2% of the tested cattle were Kola. The proportion of positive animals in Woldia was 1.6% in Kola compared to 3.2% in unclassified zebus (OR: 0.5; CI: 0.2; 1.0). The proportion of adults (animals older than three years) was higher in Kola (91%) than in unclassified zebus (75.3%). The sex composition was similar in the Kola as in the unclassified zebu group. Male, in Bako-Gazer accounted for 4.8% of the tested cattle. The proportion of positive cattle was higher in the Male cattle (5/78) than in the unclassified zebus (72/1528), but this effect was statistically not significant. The proportion of adult animals was higher in Male (97.3%) than in unclassified zebus, with animals older than four years accounting for 51.3% of the Male animals. Male cattle had a higher proportion of females (74.4%) than unclassified zebus (50.3%). Cross-breeds were present in all Woredas but in such low numbers (overall N = 58; 1.1%) that no analysis has been performed.

The multivariable analysis confirmed in general the findings of the univariable analyses. The results are shown in table 3. Overall, oxen and animals in good body condition were significantly associated with positive BTB reactors. This was also the case in Meskan. In contrast, none of the variables were significantly associated with positive CIDT in Woldia and only the oxen in Bako-Gazer alone were associated with positive reactors.

M. avium reaction

M. avium positive reactions were found in all 3 Woredas (overall N=188), but statistically differed by regions. No *M. avium* reaction was found in calves younger than 1 year. In Woldia the *M. avium* prevalence was nearly 3 times lower than in Meskan (data not shown). The highest prevalence was found in Meskan (4.7%, CI: 3.4; 6.3%), followed by Bako-Gazer (3.6%; CI: 2.8: 4.8%) and Woldia (1.7%; CI: 1.1; 2.6%).

The univariable analysis showed that year, sex, age and breed were not significantly associated with positive *M. avium* reaction. However, body condition was shown to influence positivity with animals in good condition having an OR=1.6 (CI: 1.0; 2.3) when compared to normal body condition as was the case with *M. bovis* tuberculine positivity. In the multivariable analysis, only animals older than 10 years were significantly associated with *M. avium* positivity (OR: 0.4; CI OR: 0.2; 0.7) rather than younger animals.

Mixed PPD reactions

Hundred and nine animals (2%) showed swellings greater than four millimeter at the avian as well as at the bovine PPD injection sites. Out of these animals, five were classified as positive BTB reactors when OIE definition applied.

Draft animals

Overall, 60% (34/57) of all BTB positive (4 mm cut-off) and 38% (72/188) of all MAC positive animals were oxen, thus draft animals.

Thirty-three animals, all oxen, were classified as draft animals and additional interview information on their stamina was available. Twenty two were BTB positive (67%) using the 2 mm cut-off. Half of these animals (n=11) were said to be weaker and less able to do field work during the last year. In the contrary, only 1 out of the 11 PPD negative oxen (9%) was said to be weak. Statistical difference was found when tabulating PPD results and animal stamina ($p = 0.02$).

Discussion

In order to embark in an efficient national BTB control and/or elimination program, infected cattle need to be identified accurately and in early stages of the disease. Failure to do so will lead to continued transmission of the disease. The detection of BTB in live animals using the comparative intra-dermal tuberculin test is recognized as official test in most countries. The test uses purified protein derivates (PPD), a cocktail of mycobacterial antigens with suboptimal specificity. In Ethiopia, Ameni and others (2008) re-evaluated the OIE cut-off recommendation of > 4 mm and suggested the > 2 mm cut-off as more appropriate for Ethiopia. In this study, the authors found an overall prevalence of less than 1% when using the >4 mm cut-off, which is much lower than what was found in previous studies from Central Ethiopia, where prevalence in zebus between 7.9% and 11.6% were described when using the same cut-off. Those latter studies, however, were carried out in an urban/peri-urban region having higher numbers of exotic breeds (Holstein cows and their cross breeds), more intensive livestock keeping systems and higher burden of BTB. In the region of Addis Abeba, BTB prevalence was shown to be between 22 % and 34% in exotics (Ameni and others 2007, Tsegaye and others in press). In contrast, our present study included only rural areas characterized by local zebu breeds and traditional smallholder mixed farming practice. Rural versus peri-urban as well as breed differences of *M. bovis* prevalence in Africa are not new and have already been described in the first half of the twentieth century (Von Ostertag and Kulenkampff 1941).

Considering the absence of effective control in most of the sub-Saharan countries of Africa, these observations show the extraordinary persistence of low level transmission of bovine tuberculosis from animal to animal in rural areas despite the wide distribution of the disease in the Woredas (high herd-village prevalence). Abattoir studies were carried out in the same study areas and confirmed our low prevalence: out of 37 000 inspected cattle only 4% showed tuberculous-like lesions with *M. bovis* isolated from 58 out of 171 acid-fast cultures (Berg and others in press). With the given data the authors could not detect a significant temporal trend, although variations over the years were observed. Within a Woreda, always the same villages were tested during the same month of each year to avoid seasonal bias and the same person performed all the tests to avoid bias

related to the testing method. The PPD reagents were all from the same manufacturer but from different batches. Batches could have been influenced by transport, temperature, and storing. Also, the variations could have simply been explained by sampling variations. However, seasonality could have influenced variation between the three different Woredas to some extend since each Woreda was tested during different time in the year.

Our observations indicate endemic stable transmission at a comparatively low prevalence, similarly to the observations made by Cleaveland and others (2007) in Tanzania. Other authors, however, have found high prevalence in zebu cattle in other African countries (Bongo and others 2009). The authors observed in Meskan Woreda over the years a general increase of Holstein cattle, originating from Addis Abeba and vicinity, thus highlighting the danger of potential spread of the disease from urban areas characterized by high BTB prevalence into low BTB prevalence rural areas either by movement of live exotic breeds or potentially through their semen (artificial insemination) (Niyaz and others 1999).

The apparent small variation in BTB prevalence over the years in Bako-Gazer, although showed to be statistically not significant could be explained by various factors varying over the years and acting on an already low existing BTB prevalence, such as elimination of positive animals, and/or presence of immunosuppressive factors such as disease epidemics, heavy parasitism, nutritional factors and involvement of MAC agents [*Mycobacterium avium* complex] (Lepper and others 1977, Doherty and others 1996, Dunn and others 2006,). However, although there is a geographical pattern of *M. avium* positivity, data suggested that *M. avium* reaction did not vary significantly during the time of the study; therefore it was unlikely to have influenced the variation in BTB prevalence over the years. Studies have shown that co-infection with paratuberculosis compromises skin test results by drastically decreasing sensitivity of the test (Walravens and others 2002, Aranaz and others 2006, Alvarez and others 2008). Similarly, Amadori and others (2002) showed in a trial that cattle sensitized by mycobacteria of the avium and intracellulare group, concealed *M. bovis* for a certain period of time. The CIDT can

fail to diagnose *M. bovis* infection because *M. avium* was shown to provide a certain degree of immunity against *M. bovis* (Hope and others 2005). No studies have been done so far on the prevalence of paratuberculosis (Johne's disease) in Ethiopian cattle and it is not know to what extend such a disease could compromise the detection of BTB in rural Ethiopia using the CIDT and thus leading to false negative animals. It is likely that also other co-infections like Fasciola spp. may affect CIDT response (Flynn and others 2006). More research is needed to assess CIDT response in relation to co-infections in Ethiopian cattle before coming to definitive conclusions regarding BTB prevalence. Co-infection and poor animal health could be a major source of false negative results indicating the need to interpret survey results with caution.

Animals in good body condition showed more CIDT positive responses than normal and thin animals (OR: 2; CI:1.5;2.9) – a finding many other authors also have shown (Delafosse and others 1995). This suggests that animals in poor body condition may have a compromised immune response in response to PPD injections. Positive *M. avium* reaction was significantly associated with positive *M. bovis* reactor (p =0.006). Mixed *M.avium* and *M.bovis* skin reactions greater than 4 mm each were found overall in 104 of the 5371 tested animals but these were classified as BTB negative animals according to the author's definition of positivity since both sites reacted equally strong. The question arises whether this phenomenon reflects a true mixed infection of both agents, thus classifying the animals as BTB positive or whether the animals have a generally sensitized unspecific immune reaction unrelated to any mycobacterial infection. Strain isolation from animals showing mixed skin reactions is warranted to answer this question.

Using a 2 mm cut-off increased the apparent prevalence 3 to 7 fold depending on the region. Reducing the cut-off increases the number of positive animals. If removed from the herd and in the absence of any compensation model in resource poor countries, the losses are to the farmer. The question remains in these countries: who should pay for a control scheme involving culling of positive animals, a strategy that has been shown to be very effective in industrialized countries where large financial means have been invested for a compensation approach? The cost of false negative diagnosis however, very likely

outweighs the cost of false positive diagnosis as undetected sick animals would contribute to the spread of the disease (Bongo and others 2009). Furthermore, the accuracy of a 2 mm skin result difference is questioned. Bias might be related to the reading of skin thickness using a cut-off as small as 2 mm, e.g. cuts caused by shaving the injection sites might lead to minor skin inflammation and thus increase skin thickness, animals are not standing still during reading, pressure on the caliper by the person reading the skin thickness

The authors observed that overall oxen were at higher risk for being positive reactors (OR: 2; CI: 1.4; 2.6). Farmers usually took extra care of their oxen by feeding them well compared to the other sex classes because oxen are essential workforce for ploughing, threshing and harvesting. This study could not explain why these risk factors varied by Woredas, for instance why neither oxen nor body condition score influenced CIDT results in Woldia. There may be an environmental or husbandry explanatory component. If oxen are more at risk for BTB, the whole agriculture, particularly crop farming, could be affected by reduced work force. It is therefore important to assess the impact of the disease on the daily working capacity of draft animals. In the current study, BTB appeared to significantly affect the stamina of oxen who are central to crop farming in Ethiopia and thus to the national economy. However the chosen sample size was too small to draw final conclusions and further research is needed in that particular field.

The authors assessed the effect of altitude on BTB prevalence in Woldia, where cattle in the highlands (> 2000 m) were more frequently tuberculin positive than in cattle from regions in lower altitude (<2000 m) but the difference was not significant statistically. For human TB, it is evidenced knowledge that TB is less prevalent in high altitude (Saito and others 2006). Though known to be linked with changes in alveolar oxygen pressure, the exact mechanism behind the effect of higher altitude on tuberculosis is not fully established yet (Saito and others 2006). Most extra-pulmonary TB cases recorded in the DOTS in the hospital of Woldia were coming from the lowlands (unpublished data). More research should be undertaken to assess the impact of altitude on BTB in cattle and

assess whether the same mechanism would apply for BTB as for TB (while considering cattle keeping practices, which can sometimes vary between lowlands and highlands).

Knowing the individual and herd/village prevalence of BTB in rural areas can contribute to the choice of a future control program of BTB in Ethiopia. In light of our results, the question arises if the CIDT alone is accurate enough to detect infected animals in rural areas which seem to be characterized by very low BTB prevalence, high herd/village prevalence and high burden of other infectious diseases and parasitism and which cut-off would be more suitable in such a situation. Should the standard skin testing be coupled with blood tests such as the IFN-γ test to increase sensitivity of detection of positive animals? Ethiopia has with 43 million heads the biggest cattle herd in Africa (CSA 2008). Therefore a mass vaccination as it was done for instance in Malawi (Ellwood and Waddington 1972) or a test and slaughter program of all herds is not feasible financially and logistically, as long as the State will not be able to compensate farmers for culled animals. However, a test and slaughter program might be economically feasible in some regions with very low BTB prevalence. It is therefore essential to know the BTB status in each region. Furthermore, "zoning" could help keeping low BTB prevalence in regions that are at low risk. A future challenge will be to avoid the spread of the disease into low prevalent rural areas, by the distribution of Holsteins or Holstein semen in order to improve milk cattle, coming from Central Ethiopia with its high BTB prevalence, in case no other measures are in place in the highlands.

M. tuberculosis was isolated in cattle from abattoir samples, thus indicating a human-animal transmission (Berg and others in press). Considering the high prevalence of human TB in Ethiopia (WHO 2008), and the shown transmission to cattle, the question arises to what extend a *M. tuberculosis* infection in cattle would affect the CIDT. This question needs further research and attention, especially when considering the zoonotic potential of both *M. bovis* and *M. tuberculosis*.

In conclusion, this study showed a very low apparent prevalence of BTB in cattle of rural Ethiopia in contrast to Central Ethiopia. The CIDT results were likely to be influenced by

many factors such as the testing method, pre-sensitization with non-tuberculous mycobacteria and other infectious diseases. Interpretation of results should therefore be done with caution. Inconsistency of results regarding risk factors for positive reactors inherent to animals by regions highlighted the danger to generalize statements and that results should be rather interpreted regionally within Ethiopia. Finally, this study suggests a possible role of MAC agents in the general tuberculosis epidemiology, which should be further investigated.

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Table 1: Prevalence of BTB in the selected study sites using 4 and 2 mm cut off (calculated using a logistic regression model with a random effect on villages)

Study sites			all years	Year 1	Year 2	Year 3
All Woredas	Number animal		5377	1736	1761	1880
	Number BTB reactors	4 mm cut-off	57	15	26	16
		2 mm cut-off	238	79	85	74
	Prevalence (LCL-UCL) in %	4 mm cut-off	0.9 (0.6;1.3)	0.7 (0.3;1.8)	1 (0.5;2)	0.8 (0.5;1.4)
		2 mm cut-off	4 (3.4;4.8)	4.1 (3.1;5.5)	4.2 (3.1;5.7)	3.7 (2.7;4.9)
Meskan	Number animal		1838	624	590	624
	Number BTB reactors	4 mm cut-off	27	4	14	9
		2 mm cut-off	111	28	47	36
	Prevalence (LCL-UCL) in %	4 mm cut-off	1.3 (0.7;2.1)	0.3 (0.02;3.3)	2 (1.0;4.4)	1.44 (0.7;2.7)
		2 mm cut-off	6 (4.9;7.3)	3.9 (2.3;6.5)	7.9 (6; 10.4)	5.7 (4.1;7.9)
Woldia	Number animal		1923	620	629	674
	Number BTB reactors	4 mm cut-off	8	2	4	2
		2 mm cut-off	49	22	13	14
	Prevalence (LCL-UCL) in %	4 mm cut-off	0.3 (0.1;1.1)	0.3 (0.08;1.3)	0.6 (0.2;1.7)	0.2 (0.07; 1.2)
		2 mm cut-off	2.2 (1.5;3.3)	3.2 (1.8; 5.5)	1.4 (0.5;3.8)	2 (1.2;3.4)
Bako-Gazer	Number animal		1616	492	542	582
	Number BTB reactors	4 mm cut-off	22	9	8	5
		2 mm cut-off	78	29	25	24
	Prevalence (LCL-UCL) in %	4 mm cut-off	1.4 (0.9;2)	1.8 (0.9;3.5)	0.7 (0.2;3.3)	0.9 (0.3;2)
		2 mm cut-off	4.6 (3.5;6)	5.8 (4;8.4)	4.3 (2.6;7)	3.5 (1.9;6.3)

Table 2: Univariable analysis of cattle variables in the study sites combining all years (logistic regression with random effect on village)

Variable		Overall			Meskan			Woldia			Bako-Gazer		
		Number (%) animals	p-value	OR (95% CI)	Number (%) animals	p-value	OR (95% CI)	Number (%) animals	p-value	OR (95% CI)	Number (%) animals	p-value	OR (95% CI)
Sex	Female	2507 (46.6)			783 (42.6)			889 (46.3)			835 (51.7)		
	Bull	1106 (20.6)	0.013	1.6 (1.1; 2.3)	784 (42.6)	0.8	1.1 (0.6; 2.0)	293 (15.2)	0.6	1.3 (0.5; 3.0)	480 (29.7)	0.005	2.2 (1.3; 3.9)
	Ox	1764 (32.8)	0.0001	2 (1.4; 2.6)	785 (42.6)	0.003	1.9 (1.2; 3.0)	741 (38.5)	0.2	1.4 (0.8; 2.7)	301 (18.6)	0.001	2.6 (1.4; 4.8)
Age	Calves (<1 yr)	257 (4.8)	0.2	0.6 (0.3; 1.3)	65 (3.5)	0.9	0.9 (0.3; 2.6)	123 (6.4)	0.6	0.7 (0.1; 2.9)	69 (4.3)	0.2	0.3 (0.04; 2.0)
	Juvenile (1-2 yr)	752 (14)	0.007	0.5 (0.3; 0.8)	290 (15.8)	0.017	0.4 (0.2; 0.8)	244 (12.7)	0.3	0.6 (0.2; 1.7)	218 (13.5)	0.2	0.6 (0.3; 1.3)
	Breeder (=>3-10 yr)	3371 (62.7)			1175 (63.9)			1268 (66)			928 (57.4)		
	Old (>10 yr)	997 (18.5)	0.6	1.1 (0.8; 1.5)	308 (16.8)	0.7	1.1 (0.7; 1.8)	288 (15)	0.3	1.5 (0.7; 3.0)	401 (24.8)	0.7	0.9 (0.5; 1.6)
Body condition	Normal	2269 (61.3)			657 (54.1)			993 (74.5)			619 (53.7)		
	Emaciated to thin	335 (9.1)	0.7	0.9 (0.4; 1.7)	73 (6)	0.4	0.5 (0.1; 2.3)	168 (12.6)	0.8	0.9 (0.2; 3.0)	94 (8.2)	0.5	1.4 (0.5; 3.8)
	Musculous to fat	1095 (29.6)	0.004	2 (1.5; 2.9)	484 (39.9)	0.002	2 (1.3; 3.3)	172 (12.9)	0.5	1.4 (0.5; 3.9)	439 (38.1)	0.1	1.5 (0.8; 2.8)
Altitude	Continuous	5377	0.35										

Table 3: Multivariable analysis (calculated using the 2 mm cut-off and logistic regression with random effect on village)

		Overall			Meskan			Woldia			Bako-Gazer		
Variable		Number (%) animals	p-value	OR (95% CI)	Number (%) animals	p-value	OR (95% CI)	Number (%) animals	p-value	OR (95% CI)	Number (%) animals	p-value	OR (95% CI)
Sex	Female	2507 (46.6)			783 (42.6)			889 (46.3)			835 (51.7)		
	Bull	1106 (20.6)	0.5	1.2 (0.7;2.0)	784 (42.6)	0.4	1.3 (0.6;2.9)	293 (15.2)	0.7	0.7 (0.1;3.5)	480 (29.7)	0.4	1.4 (0.6;3.1)
	Ox	1764 (32.8)	0.001	2.0 (1.3;3.0)	785 (42.6)	0.02	2.0 (1.1;3.7)	741 (38.5)	0.7	1.2 (0.5;3.1)	301 (18.6)	0	3.1 (1.4;7.0)
Age	Calves	257 (4.8)			65 (3.5)			123 (6.4)			69 (4.3)		
	Juvenile	752 (14)	0.7	0.8 (0.3;2.0)	290 (15.8)	0.1	2.6 (0.8;8.5)	244 (12.7)	0.5	0.5 (0.06;4.3)	218 (13.5)	0.3	0.3 (0.04;2.7)
	Breeder	3371 (62.7)	0.056	0.5 (0.2;1.0)	1175 (63.9)	0.2	0.5 (0.2;1.5)	1268 (66)	0.6	0.6 (0.1;3.0)	928 (57.4)	0.2	0.4 (0.1;1.5)
	Old	997 (18.5)	0.7	0.9 (0.6;1.4)	308 (16.8)	0.6	1.2 (0.6;2.1)	288 (15)	0.7	1.8 (0.7;4.2)	401 (24.8)	0.1	0.6 (0.3;1.2)
Body condition	Normal	2269 (61.3)			657 (54.1)			993 (74.5)			619 (53.7)		
	Emaciated to thin	335 (9.1)	0.9	1.0 (0.5;2.0)	73 (6)	0.3	0.4 (0.09;2.0)	168 (12.6)	0.9	1.0 (0.3;3.8)	94 (8.2)	0.4	1.6 (0.5;4.7)
	Musculous to fat	1095 (29.6)	0.013	1.6 (1.1;2.2)	484 (39.9)	0.05	1.6 (1.0;2.7)	172 (12.9)	0.7	1.2 (0.4;3.5)	439 (38.1)	0.6	1.2 (0.6;2.2)

Table 4: Univariable analysis of MAC results in all study sites combining all years (logistic regression with random effect on village)

		Overall			Meskan			Woldia			Bako-Gazer		
Variable		Number (%) animals	p-value	OR (95% CI)	Number (%) animals	p-value	OR (95% CI)	Number (%) animals	p-value	OR (95% CI)	Number (%) animals	p-value	OR (95% CI)
Sex	Female	2507 (46.6)			783 (42.6)			889 (46.3)			835 (51.7)		
	Bull	1106 (20.6)	0.8	0.9 (0.6;1.4)	784 (42.6)	0.051	0.4 (0.2;0.9)	293 (15.2)	0.8	0.9 (0.3;2.4)	480 (29.7)	0.2	1.5 (0.8;2.6)
	Ox	1764 (32.8)	0.1	1.3 (0.9;1.8)	785 (42.6)	0.055	1.5 (0.9;2.4)	741 (38.5)	0.6	0.8 (0.4;1.8)	301 (18.6)	0.9	1 (0.4;2.2)
Age	Calves (<1 yr)	257 (4.8)			65 (3.5)			123 (6.4)			69 (4.3)		
	Juvenile (1-2 yr)	752 (14)	0.9	0	290 (15.8)	1	0	244 (12.7)	1	0	218 (13.5)	1	0
	Breeder (=>3-10 yr)	3371 (62.7)	0.1	0.7 (0.4;1.1)	1175 (63.9)	0.02	0.3 (0.2;0.8)	1268 (66)	0.3	0.5 (0.2;1.8)	928 (57.4)	0.3	1.4 (0.7;2.8)
	Old (=>10 yr)	997 (18.5)	0.1	0.7 (0.5;1)	308 (16.8)	0.3	0.7 (0.4;1.4)	288 (15)	0.2	0.5 (0.1;1.5)	401 (24.8)	0.4	0.8 (0.4;1.5)
Body condition	Normal	2269 (61.3)			657 (54.1)			993 (74.5)			619 (53.7)		
	Emaciated to thin	335 (9.1)	0.8	0.9 (0.4;1.8)	73 (6)	0.3	0.3 (0.5;2.7)	168 (12.6)	0.2	1.9 (0.7;4.8)	94 (8.2)	0.4	0.6 (0.1;2.4)
	Musculous to fat	1095 (29.6)	0.015	1.6 (1.1;2.3)	484 (39.9)	0.07	1.7 (0.9;2.9)	172 (12.9)	0.9	0.9 (0.3;3.1)	439 (38.1)	0.7	1.1 (0.6;2.0)
Altitude	Continuous	5377	0.16										

11. Risk factors of Bovine Tuberculosis in cattle in rural livestock production systems of Ethiopia

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Abstract

This study shows a representative stratified cluster sample survey of comparative intradermal tuberculin test apparent prevalence in cattle, using a cut off for positivity of 2 mm, from four regions in Ethiopia and assesses possible risk factors for tuberculin positive reaction in cattle. Seventy-three villages in 24 Kebeles (administrative units) were randomly selected, within which 2216 cattle from 780 owners were tested. In addition, 450 of these cattle owners were interviewed for risk factor assessment. Ninety-nine percent of the tested cattle in this rural livestock production system were traditional zebus. The individual overall cattle BTB prevalence was 3%, with the highest prevalence found in Meskan Mareko, in Central Ethiopia (7.9%) and the lowest in Woldia, in the North East edge of the Rift Valley (1.2%). Generalized Linear Mixed Models (GLMM) with random effect on Kebeles was used to analyze risk factors of cattle reactors and human tuberculosis infection. Purchase of cattle and presence of other livestock in the herd were statistically significant, with OR: 1.7, p-values of 0.03 and OR: 2, p = 0.05, respectively. Family members diagnosed with TB or showing clinical signs of extra pulmonary TB (EPTB) were reported in 86 households (19%). None of the assessed potential risk factors of disease transmission between cattle and human (food consumption, livestock husbandry, presence of BTB positive cattle) were statistically significant.

Key words: Ethiopia, bovine tuberculosis, *Mycobacterium bovis*, prevalence, cattle, risk factors

Introduction

Bovine tuberculosis (BTB) is an infectious disease caused by *Mycobacterium bovis*, a member of the *Mycobacterium tuberculosis* complex (MTC), which also comprises the closely related *M. tuberculosis*, the major causative agent of human tuberculosis (TB). (Van Soolingen et al., 1994). Around 9 million new cases of human tuberculosis and 2 million deaths are reported annually worldwide (CDC 2007), with sub-Saharan Africa displaying the highest annual risk of infection with tuberculosis probably catalyzed by the HIV/AIDS pandemic (Corbett et al. 2003). It has been estimated that *M. bovis* accounts globally for 3.1% of all human TB cases (2.1% of all pulmonary and 9.4% of all extra pulmonary TB cases) (Cosivi et al., 1998). However, the extent of *M. bovis* involvement in the global TB burden in Africa is still largely unknown. This can be partly explained by the fact that in humans, TB due to *M. bovis* is indistinguishable from that due to *M. tuberculosis* in terms of clinical signs, radiological and pathological features (Grange 2001). In addition, most laboratories in sub-Saharan Africa have not the capacity to differentiate *M. bovis* from *M. tuberculosis (*Ayele et al. 2004*).*

The epidemiology of *M. bovis* was described by numerous authors in extensive detailed reviews, however they tend to focus mainly on experiences from industrialized countries, where control and/or eradication programs have been implemented since a long time (O'Reilly and Daborn 1995, Cosivi et al., 1998, Pollock and Neill 2002, Wedlock et al., 2002, Phillips et al., 2003, Neill et al., 2005). Although cattle are considered to be the main hosts of *M. bovis*, the disease has been reported in many other species, including humans, other domesticated animals and wildlife (De Lisle et al., 2002). Infectious animal shed *M. bovis* via milk, saliva, feces/urine and discharging lesions (Phillips et al., 2003). It is generally accepted that human beings get infected either by inhalation of bacteria-containing dust-particles and aerosols shed by infected animals or by ingestion of contaminated animal products (e.g. raw milk) (Cosivi et al.1998). The main route of infection in cattle is through aerosol exposure, facilitated by close contact between animals (Neill et al.1991). In cattle, ingestion of contaminated products (e.g. pasture, water) is generally considered to be rather a secondary less important route of transmission (Menzies and Neill 2000). A high prevalence of *M. bovis* were found in a

recent abattoir survey in Chad in culture positive mammary glands and in young tuberculin positive animals (Müller B. personal communication). Jha et al (2007) isolated *M. bovis* in milk and feces from milking buffaloes and cattle in Nepal. This indicates that transmission to young animals by milk should not be neglected. Recent publications from Africa also suggest that ingestion of *M. bovis* might be an important mode of disease transmission in cattle, since mesenteric lymph nodes were shown to be more affected than mediastinal lymph nodes (Cleaveland et al. 2007, Ameni et al. 2007). Therefore, also contaminated environment might play a bigger role in the epidemiology of BTB than assumed until now, thus showing that our understanding of the epidemiology of *M. bovis* in sub-Saharan Africa is still limited.

Indeed, also little information on risk factors of disease transmission to cattle, between cattle and from cattle to humans is available from the African context and most information is extrapolated from experiences in the industrialized world. For Africa, the most comprehensive epidemiological studies have been done so far in Tanzania (Kazwala et al. 2001, Cleaveland et al. 2007, Mfinanga et al. 2004) and Uganda (Oloya et al. 2007). However, despite the paucity of information, it is generally accepted that besides causing major economic losses, BTB poses also a serious zoonotic threat in Africa (Ayele et al. 2004).

Bovine TB has been shown to be endemic in cattle from Ethiopia. However, most published data were obtained from central Ethiopia (Ameni et al. 2003, Asseged et al. 2004, Teklul et al. 2004, Ameni et al. 2007).

In this paper, we mainly attempt to assess possible risk factors for bovine tuberculosis in cattle and humans in rural Ethiopia. We also present briefly the results of a representative multi-stage cluster sample survey on BTB prevalence in cattle from four regions of Ethiopia for better understanding of the context.

Material and methods

Study sites

The cross-sectional study was carried out between November 2006 and May 2007 in the frame of a North-South BTB research collaboration, in 3 out of the 7 regional zones of Ethiopia (Oromia, Amhara, and Southern Nations, Nationalities and People Region (SNNPR)) between the latitudes of 5.1°N and 11.5°N and the longitudes of 36.1°E and 40.1°E. Within these regions, six Woredas (districts) were selected according to the further requirements of the study as a whole (e.g. presence of abattoirs, hospitals, wildlife), namely Woldia (Northern highlands), Meskanena Mareko (Rift Valley), Bako Gazer (Southern middle lands), Dinsho, Robe and Goro. The latter three Woredas were combined into one study area, the Bale Mountains, which is a highland area adjacent to a national park. Altitude of the study sites varied between 1300 m and 4200 m above sea level. Although belonging to different ethnic groups with different culture and religion, all farmers were sedentary small holders with similar mixed livestock-crop farming system.

Tuberculin survey in cattle

Sampling of cattle

Cattle herds were selected by a stratified cluster sampling proportional to the size of the cattle population, in which the unit village was considered as a cluster. Sample size was calculated using the formula described by Bennet et al (1991).

The standard error $s.e._x$ which measures the precision of our estimate is given by (1):

$$(1) \qquad s.e._x = \sqrt{\frac{pqD}{n}} = \sqrt{\frac{pqD}{cb}}$$

Roh (ρ), which describes the rate of homogeneity, thus the variability is given by ¨(2):

$$(2) \qquad \rho = \frac{(WithinHerdVariation)}{(TotalVariation)}$$

$$(3) \qquad D = 1 + (b-1)\rho$$

We take roh (ρ) as 0.2, and using formula we obtain a design effect D of 6.8 (3). Choosing 30 animals per cluster, with a disease prevalence of 5% and 17 clusters (total sample size per Woreda = 510 animals) gives us an estimate of the standard error (1) or precision of 0.025. The total sample size per Woreda is given by n=b*c, thus 510 animals, which gives us a total number of required animals of 2040 for all 4 Woredas. A complete list of Kebeles and villages within the Kebeles was obtained from each Woreda agricultural office. Kebeles within the Woreda were selected randomly using random numbers generated in Microsoft Excel®; villages were selected randomly and proportionally to their number within a particular Kebele. Since cattle of all villagers are kept together, at least during the day on grazing areas and for drinking, each village was considered as one big cattle herd for assessment of tuberculin reactivity status. Thirty animals were selected from a minimum of five and a maximum of 15 owners per village. In general, not all animals per owner were tested since as many owners as possible per village were included in the study either randomly from a list of all owners (where owner number higher than 15) or including the total number of owners gathered at the place where the tuberculin testing was performed (where owner number was less than 15). Animals younger than 6 months, late stage pregnant cows and clinically sick animals were excluded from the testing. Possible sampling bias was introduced by the process that the owner himself decided which animals fulfilling the inclusion criteria were tested. Farmers were asked to gather their animals at a certain point in or in the proximity of the village (e.g. communal pasture, water point, middle of the village) for testing and reading. If animals were not at the meeting point during the reading day, house to house visit was conducted. As compensation and incentive for farmer's participation, all tested cattle were dewormed on the reading day with Albendazol boli (Ashialben 2500, Ashish Life Science PVT, Mumbai, India).

Tuberculin testing of cattle

Always the same person conducted the tuberculin testing and reading of the result to avoid bias related to injection and reading technique. The comparative intradermal tuberculin test was conducted in all cattle using both avian and bovine purified protein derivates (PPD) supplied by the Veterinary Laboratories Agency, Weybridge, UK. Intradermal injections of 0.1 ml (2,500 IU/ml) bovine PPD and 0.1 ml (2,500 IU/ml)

avian PPD were made in two shaved sites, 12 cm apart from each other in the middle neck region, after having recorded skin thickness with a caliper. Skin thickness was measured again at both injection sites after 72 hours. The reaction at each site was derived as the difference of the skin thickness after 72 hours minus the thickness before injection. An animal was considered positive if the bovine minus the avium reaction was greater than 2 mm (Ameni et al. 2008). A village herd was considered positive if it had at least one positive tuberculin reactor.

Interview of cattle owners

Cattle owners were interviewed according to their willingness to participate and given verbal consent, on the same day their cattle were tested for BTB. Interviews were made in all sites in Amharic by a trained interviewee. Questionnaires included closed and open questions on livestock husbandry/management and household characteristics, such as herd size and structure, presence of other livestock, vaccination /deworming of cattle, mixing of cattle and other livestock at night, cattle housing at night, watering and grazing system, reproduction, cattle contact with other cattle herds, purchasing of animals. Furthermore, questions were asked related to human consumption habit, contact between humans and cattle, TB knowledge and known TB status in the household. A household was considered positive for TB if at least one member in the last five years had been diagnosed with pulmonary TB or showed clinical signs of extra pulmonary TB (EPTB) (e.g. cervical lymphadenitis). In addition, focus group discussions were conducted in the villages.

Geographic coordinates and altitude were registered at the central point of each village by Global Positioning System (GPS).

Statistical analysis

Data were double entered in Access, validated with EpiInfo (version 3.3.2) and analyzed with the software package STATA/IC 10.1 (StataCorp, Texas, USA). Analysis of potential risk factors for cattle being positive for BTB and variance component estimation, were performed using Generalized Linear Mixed Models with binary outcome and logit link function (GLLAMM add-on). The exploration of the different variance components of each stage of the multistage cluster sampling indicated that the random

effect variances were mainly associated with Woreda and Kebele. In contrast, the variance components of owner and village level were <0.0001. Therefore, we included in all models Kebele as random and Woreda as fixed effect.

Results

Prevalence of tuberculin positive cattle

Seventy-three villages in 24 Kebeles in the 4 Woredas were selected, of which a total of 2216 cattle from 780 cattle owners were tested for BTB. Ninety-nine percent of the tested animals were traditional zebus, 1% accounted for exotic Holstein breed and cross breeds (Holstein x zebu). The overall apparent individual animal prevalence of tuberculin reactors was 3.1% (95%CI: 2; 4.8) but varied significantly between the Woredas with the highest prevalence in Meskan Mareko and the lowest in Woldia (table 1). Forty-nine out of 73 tested village herds had at least 1 positive tuberculin reactor, giving an overall herd prevalence of 67%.

Descriptive epidemiology

Four hundred and fifty farmers were interviewed. Fifty-six owners (12.4%) had PPD positive cattle, and among these farmers 24% reported tuberculosis cases in their household. Cattle fodder consisted of 90% pasturing and crop residues after harvest. Fifty-four percent and 31% of the farmers vaccinated their cattle against blackleg and/or pasteurellosis and dewormed on a regular basis.

Livestock other than cattle were kept in mixed herding systems in 70% of the interviewed farms interviewed (31% goats, 45% sheep, 25% donkey, 9% horses and 2% camels). Sixty-two percent of the cattle herds grazed on communal pasture either full time or part time, 81% were watered at the river and 99% of the herds had year long close contact with other cattle herds during communal grazing and/or watering, veterinary campaigns, communal harvesting-ploughing and/or threshing. Overall, nearly half of the farmers (46%) kept cattle inside the living housing at night. Natural service for cow fertilization was used in 92% of the farms with 54% of the farmers using a bull from a neighboring farm for reproduction. Encounters between wildlife and cattle were overall rare (observed and reported by 19% of interviewees with the exception of the Bale mountains national park, where 59% of farmers stated that wildlife share common habitat with cattle.

Herding was mainly done by children (37%) or children and men combined (50%). Twelve percent of the herds were looked after by adult men, whereas women were rarely involved in herding (0.4%). Herds were not looked after at all in 0.7% of the interviewed households. In contrast, milking of cows was mainly carried out by women (45.5%) or adult men and women combined (44.5%). In 9.5% of the interviewed households adult men were milking cows. Children were rarely involved in milking tasks (0.5%). Eighty-one percent of the farmers did not boil the drinking milk and 74% ate raw meat. Farmer's knowledge of clinical signs of TB either in humans or in cattle was in general high (70%). However, we noticed during focus group discussions that farmers often did not know that the disease could be transmitted from cattle to humans.

Risk factors for positive PPD in cattle

We assessed 23 potential risk factors for BTB positivity in cattle. Variables with more than 10% missing values and variables, which are assumed to be redundant, were excluded from the analysis to ensure sufficient power and to avoid co-linearity in the multivariable model. In case of co-linearity, we included the biologically more plausible variable in the multivariable model. The significant variables resulting from the univariable analysis were purchase of cattle (OR: 1.7, CI OR: 1; 2.9, p= 0.04) and the presence of other stock (OR: 2, CI OR: 1; 4, p= 0.05) (table 2).

We excluded the variable "presence of sheep" for the multivariable model due to co-linearity with the variable "presence of other stock". We included following variables in the multivariable analysis according to the criteria mentioned above: presence of other stock, purchase of cattle, not dewormed cattle and communal grazing. All these variables showed a higher risk for having positive cattle reactor, although none of them were statistically significant (table 3).

Potential risk to people

Tuberculosis (confirmed clinical diagnosis, clinical signs of cervical lymphadenitis) was reported in 86 households (19%) At least 20% of the reported cases were EPTB (cervical lymphadenitis). None of the assessed 8 variables was statistically significant in the univariable model for having a TB case in a household (table 4).

Discussion

Our study reports a representative estimate of BTB prevalence in rural Ethiopia. The overall prevalence found in our study is consistent with the results found in some African countries. In Tanzania, Shirima et al. (2003) and Cleaveland et al. (2007) found individual cattle prevalence of 1.3% and 0.9%, respectively. We present apparent prevalence using a cut off for skin test reaction of 2 mm as suggested by Ameni et al (2008) who stated that maximum sensitivity can be achieved in central Ethiopia using a 2 mm cut off without affecting specificity. However, our prevalence is still low compared to previous results published from central Ethiopia, where prevalences between 7.9% and 11.6% were observed in local zebus, using a 4 mm cut-off (Ameni et al., 2003, Ameni et al., 2007). This suggests that BTB epidemiology in rural extensive systems very likely differs from peri-urban livestock-production systems (central Ethiopia) in the country. This could be explained by different husbandry practices and a higher number of cross breeds and exotic breeds (considered to be more susceptible to BTB) found in peri-urban settings. Bovine TB seems to be endemic and widespread in rural Ethiopia, with 67 % of the tested village-herds having at least one positive reactor. Regions located in higher altitude seem to have a lower prevalence (table 1), however, there was no statistical association in our study. Still, the role of altitude seems worth pursuing in further research, since it has been shown that human tuberculosis, altitude was negatively correlated with tuberculosis prevalences (Vargas et al., 2004, Saito et al., 2006). Although the situation in the sampled villages is favorable for BTB transmission between animals, such as very close contact throughout the year in poor ventilated houses, communal grazing/ploughing/threshing/watering, overcrowded pastures, only few animals per herd were found positive with an overall individual prevalence of 3%. This indicates a low transmission in the investigated livestock systems, which are characterized by small herd size, mostly communal grazing with some crop residue supplementation, if housing, then only at night but not during the day and cattle that are often kept together with small ruminants.

In contrast to Cleaveland et al. (2007) and Ameni et al. (2003), herd size did not play a significant role as risk factor for tuberculin reactivity. This could be explained by the high proportion (81%) of small herds with less than 10 animals in our study, making a comparison of different herd size difficult.

There seem to be very little transmission during extensive communal grazing, even on crowded pasture. Similarly, Francis (1947) observed that if young stock grazes with heavily infected older stock, the infection rate would remain low until they are brought back into stabling. This could also indicate that among others, transmission might be maintained by a few cows shedding mycobacteria in their milk, early infection of young animals of which a low proportion become themselves infectious in later stage. Our result suggests that spread of disease may not necessarily be linked with the daily gathering of many animals from different herds at one site as often suggested in the literature and definitely needs further investigation, including also other ways of gathering such as watering of animals. We could not do a proper statistical analysis of the effect of different watering sources since only 3% of the animals were not watered at communal sources. Cattle that were not regularly dewormed were nearly twice at risk for being a reactor, but the result was not statistically significant (OR: 1.9, p = 0.1). However, this result suggests that high parasitic loads may decrease the animal resistance and make it more susceptible to BTB.

In our study, and in contrast of recent findings in Tanzania with similar settings (Cleaveland et al., 2007), keeping cattle inside at night, although increasing the risk for having reactors, was not statistically significant for BTB positivity in cattle (OR: 1.9, p=0.2), despite prevailing poor ventilation and very close animal contact. This could be explained by the low number of animals kept indoors. We did not investigate any direct environmental risk factors. However, recent studies in Tanzania (Cleaveland et al. 2007) and Uganda (Oloya et al., 2007) suggested that flooding may play an important role in disease transmission. Environmental source of infections should be more thoroughly investigated in future research since their importance in the epidemiology of BTB transmission remains elusive.

Animals older than 10 years were at higher risk of infection (OR:1.9), which is in line with findings by Cleaveland et al, (2007) from Tanzania. Phillips et al. (2002) also suggested that older animals were more susceptible for *M. bovis* infection. However, in our case, the result was not statistically significant (p = 0.3). This might have been due to the low number of old animals in our study (N= 35; 7%). These findings suggest nevertheless, that removing old animals from a herd and avoiding purchasing old animals from markets might help decreasing within herd prevalence and risk of introduction of

infectious animals, respectively. Purchase of animals were significantly associated with BTB positivity (OR: 1.7; p = 0.046), suggesting that the disease is likely to be spread regionally by animals movements.
Having other livestock than cattle in the herd increased the risk of positive tuberculin reaction in cattle (OR: 2 p= 0.05). Keeping sheep in the farm although not being statistical increased the risk of finding positive PPD cattle (p=0.07; OR: 1.7). Nearly half the farmers having other stock than cattle, owned sheep, which are kept in mixed herding with cattle. Bovine TB in sheep is rare but has been nevertheless described in Europe (Malone et al., 2003) and Sudan (Tag el Din and Gamaan, 1982) and should be further investigated considering the large sheep population in Ethiopia.

Reported human tuberculosis in our study would comprise all forms of TB and no differentiation was made between *M. tuberculosis* and *M. bovis*. Considering the high percentage of extra pulmonary cervical lymphadenitis recorded (at least 20% of all reported TB cases in households), it is plausible that *M. bovis* might play a role. Farmer consumption habits (raw milk, raw meat) did not show any statistical significance, in the opposite of previous findings in Ethiopia (Ameni et al., 2003). In contrast to Regassa et al. (2007) and Ameni et al. (2007), we did not find any correlation between having PPD positive cattle and having a human case of TB in the household, even though people and cattle often shared the same house. However, statements on the zoonotic potential of BTB require confirmed *M. bovis* cases to address their risk factors specifically. Vice-versa, having confirmed TB cases in a household was not associated with cattle reactors (OR: 1.1; p = 0.7).

Such high household interview numbers (450) as conducted in our study have rarely been done in the past. However, considering the very low prevalence of the disease in the country, the power of the study should be further increased in future research. Because of the clustered distribution of livestock, random effects models are more appropriate and risk factor assessments are more conservative. Further studies on risk factors of BTB in humans require case-control studies with confirmed *M. bovis* infection. “Classical” risk factors have been investigated to a certain extend in small studies in the Ethiopian Highlands, showing sometimes diverging results. However, in order to embark in a

national BTB control program, thorough knowledge of possible risk factors is an essential prerequisite and should therefore also include risk factors associated with environment and milk, as well as the role of co-infection in cattle with diseases highly prevalent in some areas (e.g. trypanosomiasis, fasciolosis, Chronic Contagious BronchoPneumonia), nutritional challenges and the genetic role of different cattle breeds to BTB susceptibility It appears that the epidemiology of BTB varies between different African countries but also between different regions in Ethiopia depending on livestock systems (extensive, intensive), breeds (local, exotic, cross-breed) but also ecological and geographic factors. Further research is needed to better understand BTB transmission in extensive livestock systems of Ethiopia as well as the true potential of zoonotic risk of transmission and finally to address the potential of control options.

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Table 1. PPD prevalence in cattle in the four different Woredas using a cut-off of 2 mm (calculated using a logistic regression model with a random effect on Kebele)

Woreda	**Number Kebele**	**Altitude range (meter)**	**Number village**	**Number positive village***	**Total tested animal**	**PPD positive reactors**	**Percentage of reactor animals (prevalence)**	**95%CI**
Meskanena								
Mareko	5	1800-2170	21	20	590	47	7.9	5.8-10.5
Woldia	6	1460-3500	22	8	629	13	1.2	0.3-3.9
Bako Gazer	7	1330-1640	19	14	542	25	4.3	2.3-7.7
Bale Mountains	5	2120-3500	11	7	455	9	2.0	1.0-3.8
Total	**23**		**73**	**49**	**2216**	94	**3.1**	**2.0-4.8**

* A village is positive if it has at least 1 positive reactor

Table 2: Univariate analysis of risk factors for cattle tuberculin reactor using GLLAMM model with random effect on Kebele

Risk factor		Proportion %(Nb/Total)	OR	95% CI for OR	p-value
Presence of other stock		70 (313/450)	2	1; 4	0.05
Presence of sheep		45.3 (204/450)	1.7	1; 3	0.07
Purchase of cattle		38 (172/450)	1.7	1; 2.9	0.04
Communal grazing		62 (265/428)	1.5	0.9; 2.6	0.1
Not dewormed cattle		24.3 (109/449)	1.8	0.9; 3.8	0.1
Presence of old animals (> 10 yrs)		7 (35/450)	1.5	0.7; 3.3	0.3
Cattle housing night	base: free-roaming				
	outside shed	11 (48/449)	1.4	0.6; 3.4	0.4
	indoor with people	46 (209/449)	1.9	0.7; 5.2	0.2
Herd size	base: <5 cattle				
	<10 cattle	39 (176/450)	1.5	0.8; 2.9	0.2
	>10 cattle	22 (99/450)	1.5	0.6; 3.2	0.3
Presence of donkeys		25 (112/450)	1.3	0.7; 2.3	0.4
Presence of oxen		80 (357/450)	0.8	0.4; 1.7	0.6
Presence of camels		2 (10/450)	1.7	0.2; 14.7	0.6
Not vaccinated cattle		20.4 (92/450)	1.2	0.6; 2.5	0.6

Contact with wildlife	19 (86/450)	0.9	0.4; 1.8	0.7
Not own bull for reproduction	54 (216/400)	1.1	0.6; 2.2	0.7
Human TB cases	19 (86/449)	1.1	0.6; 2	0.7
Presence of horses	9.3 (42/450)	1.3	0.4; 4.3	0.7
Presence of adult breeders (<10 yrs)	90.4 (407/450)	1.1	0.4; 2.9	0.8
Presence of calves (<1yr)	59.5 (268/450)	1	0.6; 1.8	0.9
Presence of juveniles (1-3 yrs)	61 (274/450)	1	0.5; 1.7	0.9
Presence of goats	31 (141/450)	1	0.6; 1.8	0.9

Table 3: Multivariable analysis of potential risk factors for positive cattle reactors using GLMM with Kebele as random effect

Variable	OR	95%CI OR	p-value
Purchase	1.5	0.9; 2.7	0.1
Deworming	1.8	0.8; 3.9	0.1
Communal grazing	1.3	0.7; 2.3	0.4
Other stock	1.7	0.8; 3.5	0.1

Table 4: Univariable analysis for risk factors for perceived TB cases in humans using GLMM with Kebele as random effect

Risk factor		Proportion %(Nb/Total)	OR	95% CI for OR	p-value
Cattle housing at night	base: free-roaming				
	outside shed	11 (48/449)	1.7	0.7; 3.9	0.4
	indoor with people	46 (209/449)	1	0.4; 2.6	0.2
Raw milk consumption		68.5 (307/448)	0.3	0.5; 1.8	0.7
Raw meat consumption		74.4 (334/449)	1.1	0.6; 2	0.6
Keeping other livestock		70 (313/449)	1	0.6; 1.8	0.8
Cattle herd size	base: <5 animals				0.2
	<10 animals	33.6 (151/449)	1.4	0.8; 2.5	
	<20 animals	13.6 (61/449)	1.5	0.7; 3.3	
	>20 animals	2.9 (13/449)	0.3	0.03; 2.8	
Number of cattle reactors	base: none				0.8
	1	11.5 (52/450)	1.2	0.6; 2.4	
	2	0.9 (4/450)	1.4	7.2; 14.9	
Shepherding	base: mixed shepherding				
	children	36.3 (94/259)	0.7	0.3; 1.7	0.4
	adult	13 (33/259)	1.7	0.6; 4.4	0.3
Continuous altitude					0.3

12. Bovine tuberculosis in Ethiopian wildlife

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Abstract

Bovine tuberculosis (BTB) is known to be endemic in Ethiopian cattle. However, the status of the disease in wildlife populations, that often share same habitat as livestock, is unknown. This study screened for bovine TB in wildlife in five regions in Ethiopia. Blood and tissue samples from 133 animals that belonged to 28 mammal species were collected from 2006 to 2008. We used a rapid serology test (RT), based on lateral flow technology, and performed culture of lymph node specimens inoculated on Lowenstein-Jensen and Middlebrook 7H11 media. Acid-fast colonies were further analyzed using molecular typing methods. Twenty out of eighty-seven serologically tested animals (23%) were positive for BTB using the RT, and acid-fast bacilli were cultured from 29 out of 89 animals (32.5%). None of the positive cultures yielded mycobacteria from the *Mycobacterium tuberculosis* complex while many environmental mycobacteria were isolated. Among the latter, *M. terrae* was the most common agent. In conclusion, this study highlighted the high prevalence of environmental mycobacteria in wildlife, which role is still unknown and suggested that flagship endemic rare species such as the Mountain Nyala and the Ethiopian wolf might be at risk for BTB. It also assessed the RT for field purpose in an attempt to validate this serology test.

Key words: Bovine tuberculosis, *Mycobacterium bovis*, Mycobacteria spp, Ethiopia, wildlife

INTRODUCTION

Bovine tuberculosis (BTB) is a multi-host disease caused by *Mycobacterium bovis*. This pathogen belongs to the *Mycobacterium tuberculosis* complex, a group of genetically closely related mycobacteria, which also includes the human pathogen *M. tuberculosis* (Brosch et al., 2002; Mostowy et al., 2005). Cattle are considered to be the main host for *M. bovis* and the disease has thus considerable economical impacts on the agricultural and trade sector (Zinsstag et al., 2006). BTB is a potential zoonotic threat to human beings who can get infected by consuming raw meat and milk products, derived from BTB positive cattle (Grange, 2001; Neill et al., 2005). Wildlife is also increasingly described as a source for BTB in humans in close contact with infected animals, such as hunters (in North America) and game farmers (Liss et al., 1994; Wei et al., 2004; Baker et al., 2006; Wilkins et al., 2008).

The list of wildlife species around the world in which *M. bovis* has been isolated is long and reports in the literature of new susceptible species have been increasing in recent years.

Some wildlife species have been known since a long time to be maintenance hosts. The classical examples are the brushtail possums (*Trichosurus vulpecula*) in New-Zealand (Coleman et al., 2006), the white-tailed deer (*Odocoileus virginianus*) in the United States (O'Brien et al., 2002), the Eurasian badger (*Meles meles*) in the UK and Ireland (Griffin et al., 2005), the African buffalo (*Syncerus caffer*) in South-Africa (de Vos et al., 2001; Rodwell et al., 2001), and described more recently, the wild boar (*Sus scrofa*) in Spain (Naranjo et al., 2008) and Wood bison (*Bison bison*) in Canada (Nishi et al., 2006). These wildlife species can maintain the disease in the absence of infected cattle. They are therefore a source of infection for livestock, thus hampering costly national control and eradication programs in developed countries. For instance, the incidence of BTB in the UK is increasing by 18% per year and the existing of an uncontrolled BTB wildlife reservoir is a strong contributing factor to this rising figure. Control of the disease in livestock cost the British government as much as 74 million GBP per year (Mathews et al., 2006).

These maintenance species are also a source of infection for other wildlife species sharing same ecosystems, thus having the potential to threaten the status of valuable or

endangered species. This has been shown recently in the Krueger National Park where lions preyed on BTB infected buffaloes, which lead to a severe mortality in the park's lion population (Keet et al., 2000; De Vos et al., 2001). Wildlife species other than maintenance species are known as spill-over hosts or dead-end host. They cannot sustain the disease in the absence of an infectious source. However, their role in the complex epidemiology of BTB is unknown as more and more species are detected with BTB, and new interactions and new maintenance hosts are described (Renwick et al., 2007).

In Sub-Saharan Africa, most detailed data on BTB in wildlife has been collected in Southern Africa (Bengis et al., 1996; Keet et al., 2001; Michel et al., 2006; Michel et al., 2009), Kenya (Tarara et al., 1985; Sapolsky and Else 1987), Uganda (Woodford, 1982) and Tanzania (Cleaveland et al., 2005).

To this date, no data has been collected from Ethiopian wildlife. Ethiopia is a country known for its rich bio-diversity and various ecosystems and is home to two-hundred-fifty-five different mammals, of which thirty-one are endemic and thirty-eight are listed in the International Union for Conservation of Nature- Red List of Threatened species (IUCN, 2008). However, the rapid intensification of the human-livestock-wildlife interface in Ethiopia, which is mainly fuelled by the rapid population growth, massive land degradation and recurrent draught, is a potential risk of disease transmission at the interface.

This study was done in close collaboration with the Ethiopian Wildlife Conservation Authority, which approved and supported the research. The study aimed primarily at screening wildlife species from different parts of Ethiopia for BTB by means of serology, strain isolation by culture and molecular typing, in order to assess disease prevalence in wildlife and survey which animal species and ecosystems are affected. The second aim of the study was to assess a rapid serological test to attempt to validate it for field purpose.

MATERIALS AND METHODS

Study sites

The study was carried out in the following five regions in Ethiopia: Welega (Oromia), Awash (Afar), Babille (Harari), Bale Mountains (Oromia), and South Omo (SNNPR) (Fig 1), between the latitudes of 5°-11.5° N and the longitudes of 35°-43° E, thus

covering different ecological zones, with altitudes ranging between 400 m and 3800 m above sea level. The majority of the samples were collected in hunting controlled areas (74%), followed by the Bale Mountains National Park (19.5%) and the Babille elephant sanctuary (6.5%).

Sample collection

All wildlife samples of this study were collected between 2006 and 2008. Animal tissues were obtained from two sources, including harvests from licensed hunters and opportunistically from fresh carcasses (e.g. road kill). No animals were killed for the purpose of the study.

Animals were examined and body condition assessed. Mediastinal, mesenteric and submandibular lymph nodes were collected from the animals, as well as suspect tuberculous lesions from any organ, if present, and tonsils from carnivores. All sample specimens were collected using disposable sterile surgical instruments (forceps, scalpel blades – a different set for each animal) and then kept in universal tubes containing sterile phosphate buffer saline (pH 7.2).

Blood was collected either from fresh carcasses (animals killed by licensed hunters, road kills) or from anaesthetized live animals (captured/anaesthetized and released in the frame of other projects for other purposes). In the case of live animals, blood was drawn by veno-puncture (choice of vein and syringe/needle size was species dependent). In the case of a carcass, veno-puncture was often not feasible due to the rapid clotting of blood. Blood was drawn after opening the carcass, either by heart puncture or by severing a big vessel (e.g. vena cava) and collecting the blood directly in a sterile collecting tube. Blood tubes were stored upright for 6-12 hours; sera were then pipetted from the tubes and transferred into 1 ml cryo-tubes.

All tissue and serum samples were kept at 4°C from the collection sites and during transport to the AHRI laboratory in Addis Abeba, where they were stored at -20°C until further processing.

Sample analysis

Serology for specific antibody detection was performed using the rapid test (RT) developed by Chembio Diagnostic Systems, Inc., which is a colored latex-based lateral

flow technology using a cocktail of selected *M. bovis* antigens including ESAT-6, CFP10, and MPB83 (Lyashenko et al., 2008). Results were read 20 minutes after adding the rapid test buffer solution to the serum. Animals were considered positive if any visible line appeared in the test area of the RT in addition to the control line. Results were negative if no band was visible in the test area in addition to the visible control band.

Submandibular and mediastinal lymph nodes were pooled together, whereas mesenteric lymph nodes were processed and cultured separately. After homogenization and neutralization according to standard methods (Roberts and Koneman, 1991), sediments were inoculated on three different media slants: Lowenstein-Jensen media supplemented with glycerol or pyruvate, and Middlebrook 7H11 medium supplemented as previously described (Gallagher and Horwill, 1977). The slants were incubated horizontally at 37°C for one week then vertically for an additional five weeks. Cultures were considered negative if no visible growth was seen after six weeks of incubation. Positive culture were stained according to the Ziehl-Neelsen method and examined under the microscope for detection of Acid-Fast Bacilli (AFB).

Molecular typing

Heat killed AFB positive samples were investigated by multiplex PCR, including primers specific for the *Mycobacterium* genus and the *M. tuberculosis* complex (Wilton and Cousins, 1992). Strains were investigated for the *M. tuberculosis* complex by PCR using protocols for RD4 typing (Gordon et al., 1999) and RD9 typing (Berg et al., 2009). Isolates that did not belong to the *M. tuberculosis* complex but tested positive for the genus were sequenced over the 16S rRNA locus (Han et al., 2002). Analysis of the sequences was performed by BLAST search (Altschul et al., 1997) of databases at NCBI and RIDOM (http://rdna.ridom.de)(Harmsen et al., 2002); particularly, the sequences of the variable Region A and Region B (Kirschner et al., 1993) were considered when determining the *Mycobacterium* species.

Data analysis

Data were double entered in an Access database and validated using Epi Info (version 3.3.2). All statistical descriptive analysis was done using software package STATA 10.1

(StataCorp, Texas, USA). Molecular typing results of the isolated strains were used as basis for estimating disease prevalence in wildlife.

RESULTS

Samples from a total of a hundred-thirty-three animals belonging to twenty-eight different mammal species were investigated during the study. The majority were male (n=116; 87%) and adult animals (n=128; 96%). Body condition was good in a hundred-twenty-four animals (93%), whereas eight animals (6%) were found to be thin and one (1%) was emaciated.

Serology

The rapid test (RT) was performed on serum collected from eighty-seven animals (thirteen live animals and seventy-four fresh carcasses). Results of the species involved are shown in table 1. Twenty of these animals (23%), belonging to ten different species, were sero-positive on RT. The lymph nodes of forty-seven of these animals were further processed and cultured (see below).

Clinical examination and gross visible lesions

Out of a hundred-thirty-three animals sampled, six showed various clinical signs: one Grant's gazelle was lame due to an old injury and five animals (Gerenuk, warthog, Grant's gazelle and Hartebeest) had enlarged head lymph nodes. Upon carcass examination, gross visible nodules varying between pea-size to 5 cm diameter were seen in five animals. Nodules in the lung were seen in Menelik bushbuck and Hartebeest. Multiple nodules were observed on the liver in a warthog and on the mesenteries in a Grant's gazelle.

Culturing and molecular typing

Lymph nodes from eighty-nine animals (67%) were subjected to culture. Forty slants representing twenty-nine animals (32.5%) showed colony growth. AFB positive samples were further analyzed by *Mycobacterium* genus and deletion typing (Table 1). None of the samples were characterized as belonging to the *M. tuberculosis* complex. However, seventeen strains were identified as non-tuberculous mycobacteria (NTM) with *M. terrae*

complex being the most prevalent agent (N = 19; 65.5%) followed by non tuberculous mycobacteria strains other than M.terrae complex and MAC (N = 9; 31%). Strains from the *Mycobacterium avium* complex (MAC) were isolated from two Grants' gazelles, and one isolate from a gerenuk (*Litocranius walleri*) was typed as Nocardia spp. The above mentioned species showing nodules on post-mortem examination were all culture negative. Among the forty-seven animals that were examined with serology and tissue culture, fifteen were AFB positive and sixteen were serology positive. However, only five of these animals were positive with both serology and culture.

DISCUSSION

This study provides the first piece of information on tuberculosis in Ethiopian free-ranging wildlife.

In this study, no *M. bovis* was isolated from wildlife; in contrast to the neighboring East African countries where the pathogen was reported in buffaloes in Uganda (Woodford, 1982) and in baboons in Kenya (Tarara et al., 1985; Sapolsky and Else, 1987). Recent studies in Tanzania also showed that BTB was prevalent in the Serengeti-Ngorongoro ecosystem, affecting buffaloes, lions and wildebeest (Cleaveland et al., 2005).

Wildlife habitats in Ethiopia are not fenced. This allows an intensive interaction between a fast growing human population, its livestock and wildlife competing over scarce grazing land. Livestock are also grazed in various protected habitats especially during times of fodder shortage (Stephens et al., 2001; Jacobs and Schloeder, 2001). In our study areas, wildlife remained geographically localized with no major migration patterns.

Bovine TB is endemic in the Ethiopian cattle population with prevalence ranging between 0.8% and 13.5 % in the rural areas (Ameni et al., 2007; Tschopp et al., 2009; Berg et al., 2009). Wildlife, and in particular herbivores sharing same pastures as cattle, might therefore be at risk for BTB transmission.

The "classical" African species described in the literature as being reservoirs for BTB such as buffaloes in the Republic of South Africa (RSA), Uganda and Tanzania (Woodford, 1982, Cleaveland et al., 2005, Michel et al., 2007), or potential reservoirs

such as kudus (Bengis et al., 2001), exist in large numbers in our study sites. *M. bovis* was not isolated from any of these species suggesting that they may not be major reservoirs for the disease in Ethiopia. However, a definitive conclusion is difficult to be drawn due to the small number of animals examined in this study.

The concern regarding potential disease transmission from livestock to some high profile species such as the Mountain Nyala (*Tragelaphus buxtoni*) and the Ethiopian wolves (*Canis simensis*) is high. Both species are listed in the IUCN Red List of endangered species and often share same habitat as humans and their livestock. Furthermore, Mountain Nyalas belong to the Tragelaphus family, like the kudu, which was shown to be a susceptible and potential maintenance species for BTB (Bengis et al., 2001; Keet et al., 2001). In our study, forty percent (2/5) of the Mountain Nyalas were sero-positive on the rapid test. However, only a very few number of animals were tested and further surveillance is warranted. Twenty rat species from the Web Valley (Bale Mountains national Park) were examined for BTB and one grass rat (*Arvicanthis blicki*) was sero-positive; unfortunately no strain isolation by culturing was performed because of logistic constraint (e.g. remoteness, cold chain availability) to confirm the serological finding. But this result is however worrisome from a conservation point of view, since Ethiopian wolves mainly prey on afro-alpine rodents (Sillero-Zubiri and Gottelli, 1995). Various rodents have been shown to host BTB in the UK (Delahay et al., 2007; Matthews et al, 2006). Furthermore, as seen in the Krueger National Park (RSA), carnivores might be at high risk for contracting BTB if the disease is present in their preys (Michel et al., 2006).

In a country such as Ethiopia, characterized by often small wildlife populations, a high number of endemic rare species, and remoteness and/or inaccessibility of wildlife areas, the rapid test would be a valuable and important tool to screen live animals in a practical, easy and cheap way that is not constraint by difficult logistics seen during the collection of animal lymph nodes. However, the sensitivity of serology tests is in general poor (Cleaveland et al., 2005). Lyashenko et al (2008) found an overall sensitivity of the RT of 77%, which increased to 97% in deer. However, their study only included four species: deer, wild boar, possum and badger. The test was so far not used for most African species. We initially aimed in our study at validating the rapid test kit for the wildlife

species; unfortunately the number of animals sampled was too little to get conclusive results. Lyashenko et al (2008) found that sensitivity increases manifold with the presence of gross lesions. In our study, very few "tuberculosis-like" gross lesions were observed in the lymph nodes of tested animals, which could also partly explain the low sensitivity of the RT. The performance of the test seems also to vary depending on the species tested. We used the RT in twenty-two different species and nine species reacted positive to the test. Twenty of the tested animals (23%) were reactive on the rapid serology test. The highest positive rates were found in Guenther's Dik-Diks (33%), Lesser Kudus (60%), Bushbucks (25%), Buffaloes (33%) and Mountain Nyalas (40%). Two out of eight elephants tested (25%) were RT positive, which is worrying since the test has been validated for this species (Lyashenko et al., 2006). However, no elephants were post-mortem examined in this study. BTB has been shown to be an increasing problem in domestic elephants in the Indian subcontinent (Sreekumar et al., 2007). In Ethiopia, only a small population of elephant remains in the wild, and it can not be ruled out that BTB is not prevalent in this endangered population.

Large numbers of atypical mycobacteria were isolated by culture. It is possible that some of these isolates have given rise to cross-reaction with the antigens contained in the RT, thus generating false positive results, which could explain the high prevalence of BTB sero-positivity. However, experimental infection with *M. paratuberculosis, M. avium* and other non-tuberculous mycobacteria carried out in the study by Lyashenko et al (2008) did not show any reaction in the rapid test, thus suggesting that our positive serology results may be consistent with infection of species from the *M. tuberculosis* complex. No strains of the *M. tuberculosis* complex were isolated from any of the samples that were processed for culture. Unfortunately, only forty-seven of the samples that underwent serology were matched with the gold standard method of culturing. It is therefore possible that some serologically positive animals, for which no culture was done, were indeed positive for BTB. Furthermore, we may have failed to detect some infected animals, which could have been in the early stage of infection with no visible lesions or if lesions were present in tissues that were not examined. This could explain the high serology positivity while cultures were negative. Serology was performed on a large number of species but only on a few animals per species. Sensitivity and specificity of the RT for a particular species could therefore not be evaluated. More sensitive and

specific tests are needed to screen African wildlife and future research on test validation should probably focus on possible maintenance species or highly endangered species for the Ethiopian context.

The isolation of seventeen different strains of non-tuberculous mycobacteria (NTM) is a major result of this study. *M. terrae* was one of the most frequently isolated species in this current study. It was also isolated in wildlife in Tanzania (Cleaveland et al., 2005) and in RSA (Michel et al., 2007). These reports suggest that *M. terrae* may be a ubiquitous NTN across Africa. In the opposite of other NTM strains that may be more region-specific: in South Africa, reports show the presence in wildlife of *M. vaccae* and *M. engbaeckii (*Michel et al., 2007) and *M. goodie* (Van Helden et al., 2008); while in Tanzania, *M. phlei* and MAC were the NTM isolated besides *M.terrae* (Cleaveland et al., 2005). None of these NTM-with the exception of *M.vaccae* were isolated in our study. While in the published reports mentioned above, only a few numbers of NTMs were isolated in wildlife, a wide range of different strains of NTM were isolated in our study. Some of these NTMs have been described in captive wildlife before, such as *M.asiaticum* in a red-handed tamarin (*Saguinus midas*) (Siegal-Willott et al., 2006), and *M.gordonae* and MAC in captive pumas (*Felis concolor*) (Traversa et al., 2009). In our study, *M.asiaticum* was isolated in free-ranging Grants gazelle (*Nanger granti*). This NTM is also known to be a human lung pathogen (Taylor et al., 1990). In Tanzania and Ethiopia, *M.terrae* was shown to be a pathogen, associated with granulomatous lesions in cattle and in humans (Kazwala et al 2002-cited in Cleaveland et al., 2005; Berg et al., 2009). Most of the atypical mycobacteria in our study were isolated from the mesenteric lymph nodes suggesting environmental exposure via fodder or water. However, they were also isolated from lungs in half of the animals (N=16), suggesting a possible direct animal-to-animal aerosol transmission, possibly associated with species behavior. To this date, it is unclear what the transmission pathway of NTMs is between domestic livestock, humans and wildlife.

In conclusion, this is the first study that investigated the prevalence of tuberculosis in Ethiopian wildlife and the results suggest that BTB may not be endemic. However, the current study can not rule out that BTB does not occur in wildlife in Ethiopia since the

serology test, despite possible false positive results suggested that BTB may be prevalent in wildlife. The study therefore highlights at this stage the need for complementary testing diagnostics, especially if tissue culture as gold standard cannot be performed (e.g. on live animals). It also highlights the need for further research to increase sensitivity and specificity of serological tests and to validate such a rapid test for individual African wildlife species.

The high number of non-tuberculous mycobacteria found in the tissue samples needs further investigation regarding their pathogenicity, their role and possible interaction with the pathogenic *M. bovis* and their effect on the animal's immune system.

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Fig 1: Map illustrating the sites where wildlife samples were collected (black circles). Hatched areas represent protected wildlife habitat, black lines represent rivers and filled grey areas represent lakes.

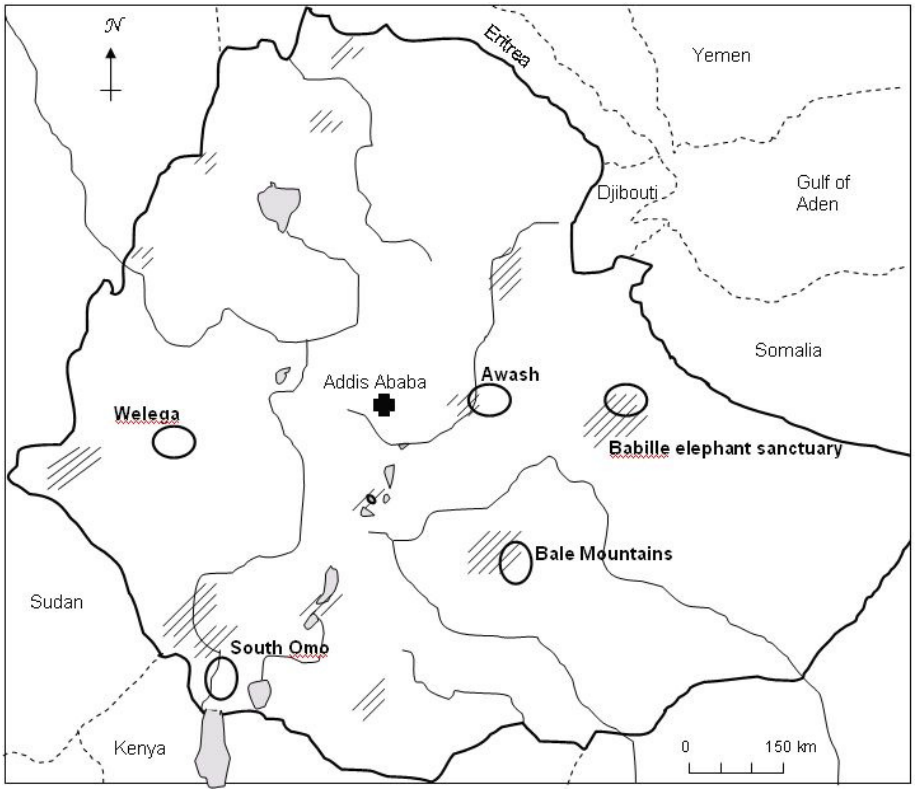

Table 1: Mycobacteria in wildlife in Ethiopia: results of serological, cultural and molecular typing investigations for bovine tuberculosis and other mycobacteria, 2006-2008

Species	Total number of animals examined	Positive serology	AFB + cultures	Molecular typing (16S rDNA gene sequencing)
Bushbuck (*Tragelaphus scriptus*)	11	1/4 (25%)	2/10 (20%)	MTC*
Soemmering gazelle (*Gazella soemmeringi berberana*)	1	0/1 (0%)	0/1 (0%)	
Guenther's dik-dik (*Madoqua gunther*)	8	1/3 (33%)	3/8 (37%)	MTC, *M.moriokaense*
Grant's gazelle (*Nanger granti*)	17	2/12 (17%)	10/17 (59%)	MTC, *M.wollinsky*, MAC**, *M.asiaticum, M.moriokaense*
Gerenuk (*Litocranius walleri*)	5	0/4 (0%)	3/5 (60%)	MTC, *Nocardia testacea*
Greater kudu (*Tragelaphus strepsiceros*)	1	NA	0/1 (0%)	
Lesser kudu (*Tragelaphus imberbis*)	7	3/5 (60%)	3/7 (43%)	MTC, *M.flavescens spp*
Tiang (*Damaliscus lunatus*)	4	0/3 (0%)	2/4 (50%)	MTC
Hartebeest (*Alcelaphus buselaphus*)	4	0/3 (0%)	1/2 (50%)	MTC
Mountain nyala (*Tragelaphus buxtoni*)	11	2/5 (40%)	1/9 (11%)	*M.vaccae, M.vanbaalenii*
Buffalo (*Syncerus caffer*)	5	1/3 (33%)	1/3 (33%)	*M.gordonae*
Bohor reedbuck (*Redunca redunca*)	3	0/1 (0%)	1/2 (50%)	*M.vaccae, M.vanbaalenii*
Waterbuck (*Kobus ellipsiprymnus*)	2	1/2 (50%)	0/2 (0%)	
Bush duiker (*Sylvicapra grimmia*)	1	NA	0/1 (0%)	
Warthog (*Phacochoerus africanus*)	6	0/2 (0%)	0/6 (0%)	
Bushpig (*Potamochoerus larvatus*)	1	NA	0/1 (0%)	
Giant forest hog (*Hylochoerus meinertzhageni*)	2	0/2 (0%)	0/2 (0%)	
Elephant (*Loxodonta africana*)	8	2/8 (25%)	NA	
Rock hyrax (*Procavia capensis*)	1	0/1 (0%)	0/1 (0%)	
Hippopotamus (*Hippopotamus amphibius*)	1	0/1 (0%)	0/1 (0%)	
Ethiopian wolf (*Canis simensis*)	5	0/5 (0%)	NA	
Serval cat (*Leptailurus serval*)	1	NA	1/1 (100%)	MTC, *M.gilvum*
Leopard (*Panthera pardus*)	3	0/1 (0%)	0/2 (0%)	
Black-backed jackal (*Canis mesomelas*)	1	0/1 (0%)	1/1 (100%)	MTC
Anubis baboon (*Papio anubis*)	3	NA	0/3 (0%)	
Black-clawed brush-furred rat (*Lophuromys melanonyx)*	14	0/14 (0%)	NA	
Blick's grass rat (*Arvicanthis blicki*)	7	1/7 (14%)	NA	
Total	133	20/87 (23%)	29/89 (32.5%)	

MTC*: *Mycobacterium terrae* complex (include: *M.priferae, M.confluentis, M.brasiliensis, M.senuensis, M.terrae, M.kumamotonense, M.arupense*)
MAC**: *Mycobacterium avium* complex

13. L'interface faune sauvage – élevage – homme de la tuberculose bovine en Afrique

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Importance du complexe *tuberculosis*

Le complexe *tuberculosis* est composé de plusieurs espèces de mycobactéries, dont *Mycobacterium tuberculosis*, l'agent principal de la tuberculose humaine, *M. africanum*, *M. microti*, *M. canetti* et *M. bovis*. Les bovins sont l'hôte principal de *M. bovis*, mais un grand nombre d'autres ruminants et d'autres animaux, notamment de la faune sauvage sont atteint (Ayele et al., 2004). La tuberculose (TB) est responsable de 9.2 millions de nouveaux cas et 1.7 millions de décès en 2006. Plus de 95% des cas de tuberculose sont détectés dans les pays on voie de développement, dont un tiers en Afrique (WHO, 2008). La tuberculose reste la plus importante cause de mortalité chez les personnes infectées par le VIH (Virus de l'immunodéficience humaine), et est responsable de 32% des décès de patients infectés par ce virus en Afrique (Guleria et al., 1996). Environ deux millions des nouveaux cas de tuberculose surviennent chaque année en Afrique sub-saharienne, et nous ne connaissons toujours pas suffisamment le rôle que joue *Mycobacterium bovis*, membre du complexe *tuberculosis* dans l'épidémie de la tuberculose.

La tuberculose bovine causée par *M. bovis* est avant tout une maladie pulmonaire des bovins (figure 1) mais peut se localiser aussi dans d'autres organes, notamment les ganglions mammaires. Elle se transmet par voie aérogène et par le lait à d'autres animaux et à l'homme. Elle est donc une zoonose importante aux plans santé publique et socio-économique car elle peut affecter le commerce international du bétail et des produits animales. La tuberculose bovine a déjà été observée au début du 20^{ième} siècle en Afrique, indiquant par exemple une prévalence individuelle faible de 1 à 2% en zones rurales et une prévalence entre 10-40% dans les élevages intensifs autour des grandes villes (von Ostertag and Kulenkampff, 1941). La question de la provenance de la tuberculose bovine soit par introduction pendant la période coloniale et/ou sa présence autochtone reste un débat ouvert et fait l'objet d'études d'épidémiologie moléculaire (Cousins et al., 2004b; Muller et al., 2008; Njanpop-Lafourcade et al., 2001). Aujourd'hui, la tuberculose bovine est rapportée dans au moins 33 des 43 pays Africains (Ayele et al., 2004) (Figure 2), mais elle est probablement plus répandue. Vu l'importance soupçonnée de *M. bovis* pour la santé publique, l'Organisation Mondiale de la Santé (OMS) a organisé une réunion en 1993 et en 2005 afin de faire le point sur les connaissances acquises de la transmission animal-homme. Entre-temps plusieurs revues de littérature ont donné une image assez complète de l'épidémiologie de la tuberculose bovine, mais démontrent un important manque de

connaissances de la situation épidémiologique de toutes les espèces concernées (Ayele et al., 2004;Cosivi et al., 1995;Daborn et al., 1996; Zinsstag et al., 2006) . Ce chapitre fait état des connaissances de la tuberculose bovine dans l'élevage, en santé publique et dans la faune sauvage ainsi qu'à leurs interfaces.

Elevage

Comme mentionné plus haut, la tuberculose bovine a déjà été détectée en Afriquedans l'élevage bovin au début du 20^{ième} siècle (von Ostertag and Kulenkampff, 1941). Poulton, cité par (von Ostertag and Kulenkampff, 1941) rapportait en 1935 des différence importantes de prévalence entre les races « Longhorn » (34%) et Zébu (2.4%) en Ouganda. Les isolements de mycobactéries d'origine animale faites par Gidel (Gidel et al., 1969) au Burkina Faso sont parmi les premières faites en Afrique. Des essais de vaccination au B.C.G. (Bacille Calmette Guérin) du bétail bovin ont été effectués au Malawi sans succès (Ellwood and Waddington, 1972), mais des nouveaux essais sont prévus en Ethiopie (Hewinson communication personelle, 2008). *M. bovis* a été détectée dans les eaux usées de l'abattoir de Yaoundé au Cameroun (Wekhe and Berepubo, 1989). La tuberculose a été décrite au Burundi (Rigouts et al., 1996) et dans la zone du lac Victoria en Tanzanie (Jiwa et al., 1997). Des mycobactéries ont été isolés du lait cru de bovins tenus par des éleveurs pastoraux dans les haut plateaux du sud de la Tanzanie (Kazwala et al., 1998). De même au Burkina Faso, des mycobactéries on été isolés en proportion importante des échantillons de lait (Vekemans M. et al., 1999). Par contre, presque aucun signe de la tuberculose bovine n'a été trouvé dans des études représentatives au Sénégal, en Guinée Conakry, Guinée Bissau et en Gambie (Unger et al., 2003). Les différentes races bovines et les différents systèmes d'élevage influencent fortement la prévalence de *M. bovis* en Ethiopie (Ameni et al., 2006). La tuberculose bovine est fortement présente dans la production laitière périurbaine au Kenya mais les éleveurs ne connaissent pas de mesures pour se protéger (Kang'ethe et al., 2007).

Dans les dernières années les nouveaux outils moléculaires utilisés pour la caractérisation de la tuberculose bovine ont permis de faire de grands progrès dans une meilleure compréhension de l'épidémiologie moléculaire en Afrique (Hilty et al., 2005). Il s'avère donc que par exemple les souches isolées au Nord du Cameroun, Nigeria et au Tchad sont assez homogènes (Cadmus et al., 2006;Diguimbaye-Djaibe et al., 2006a;Njanpop-Lafourcade et al., 2001), ce qui indique à la

fois une transmission intense et une expansion clonale du bacille dans le bétail bovin. Un grand nombre de mycobactéries ne faisant pas partie du complexe *tuberculosis* a été rapporté au Tchad mais leur rôle et interaction potentielle avec la tuberculose bovine n'est pas connu (Diguimbaye-Djaibe et al., 2006b). Les outils moléculaires sont particulièrement importants pour différencier rapidement *M. bovis* de *M. tuberculosis* et pour obtenir des empreintes des souches de mycobactéries. Ainsi, en Tanzanie, des souches similaires de *M. bovis* ont été trouvés aussi bien chez l'homme que chez les bovins (Kazwala et al., 2006). Les auteurs recommandent d'harmoniser les approches de lutte contre la tuberculose entre le secteur de santé publique et de santé animale. Le programme national de lutte contre la tuberculose en Tanzanie, premier pays en Afrique, a adopté cette proposition de collaboration étroite entre les secteurs de santé publique et santé animale. La Tanzanie est le premier pays africain a avoir adopté, dans le cadre du programme national de lutte contre la tuberculose, cette approche d'étroite collaboration entre les secteurs de santé publique et santé animale. La première caractérisation moléculaire de *M. bovis* au Mali fait état de deux groupes de souches (Figure 3), dont l'un (I) est comparable aux souches isolées en Afrique Centrale et l'autre est propre au Mali (II) (Muller et al., 2008). Des observations de *M. bovis* chez le dromadaire on été faite en Mauritanie et au Tchad (Chartier et al., 1991). Sa présence chez les petits ruminants est encore très mal connue.

En conclusion, la tuberculose bovine est largement présente dans l'élevage bovin Africain, mais à des taux très variés selon la race et le système d'élevage existant. La lutte actuelle contre la tuberculose bovine se limite, avec quelques exceptions, qu'à l'inspection de viande dans les abattoirs. Bien que contaminée, une bonne partie du lait est consommée sans pasteurisation. A long terme, une lutte ciblée visant à l'élimination de la tuberculose bovine et à la pasteurisation systématique du lait sera nécessaire en Afrique, afin d'assurer un élevage plus intensif caractérisé par une meilleure production laitière qui puisse répondre à la demande croissante de la population en viande et en produits laitiers (Ayele et al., 2004).

Santé Publique

Des mycobactéries autres que *M. tuberculosis* on été rapportés à Kinshasa (Congo) (Pattyn et al., 1967). Au Niger, parmis plus de 150 souches isolées du complexe *tuberculosis*, aucune n'était *M. bovis* (Rey et al., 1982). Enfin, à Lagos au Nigeria 4% des souches isolées du complexe tuberculosis étaient *M. bovis* et 11% des mycobactéries

atypiques (Idigbe et al., 1986). Des études au Malawi démontrent que des zones de tuberculose bovine ne correspondent pas forcément à une forte prévalence chez l'homme. Cependant il semble que la transmission animal-homme serait importante chez des communautés pastorales (Mposhy et al., 1983). La tuberculose bovine chez l'homme semble être absente à Djibouti (Auregan et al., 1988) et présente à faible échelle en Guinée Bissau (Hoffner et al.,1993) ainsi qu'au Burundi (Rigouts et al., 1996). Une analyse rétrospective des registres de la tuberculose à Bobo Dioulasso (Burkina Faso) indique une corrélation entre la prévalence de la tuberculose et l'exposition au bétail bovin. Le groupe ethnique des Peuls, éleveurs traditionnels, souffraient plus souvent de la tuberculose que les autres groupes éthniques (Vekemans M. et al., 1999). *M. bovis* a été identifié dans des cultures positives de tuberculoses pulmonaires et extrapulmonaires en Tanzanie (Kazwala et al., 2001). Enfin, au Madagascar, 1.25% des patients ayant un crachat positif en microscopie souffraient d'une infection à *M. bovis* (Rasolofo-Razanamparany et al., 1999). Considérant l'association du VIH avec le risque accrue d'une tuberculose ouverte, cette association pourrait aussi s'appliquer à *M. bovis*, cependant la plupart des pays manquent la capacité de différencier *M. bovis* des autres agents du complexe tuberculosis (Daborn et al., 1996). La tuberculose a été reconnue comme un problème majeur des éleveurs nomades et de leur bétail au Tchad (Diguimbaye, 2004;Diguimbaye-Djaibe et al., 2006a), cependant dans les premières études, aucune souche à *M. bovis* n'à été détectée chez l'homme (Diguimbaye et al., 2006). D'autres rapports de transmission du bétail à l'homme existent pour l'Ouganda (Oloya et al., 2007) et le Ghana (Addo et al., 2007).

En conclusion, la tuberculose bovine est bien présente chez l'homme et la présence du virus du VIH semble favoriser sa transmission. Vu l'étendu de sa présence chez le bétail et le manque d'infrastructure permettant la pasteurisation du lait, la présence relativement faible de la tuberculose bovine chez l'homme est surprenante parce qu'en Europe, la tuberculose bovine a été répandue chez l'homme avant l'introduction de la pasteurisation du lait à grande échelle. Nous constatons de fortes variations entre pays, avec certains pays ayant une forte prévalence dans l'élevage, mais pratiquement aucun cas décrit chez l'homme dans les pays Sahéliens. Par contre dans les pays subhumides et côtiers, la tuberculose bovine chez l'homme semble être plus accentuée. D'autres conclusions seraient prématurées car les études existantes sont très différentes les unes des autres. Une comparaison approfondie nécessite d'abord un protocole de diagnostic de terrain standardisé, l'établissement de la capacité de diagnostic par culture des

différentes espèces du complexe *tuberculosis* et ensuite une approche concerté d'études ciblées aux personnes à risque dans plusieurs pays.

Faune sauvage

Bien que les bovins sont l'hôte de maintenance principal, *M. bovis* a été isolé dans plusieurs espèces de la faune sauvage, dont le phalanger renard (*Trichosurus vulpecula*) et le daim (*Dama dama*) en Nouvelle Zéalande, le blaireau (*Meles meles*) en Angleterre, le bison (Bison bison) et le cerf élaphe (*Cervus elaphus*) et le cerf de Virginie (*Odocoileus virginianus*) aux Etats Unis sont également des hôtes de maintenance (Cousins et al., 2004b). En Afrique, *M. bovis* a été trouvé dan le cobe de Lechwe (*Kobus leche*) (Clancey, 1977) et dans le phacochère (*Phacocerus aethiopicus*) dans le parc national du Ruwenzori (Woodford, 1982). Tarara et coll. (Tarara et al., 1985) rapportent *M. bovis* chez le babouin olive (*Papio anubis*) dans la réserve faunique du Masai Mara. En étudiant *M. bovis* dans des babouins au Kenya, Sapolsky et Else (Sapolsky and Else, 1987) ont conclu que l'origine de l'infection provenait de déchets d'abattoirs des villages voisins dont se nourrissaient les babouins. *M. bovis* a été isolé pour la première fois dans le buffle d'Afrique (*Syncerus caffer*) en Afrique du Sud dans le Parc National Krüger (Bengis et al., 1996) et peu de temps après la tuberculose bovine a été détéctée dans le guépard (*Acinonynx jubatus*), le lion (Panthera leo) et le cynocéphale de Chacma (*Papio ursinus*) dans la même réserve faunique (Keet et al., 1996). Il est supposé que les bovins proches du Parc National Krüger transmettaient la tuberculose bovine à des buffles d'Afrique qui par la suite la transmettaient aux grands carnivores. Des lésions granulomateuses dans les poumons étaient présents chez tous les animaux de la chaine de transmission. L'établissement permanent de la tuberculose bovine et son augmentation rapide dans la faune sauvage sud-africaine sont très sérieuses car la maladie peut se transmettre parmis les différentes espèces de la faune par transmission directe et ceci tout au long de la chaîne alimentaire. De plus elle pose un danger de par son potentiel de transmission dans d'autres pays. Ceci a été démontré par la détection de la maladie dans la faune sauvage d'un ranch de gibier en Zambie (Zieger et al., 1998). Une étude en Zambie indique un risque de transmission de la faune sauvage au bétail (Munyeme 2008). En l'occurrence les projets transfrontaliers dans le cadre des « Peace Parks » des parcs de la paix, sont menacés par la transmission de la tuberculose bovine par la faune sauvage (Kriek, 2006).

Les premiers rapports de la tuberculose bovine dans la faune sauvage des réserves fauniques du Nord de la Tansanie ont décrit la maladie entre autres dans le Gnou bleu (*Connochaetes taurinus*) et le topi (*Damaliscus lunatus*) (Cleaveland et al., 2005). Dans la même étude des anticorps contre *M. bovis* ont été trouvés dans 4% des lions et 6% des buffles d'Afrique à Tarangire. Dans des zones où la faune sauvage est infectée par la tuberculose bovine, la recherche scientifique doit viser à caractériser la pathogénicité de la tuberculose bovine pour chaque espèce, ainsi que les mécanismes et taux de transmission entre les différents espèces, afin de pouvoir développer des modèles multi-hôtes pour mieux comprendre son évolution et dispersion (Renwick et al., 2007). Cependant cela s'étend aussi à l'élevage et à l'homme. Des meilleures connaissances épidémiologiques nécessitent donc des approches conjointes de la santé humaine et animale, ainsi que de l'écosystème « une santé unique », afin de connaître l'importance globale de la circulation du bacille (Figure 3) (Zinsstag et al., 2005).

Interfaces faune sauvage – bétail - homme

La faune sauvage et le bétail bovin, se rencontrent et pâturent ensemble de manière régulière surtout dans les alentours de réserves naturelles en Afrique qui ne sont pas pour la plupart cloturée. En ce moment nous avons la preuve d'une transmission de la tuberculose bovine du bétail bovin au buffle d'Afrique et au cobe de Lechwe au sud de l'Afrique. En revanche, la faune sauvage infectée représente un risque croissant de dispersion de la maladie à travers des frontières nationales mais aussi une transmission possible au bétail surtout autour des points d'eau. L'homme peut acquérir la maladie avec le bétail bovin à des taux très variables et ceci aussi bien par voie aérogène qu'alimentaire. Apparemment la localisation pulmonaire chez l'homme est plus courante dans les zones humides que semi-arides. De manière globale, la lutte contre la tuberculose bovine en Afrique doit viser d'abord les bovins et contenir en même temps la maladie dans les réserves de faune agissant comme potentiel reservoir (Cousins et al., 2004a) Des études approfondies et participatives sur les contacts sociaux, routes de transhumance et marchandes, ainsi qu'une analyse moléculaire de pointe sont nécessaire pour identifier les déterminantes de transmission dans un contexte spécifique, ce qui permettra de développer de stratégies de lutte adaptés au contexte environnemental, social et politique (Zinsstag, 2007).

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Figure 1: Lésions granulomateuses (taches jaunâtres) suspects de tuberculose bovine d'un poumon bovin à l'abattoir de Sarh (Tchad) (Image: Ngandolo Bongo Naré)

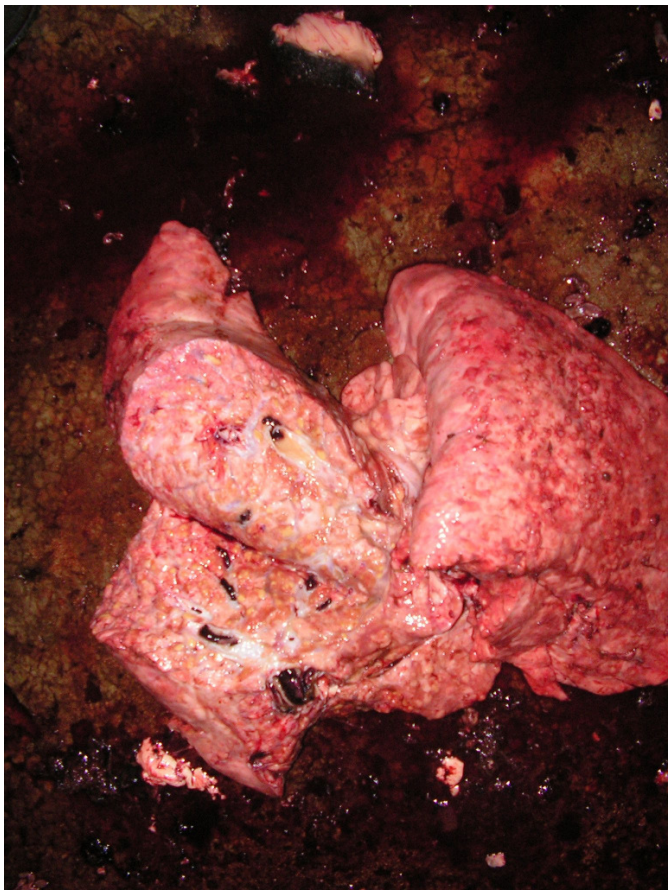


Figure 2. Présence de la tuberculose bovine en Afrique entre 1992-2001: En couleur verte, présence chez le bétail ou faune sauvage. Traits horizontales : présence chez le bétail mais quasiment absent chez l'homme. Traits verticaux : présence chez le bétail et chez l'homme (L'Ethiopie et l'Eritrée sont représentés ensemble). (Ayele et al., 2004)

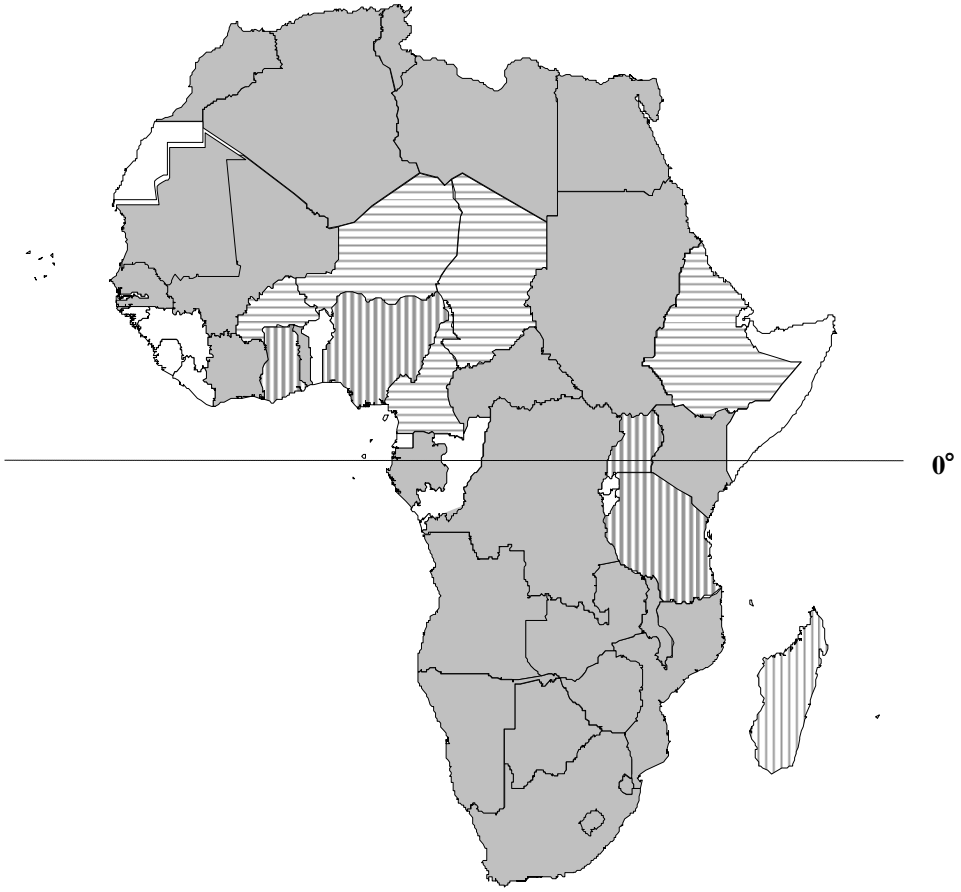
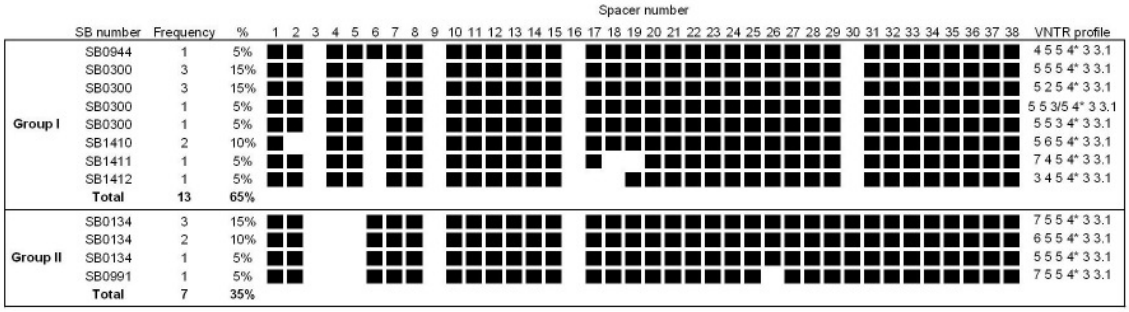


Figure 3 : Empreinte moléculaire (Spoligotype et VNTR) des premières souches de M. bovis isolés au Mali (Müller et al. 2008)



14. Farmer's perception towards agriculture, livestock and natural resources in rural Ethiopian Highlands

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Abstract

Increasing human and livestock population in Ethiopia is leading to increasing demand for feed. Cereal cropping is highly prioritized at the cost of the livestock sub-sector and the environment. Grazing land is decreasing, leading to overstocking and overgrazing of pastures, thus fuelling conflicts over scarce resources and exacerbating further land degradation.

Two independent surveys were carried out in four areas in the Ethiopian Highlands, investigating by means of questionnaires livestock husbandry as well as farmer's perception and attitudes regarding the relationship between cropping, livestock and natural resources, in the context of a broader reflection on what can help support Ethiopia's human, animal and environmental needs in a sustainable way. A total of 684 farmers were interviewed in 75 villages. The majority of animals were feeding from natural pasture and crop residue and only 1.3% of the respondents purchased supplementary feed. Overall, crop land has increased in the study area at the cost of grazing land and overstocking of pastures was seen as a major problem. Second main reason for decreased grazing land was considered to be drought in Woldia and increased human population in Gurage. No pasture management system was in place at community level in our study sites. Less than 2% of the respondents perceived and understood land degradation and subsequent decreased land fertility to be a constraint for sustainable feed production.

Strategies of de-stocking cattle herds are discussed. Cattle are intrinsically tied to cereal cropping. The more cropping land the more draft animals are needed and the more pressure is put on grazing land. Male animals account for over half of the cattle herd structure due to the need for draught animals; 80% of households kept draft oxen. Measures and priorities for future livelihoods were perceived differently by farmers from different regions. This study highlighted the lack of understanding amongst the farmers of causes and effects of land degradation and the lack of community based strategies for conservation agriculture.

Key words: Ethiopia, agriculture, livestock, sustainable land use, conflict, biodiversity, natural resources

Introduction

Ethiopia, well known for its recurrent famines, is amongst the poorest countries in the world and receives the most voluminous food aid in the world (Berry 2003).
Agriculture remains the major economic sector in the country, accounting for 43.8% of the national GDP, 90% of exports, and 85% of employments (CIA 2009). Over 90% of the agriculture—characterized by smallholder mixed farming (crop and livestock)—is practiced in the Ethiopian Highlands, which accounts for only 40% of the total territory but carries more than 80% of the human and 70% of the livestock population, making this the most densely inhabited part of the country (Demeke 2006). The Highlands are however, jeopardized by severe land degradation (Gete and Hurni 2001; Tadesse 2001; Daba et al 2003; Nyssen et al 2004; Hurni et al 2005; Nyssen et al 2008). This has direct impact on agricultural productivity, affecting both cultivated and pasture land through loss of soil and decreased soil fertility, thus constituting a major hazard to sustainable agriculture and feed resources (Hurni 1990; Yirdaw 1996; Nyssen et al 2009). The loss of agricultural value for the period 2000-2010 has been estimated at 7 billion USD (Sonneveld and Keyzer 2003).

With an Ethiopian population of over 85 million people growing at 3.2% per year (CIA 2009), the pressure on the agricultural sector is constantly increasing. Landholdings in the Highlands have an average cropland of 1.2 ha/household (CSA 2007), but are predicted to be falling to 0.6 ha/household by 2015 due to population growth (Teketay 2001). Despite an overall increase of cropland (at the cost of grazing land) and cereal production, food availability per capita has decreased in the last decade (Sonneveld and Keyzer 2003; CSA 2007).

In order to secure food availability and alleviate poverty, the Ethiopian government defined cereal intensification as a priority a decade ago (Byerlee et al 2007). But the livestock sub-sector remains, by comparison marginalized in terms of improving animal productivity and animal health and promoting better management of pastures and thus animal feed, thereby doesn't contribute to its full potential to the national economy (Ibrahim 2004; Gebremedhin et al 2004). Ethiopia has the largest livestock population in Africa, with a cattle population of 43 million head (CSA 2007). Animals are kept for milk, meat, draught power, manure and economic security. Importantly, livestock keeping is intimately linked to agriculture. Traditional farming practices in the Highlands depend on draft oxen for ploughing and threshing (Figure 1) (Goe 1987; Gebregziabher at al 2006). Draught power has been shown to be related to poverty

because farmers owning fewer oxen cultivate smaller areas and produce less labor-intensive but cheaper pulses instead of cereals (Astatke and Saleem 1996). The increasing livestock population is forced to graze on decreasing grazing land, which contributes to further land degradation(Gebremedhin et al 2004), to poorly nourished animals characterized by low productivity and to conflicts over natural grazing land (CSA 2002; CSA 2007;Nyssen et al 2009)

The present study explores by means of questionnaire surveys in four study areas in the Ethiopian Highlands, prevailing husbandry practices as well as farmer's perception on the current delicate balance between livestock, cropping and natural resources and how they outline their livelihood objectives.

Material and methods

We conducted two independent farmer household surveys using questionnaires with closed and open questions as part of a larger project assessing bovine tuberculosis in rural Ethiopia. Farmers were randomly selected within the multistage sampling framework of the tuberculosis project, according to their willingness to participate and only after they had given their oral consent. All questionnaires were translated into Amharic and back-translated into English for validation of misunderstandings and mistranslations. Interviews were carried out by a trained enumerator. The researcher was also present during all interviews to verify the accuracy of questionnaire filling. Only farmers who were fluent in Amharic (speaking and understanding) could participate in the interviewing process.

The first survey was conducted between 2006 and 2007 in four Woredas (districts) of three regions: 1) Meskanena Mareko, a Gurage area in the Rift Valley (Southern Nations, Nationalities and People Region, SNNPR) located at 8°10'N and 38°20'E (1800-2170 m a.s.l.), 2) Woldia (Amhara region) located at 11°55'N and 39°35'E (1460-3490 m a.s.l.), 3) Bako Gazer (SNNPR) located at 5°45'N and 36°40'E (1338-1634 m a.s.l.) and 4) the Bale Mountains (Oromia region) a larger geographical are located between 6°50'N and 7°10'N and between 39°40' and 40°20'E (2120-3500 m a.s.l.). In the latter zone, we regrouped three neighboring Woredas, Dinsho, Robe and Goro, in one study site (Figure 2). The study sites covered the two typical agro-ecological zones in the Highlands: "*woina dega*" between 1500-2300 m a.s.l. and "*dega*" above 2300 m a.s.l. The questionnaires for this first survey included general questions on

farm size, livestock husbandry (grazing system, fodder, farm input, keeping of other livestock), herd structure and herd turn over (exit due to death/selling and entry due to birth/purchase/gift), use of manure, and off-farm jobs.

The second survey was conducted between 2007 and 2008 in only two of the Woredas, the Eastern part of Woldia (between 1400 and 2000 m a.s.l.) and Maskanena Mareko (1800-2170 m a.s.l.). This questionnaire focused on questions related to land use, livestock and interaction between livestock and natural resources and their changes over time as perceived by farmers. Farmers were also asked about occurrences of conflicts over natural resources and their objectives regarding livestock and available land for the future.

Although the interviewed farmers in both surveys were from various ethnic, religious and cultural backgrounds, they were all livestock traditional smallholders in the Ethiopian Highlands, involved in both cropping and livestock husbandry with similar farm management.

Additional demographic data were collected from the Central Statistical Agency (CSA), Addis Ababa and Ministry of Agriculture and Rural Development, Addis Ababa. All data were doubled entered in Access and validated for entry errors with the statistical software package Epi Info (version 3.3.2). Analysis was done using the statistical software package STATA 9.1 (StataCorp, Texas, USA) and Microsoft®Excel 2002.

The study received ethical clearance from the institution and national ethical review committees (NERC, Ethiopian Science and Technology Agency).

Results

1. Livestock keeping survey

A total of 536 farmers were interviewed in four Woredas, which included 24 Kebeles (smallest administrative unit) and 75 villages. Fifty-eight percent of farmers grazed their animals on communal land, whereas the other animals grazed on farmer's own land. Animal feed mainly consisted of forage from natural pastures (free and uncontrolled grazing) and crop residues after harvest with purchased feed such as oil-cakes and molasses accounting only for 1.3% of the total feed. Veterinary services were variable, with farmers stating that 56% of their cattle were regularly vaccinated and only 33% regularly dewormed. Seventy percent of farmers were also keeping other livestock in addition to cattle.

Cattle herd structure is shown in Table 1. Adult uncastrated and castrated males (37%) exceeded the number of breeding cows kept by interviewees (30%). Nearly a quarter of the herds

consisted of oxen (22%). Regardless of age, total males accounted for 52% of herds. Considering exclusively breeding animals, there were only twice as many cows (30%) as bulls (15%), thus 1 bull for 2 cows.

Regarding herd turn-over, 38% of farmers purchased at least one animal during the previous year, which was more or less equal to the number of farmers (36%) having sold at least one animal in the same period . Birth was recorded twice as often as death, with 63% of farms having had at least one calving during the previous year and only 29% of farms recording at least one death.

Eighty percent of farmers held oxen as draught animals, accounting for 98% of all oxen (Table 2). The remaining 2% oxen were used for fattening. Twenty-three percent of all breeding males were also used as draught power. Females were rarely used as draught animals (0.4%). Seventy-nine percent of all draft animals were working more than 6 months per year.

Seventy-four percent of respondents valorized manure: 21% used it as fertilizer, but the majority (79%) used it as a source of fuel in the household and sold the remaining unused manure. Overall, 38% of respondents invested in farm improvement. As shown in Table 3, these inputs varied a great deal by region. Veterinary service is overall speaking and with the exception of Bale, viewed by farmers as the most important husbandry input to give: between 62% (Gurage) and 94% (Woldia) were seeking veterinary care, whereas improvement of breed genetics and improvement of feed, both assets contributing in increasing animal productivity were perceived as less important with a maximum of 11% and 34% of respondents being in favor of breed and feed improvement respectively. No husbandry improvements were observed in the Bale Mountains. However, only 40 farmers were interviewed, thus these figures do not necessarily reflect the reality in that region. Only 8% of farmers had alternative off-farm income source.

2. Survey of farmers' perceptions and objectives

This survey included 69 interviews in 16 villages from 5 Kebeles in the Gurage region (Meskan Mareko) and 79 interviews in 22 villages from 6 Kebeles in Woldia (total questionnaires: 148).

Cattle population

Change in herd size over time differed between the two regions: 41% of respondents in Meskan answered that their herd was now larger than in the previous ten years, due to purchase, birth and gifts from NGOs and government. Only 20% of farmers had smaller herds than in the last 10 years. By contrast, 44% of Woldia farmers stated that they had to decrease their herd size over the years due to diseases, drought and severe feed shortage. Only 18% had more cattle when compared to the past years. The majority of farmers in both regions (73% in Woldia, 56% in Meskan) stated that stocking density on communal land was too high, leading to overgrazing and degradation of pastures.

Pastures

No pasture management system was in place in Woldia and Meskan. Figure 3 (A) shows the answers of respondents from both regions concerning the current, past and future availability of pasture forage for their livestock. The majority of farmers in both regions acknowledged a current lack of grazing land, which would dramatically worsen in the future and an overstocking/overgrazing of pastures. The reasons for pasture shortage as perceived by farmers are given in Figure 3 (B). The major reason given by over 60% of respondents from both regions was clearly increased cropping land. All interviewed farmers said that land used for crops was greater in surface than the grazing land they could access. Farmers were not able to provide absolute figures for surfaces and the statements relied on their perception of changes in land use. Most Woldia farmers (91%) mentioned that they had crop land four times and more the size of pastures, while only 62% of Gurage farmers mentioned having this proportion. Only 16% and 3.7% of farmers in Meskan respectively Woldia thought that increased cattle numbers were a limitation to available pastures. In Woldia, drought was an important source of grazing shortage (31.5% of respondents). Decreased land fertility was not seen as a reason for grazing shortage in Woldia and only 1.6% of Gurage respondents observed a decrease in land fertility.

Problems and benefits of communal grazing were assessed with farmers from Meskan only (Figure 4 (A-B)). Shortage of forage and animal emaciation were seen as the main drawbacks of communal grazing by a quarter of the respondents. One third perceived easy herding as a major benefit of communal grazing.

Water resources

Rivers and lakes were the main water sources for all Woldia farmers and 60% of Gurage farmers. Because of the often long walk to watering sources, 57% of herds in Meskan and 75% of herds in Woldia were watered only once a day. Forty-four percent of Gurage farmers complained about regular shortage of water. The main complaint was that water was diverted by richer farmers for field irrigation (37.5% of respondents), followed by seasonal droughts (25% of respondents) and human water consumption (19%). During drought periods, water access for livestock was restricted in 16% of the interviewed households. Problems related to common watering were infestation of cattle with leeches (25% of respondents) during the dry season and long walking distances to water sources (21% of respondents), whereas diseases and injuries through fights were rare (4%).

Conflicts

Twenty-two percent of farmers in Meskan and 44.3% in Woldia declared that communal grazing land was also used by farmers from other villages. Conflicts over grazing land were mentioned by 23% of respondents in Meskan but only by 4% of Woldia farmers. In Meskan half of the conflicts involved fellow farmers from the same or from other villages sharing natural resources, half with Kebele authorities. Farmers reacted by oral complaints. Frictions over water resources were mentioned by 18% of Gurage respondents.

Priorities and measures for the future proposed by farmers

Figure 4-C shows farmers' priorities and objectives for the future that varied between the regions. Less than half of the farmers wished to have more pastures available. In Meskan region, 44% of Gurage farmers considered that additional grazing land should simply be provided by the government. The next two prioritized managerial improvements were improved breeds (17% of respondents) and having access to available and affordable supplementary feed (25%). Decreasing herd size was not seen as a major option by Gurage farmers (only 3% of respondents), whereas this was seen as the major measure to overcome land degradation by half of the Woldia farmers.

Discussion

Ethiopia is a country dependant on its agricultural sector, which is characterized by low productivity. Traditional production systems have lead to severe land degradation resulting in decreased feed resources for both human and livestock. But simultaneously, human and livestock populations are increasing fast, using more resources than in the past and putting increasing pressure on land (Figure 5) (Nyschen et al 2009).

Data from the Central Statistical Agency (CSA) have shown that over the last decade cropping land has increased at the expense of grazing land (CSA 1996; CSA 2000; CSA 2006). Ever decreasing grazing land combined with a fast growing livestock population of over 90 million head (CSA 2007) is likely to lead to massive overstocking and overgrazing of available pastures and increased land degradation. This national situation correlates with data from our study: 41% of interviewed Gurage farmers stated that while their herd size is larger than 10 years ago, their grazing land has decreased massively in favor of cropping land thus further increase stocking rate. All interviewed farmers, regardless of region, stated that they needed to prioritize crop land to feed their growing families.

The majority of the Gurage and Woldia farmers complained about the current situation regarding lack of pastures to accommodate their animals and about overstocking/overgrazing problems. Perception of farmers regarding the other reasons of lack of grazing land and forage in the future - besides increased crop land - differed by region. Woldia farmers considered drought to be a major constraint to both grazing land and herd size. Whereas, Gurage farmers considered the increasing human population to be a major constraint, since linked with increasing livestock and more land needed to build infrastructure.

None of the farmers in neither Gurage nor Woldia perceived land degradation and subsequent decreased land fertility as a problem. Overstocking damages land but kept in optimal numbers, livestock contribute through accumulation of manure to increased biomass production on grazing land (Tadesse et al 2002; Tadesse et al 2003). In our study, manure was collected from the fields as primary source of fuel and/or selling and thus went back only partially into soil fertilization (only 21% of the respondent used it as fertilizer). Less than 2% of the interviewed farmers understood decreased soil fertility of pastures to be one reason for feed shortage. Water shortage was perceived as a much bigger problem than grazing shortage.

Overall, farmers nearly entirely relied on forage from natural pastures to feed their animals with only 1.3% of respondents purchasing feed. Communal grazing was perceived by the interviewees as having more benefits than problems despite the associated lack of forage. Poor nutritional status contributes to low animal productivity. To compensate for low individual productivity, farmers tend to increase their herd size as shown in the Gurage study, which in turn puts more pressure on pastures. Only external factors such as severe drought, as shown in the Woldia case lead to herd depletion through selling and deaths. Such events may in turn force some of the farmers to use communal land for cereal cropping in order to survive, thus further decreasing available grazing land as well as soil fertility due to lack of manure. Farmer's solutions to the overstocking/overgrazing problem differed depending on the region: Woldia farmers stated they wished to have fewer but more productive animals to feed, which would in turn reduce the need for grazing land. On the other hand, Gurage farmers were asking local authorities for more pastures as if land was an expandable commodity. They also considered more productive breeds as priority but without having to reduce their herd size. Agriculture extensification rather than intensification was clearly the focus of these farmers. Finally, they wished to have increased water resources. In common, both Woldia and Gurage farmers saw the need for increased and affordable supplementary feed.

There was no household-level or community-based land use management in the two study areas. Our study also highlighted that interviewed farmers did not fully perceive the limitations of existing natural resources; the complete cause-effect chain and the full extend and implications of overgrazing, land degradation, future availability of feed and sustainability of natural resources. This contrasts with the attitude of rural communities in parts of Ethiopia (e.g. Tigray; pastoralist communities in Afar or Borana), who have a long tradition of restrictive regulations of grazing areas at village level and an understanding of the limitations of existing natural resources and consequently their management (Gebremedhin et al 2004; Edossa et al 2005; Abule et al 2005).

In Tigray, a region with extreme land degradation, agriculture intensification and conservation agriculture has proved to be one solution to the above described agricultural trap, when participatory approaches were used (Astatke et al 2003; Nyssen et al 2009). Conservation agriculture is a relatively new concept, especially in Africa, which combines social and economic benefits from integrating production and protection of the environment (Dumanski et

al 2006). Examples in Tigray include increased field irrigation systems (Nyssen et al 2009), slopes terracing and stone bunds (Nyssen et al 2007), catching and storing run-off water in ponds (Fekadu 2007), rehabilitation of degraded land by exclosures and thus limitation of uncontrolled and free grazing that further damages land (Mekuria et al 2007).

Decreased feed availability on overstocked/degraded communal grazing land as seen in this study are likely to have direct impacts on animal health; this is reflected by animals showing low body condition, decreased productivity and decreased resistance to diseases (Pandey et al 1993; Mishra et al 2001). Communal grazing also directly increase the risk of disease transmission between animals and increase the parasitic load on pastures (Lefèvre et al 2003). A quarter of the interviewed farmers perceived communal grazing to be associated with animal emaciation but only 6% of them saw a possible link with diseases. Increased livestock population also strains the scarce veterinary services available in the country. Official figures show that only 2 million cattle (4.6%) were vaccinated nationwide in 2007 (CSA 2007). In contrast, half of the interviewed farmers in our study stated that they vaccinated regularly their animals. This discrepancy can be explained by the fact that the latter farmers had better access to veterinary care services than farmers from other regions such as pastoralist zones. Their statement may also have only included valuable animals, such as oxen and not their entire herd. The need for increased veterinary care was clearly one the major priorities for husbandry improvement given by most respondents (62% of Gurage and 94% of Woldia farmers).

Friction over scarce natural resources exists in most part of Ethiopia. In some parts the different players manage to reach agreements (Nyssen et al 2009), whereas in other conflict and violent clashes occur, such as in the Awash river basin (Edossa et al 2005) and the Gambella region (Sewonet 2003; Reuter 2008). Verbal frictions were described in 23% of Gurage respondents in our study; half of which involved the relevant Kebele authorities in not taking seriously their need. Farmer's attitude in this study site stresses the lack of collective actions at village level to cooperate in resource management and the fact that farmers rather rely on authorities to improve the situation.

Ethiopia has the largest livestock population in Africa with one head of cattle for less than 2 people. Furthermore, within the cattle population, males account for half of the total herd

structure on national level and in our study (CSA 2007). De-stocking cattle herds seems to be the logic solution to decrease economical and ecological burden to the agriculture sector. In urban areas, farmers have switched to more productive and intensive systems including high productive exotic dairy breeds in order to supply big cities with the increasing demand for milk. However, high productive exotic breeds and their crosses account currently for only 1% of the total cattle population and milk demand is still five times higher than the supply, animal feed remains unaffordable and grazing areas are lacking (Abebe 2007; CSA 2007).

In rural areas, traditional production systems rely entirely on animal power. Increased cropping land will in turn need more draft animals and thus lead to stagnation in the agricultural system and further exacerbation of land pressure/degradation (Figure 5). Taking into account animal losses and the minimum age at which animals can start being used as draught animals, each household realistically needs to maintain a minimum herd size of 8-10 animals to permanently secure at least 2 oxen for ploughing (Sandford 1982). The majority of farmers in our study possessed 2 oxen and 12.5% of the respondents had even 3 and more oxen. Draught animals worked more than 6 months per year (ploughing and threshing), making it difficult to share working animals among farmers, and thereby decrease the number of oxen in a villages. Cows are primarily kept to produce the next male generation for draught power rather than for milk production. Improvement of breed genetic as encouraged in urban/peri-urban areas to increase productivity and thus decrease animal numbers are not a solution for these essentially ox production systems. This is also reflected in the farmer's attitudes in our study. Regardless of fodder availability, only 11% of Gurage and 7% of Woldia farmers said they would like to have improved breeds with better productivity. Moreover, our study showed that overall no efforts were made for livestock housing and feed supplementation as alternatives to natural grazing. However, even in these traditional production systems, research has shown that strategies can be used to decrease necessary oxen population. Astatke et al (2003) showed that modification of the traditional plough reduced tillage and soil erosion. The authors reported 50% less draft animals required compared to the traditional ploughing system.

Finally, increasing the export capacity of live animals and meat may help decrease the livestock surplus. Around 350'000 cattle and 1.2 million small ruminants are exported annually (FAO 2007). However, the export industry is still underdeveloped in Ethiopia and the majority of the

trade is informal (cross-border) from pastoralist areas (Little 2005). These areas are also the main suppliers of animals to export abattoirs and exporters help de-stock their herds during times of crisis (e.g. severe drought).

Conclusions

Livestock is the most important component in a farmer's life for daily survival and economical security. Yet, cereal cropping is highly prioritized at government and farm level at the cost of the livestock sub-sector, the environment and natural resources. The introduction of conservation agriculture and rehabilitation of degraded land in parts of the country has shown benefits in economical and ecological terms compared to the traditional agriculture system. But these strategies work best when community or village driven. This study showed that the perceptions and attitudes of farmers towards agriculture (cropping and livestock) and natural resources in our study sites as well as their priorities for future livelihoods diverged very much depending on the region. It also highlighted the lack of understanding amongst these farmers of causes-consequences of land degradation and subsequent sustainability of future feed sources for both human and livestock and thus, the need for increased community based awareness and participatory trials on conservation agriculture.

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Fig 1: Field ploughing in the Ethiopian Highlands using oxen pulling the traditional plough, the *maresha* (photo: Rea Tschopp).

Figure 2: Map of Ethiopia showing the different study sites

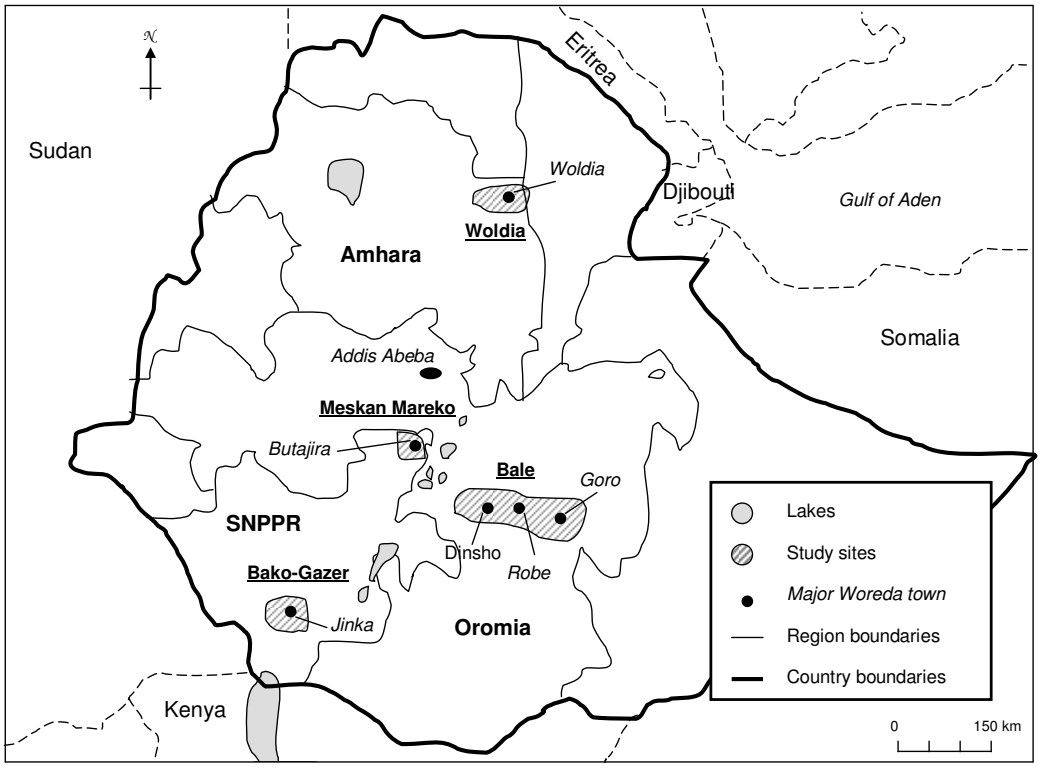


Table 1: Overall herd structure of the interviewed farms

Cattle categories	Number	Percent of total number
Calves (<1 year)	546	14.0
Juveniles (1-3 years)	730	18.7
Breeding cows	1183	30.4
Breeding bulls	581	14.9
Oxen	850	22.0
Total cattle	3890	100

Table 2: Number of oxen kept per household and percentage of households with few to several oxen.

Number of oxen	Number of households keeping oxen	Percent of households keeping oxen
None	113	21
One	148	27
Two	211	39
Three	32	6
Four and more	37	7
Total	536	100

Table 3: Farm input by region in percent of respondents

	Gurage region	Woldia	Bako Gazer	Bale Mountains
	(n = 172)	(n = 189)	(n = 135)	(n = 40)
Veterinary care	62	94	78	0
Improvement of cattle housing	11	4.6	0	0
Improvement of breeds	11	7	0	0
Improvement of feed	34	6	2	0

Figure 3: Grazing availability for livestock (A) and reasons for grazing shortage (B) as perceived by Gurage and Woldia farmers.

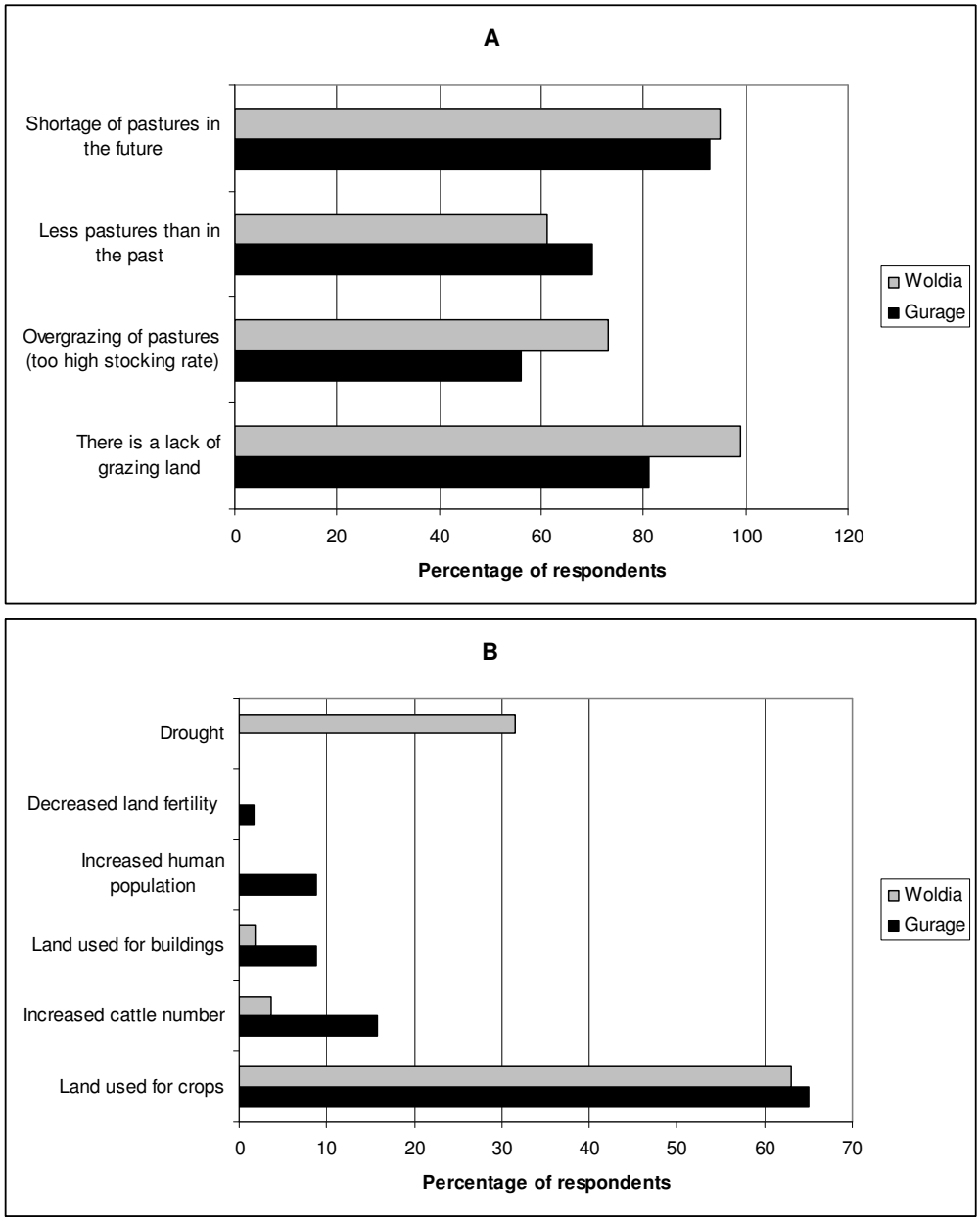
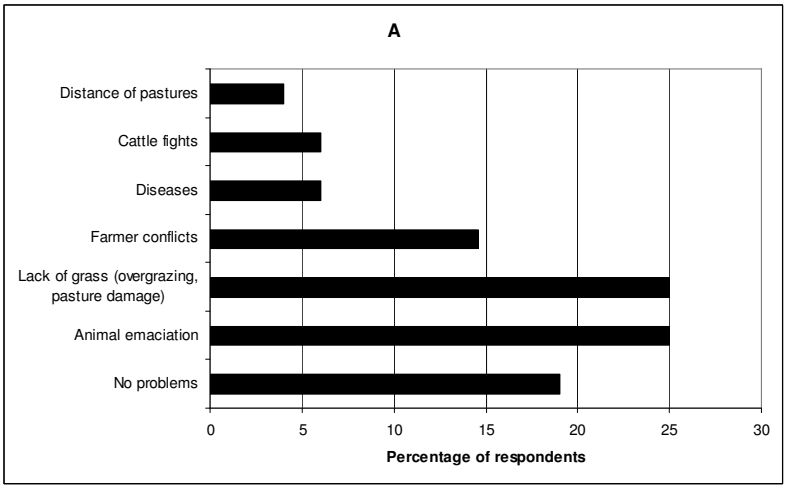
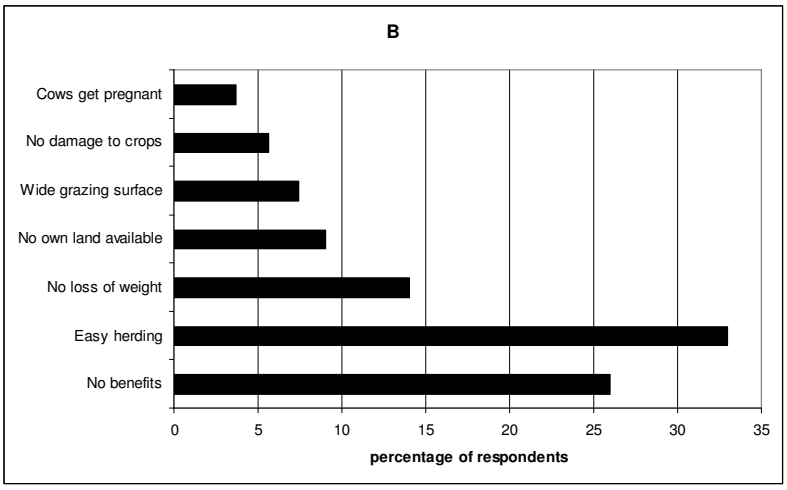


Figure 4: Problems (A) and benefits (B) of communal grazing as perceived by Gurage farmers, and measures (C) proposed by Woldia and Gurage farmers (multiple answers possible).





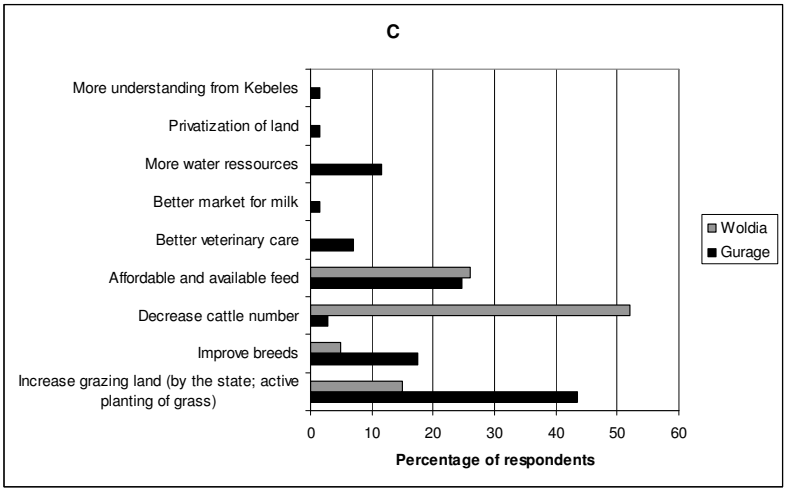
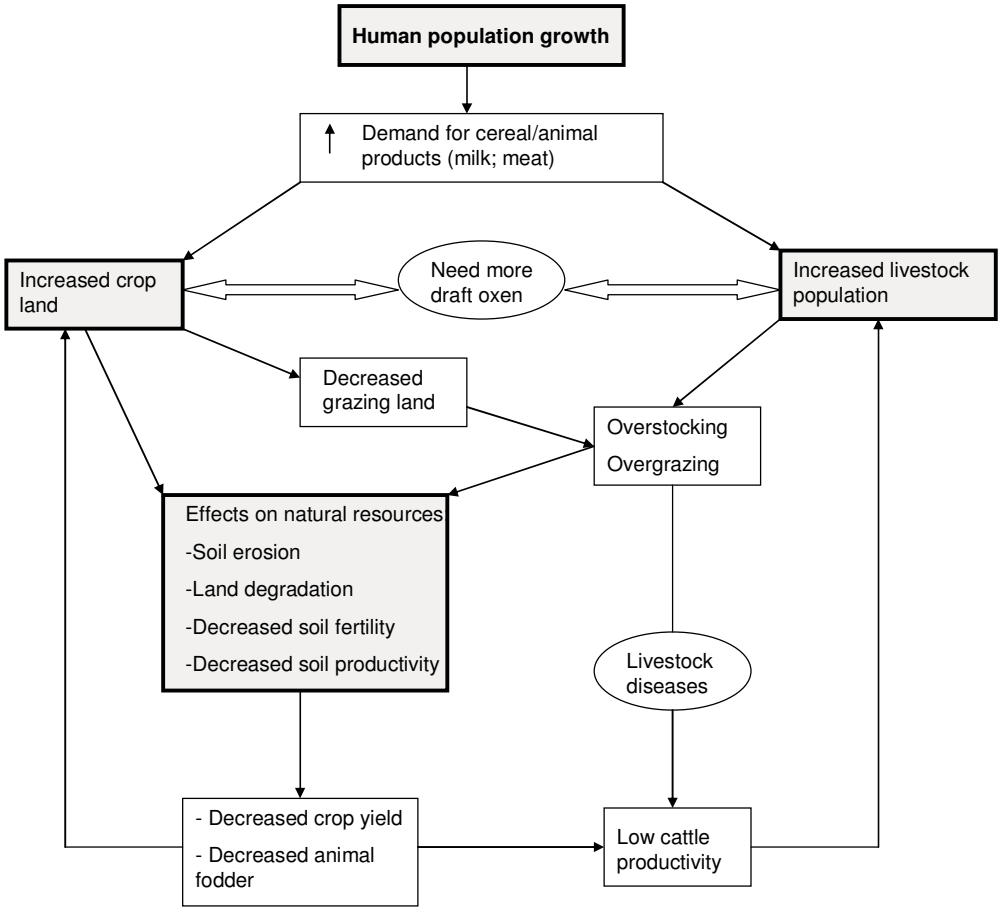


Figure 5: Flow chart summarizing in a simplified way the traditional low intensity crop-livestock system in Ethiopia and its relationship to natural resources



15. Livestock productivity studies

This chapter presents briefly some longitudinal on-going long term studies, started during this PhD but which are not finished at the time of the thesis completion. Therefore, no details on analytical methods and no results are shown at this point of time. However, these studies are essential in order to complete the ultimate goal of the original BTB project research question concerning the economical impact of BTB on livestock and to understand some discussion points in the last chapter of the thesis.

15.1. Baseline productivity analysis of Ethiopian cattle

Baseline productivity parameters are needed in order to assess possible impacts of BTB on livestock productivity. Little information was found in the literature for the Ethiopian setting, except for some intensive peri-urban farms (Mekonen et al., 2005, Gebeyehu et al., 2006, Yilma et al., 2006). However, little to no data has been collected on long term cattle productivity in herds kept under traditional management. We therefore started such a long-term study over a period of 4 years in order to collect field data on cattle productivity.

Study sites

Twenty farms were selected in Sellale (North Shoa), 200 km North of Addis Abeba and one farm in Butajira (Rift Valley), South of the capital city, both sites being located in the Central Highlands of Ethiopia. Sellale has a temperate climate and is one of the main sources for milk supply for Addis Abeba. It is one of the few regions in Ethiopia where farmers are keeping traditional zebus as well as Holstein and their cross-breeds under same traditional husbandry conditions, thus making breed comparison possible.

The study in Mercy farm (Butajira) was started only in 2007, after an initial study involving the governmental farm of Holetta (West of Addis Abeba), which kept Holsteins, failed due to stamping out of TB positive animals included in the study. Project Mercy, is a not-for-profit relief and development agency and owns a dairy farm, which supplies the nearby school and orphanage (all belonging to the project) with fresh milk. The cattle are kept under intensive husbandry and the herd is composed mainly of Boran and Fogera cattle breeds and some of their crosses. These two traditional breeds are known for higher milk production compared to other traditional zebu breeds.

Animals

Since herds undergo a dynamic turn over in time, it is difficult to give here exact static figures. At the beginning of the study in 2005 in Sellale, herd size varied between a minimum of 9 and a maximum of 28 animals. The initial stock was composed of 123 (35%) traditional zebus, 134 (38%) cross-breeds and 94 Holstein (27%). The majority of the animals were females (58%) whereas bulls and oxen made up each for 21 % of the herd composition. Animals were categorized in following age categories: 61 calves (17%), 67 juveniles (19%), 182 adult breeders (3-10 years) (52%) and 41 old breeders (>10 years) (12%).

The initial stock in Mercy farm counted 96 animals. The majority were females (N=78, 81%). The herd was composed of 36 calves (37%), 3 juveniles (3%), 25 adult breeders less than 10 years old (26%) and 32 breeders over 10 years of age (33%). The majority of the cattle were Boran (63%) followed by Fogera (28%). The remaining herd was made of 5 traditional unclassified zebu (5%), 2 cross-breed (2%) and 1 Holstein (1%).

Herd follow up

Each farm in Sellale is visited every two weeks and herd books updated after each visit. The aim of these visits is to assess herd structure, turn over and dynamics (entries and exit of animals), record data on meat gain, milk production and fertility.

Following parameters are recorded during each visit: new born calves, purchasing of animals, selling of animals, death of animals, reason for death, sick animals, and price of sold or purchased animals. New born calves are weighed 3 times with a hanging scale in 2 weeks interval since farm visits often don't coincide with the day of birth. A regression analysis will allow calculating the birth weight. The weight of these calves is then followed up once a month using an animeter during 12 months. In addition, all animals are weighed with the animeter regardless of their age, twice a year, once after the rainy season and once after the dry season in order to have an average animal weight.

Each new animal is identified by sex, age, breed, and parity for cows and a unique ID is given for each animal. Farmers did not allow us to ear-tag their animals, however they know each animal by name and we subsequently gave an ID for each name.

Follow up of milk production is started for each cow giving birth during the study period and milk production is recorded once a month until the animal is dry.

All cattle in the study are regularly dewormed twice a year with Albendazol (appendix 6).

Similar procedure is done in Mercy farm. However visits are conducted only once a month since the logistic is much easier than in the Sellale farms, e.g. all cattle were housed at night under the same roof, calves can be weighed right after birth and less animal turn over exist. In this farm, we ear-tagged all animals (initial stock, new born and purchased animals).

Data analysis

All data is doubled entered in Access table, and validated with Epi-Info. Herd book sheets are updated once a month (e.g. addition of new animal IDs, deletion of animals that left the farm) The final data will be analysed with an FAO spreadsheet called Livestock Development Planing System (LDPS2). The application helps to identify and quantify herd composition and size required for a given demand of milk and meat. It helps to identify and quantify feed and livestock constraints and also to assess various livestock development programs. The results of this study will be incorporated into the human-animal health economic model for the economic analysis of the impact of BTB in Ethiopia, similarly to what was done in a Brucellosis study in Mongolia (Roth et al., 2003).

15.2. Herd structure of cattle in Ethiopia

To complete the above LDPS2 sheet, average Ethiopian cattle herd structure has to be known. We collected the information regarding herd structure in the study described in chapter 15. Considering a herd size of 43 million cattle (CSA 2008) we get the following figures:

	Percent of total	Total numbers of animals in the country
Calves	14	6'020'000
Juveniles	18.7	8'041'000
Breeders females	30.4	13'072'000
Breeders male	14.9	6'407'000
Oxen	22	9'460'000
Total	100	43'000'000

15.3. Impact of BTB on animal weight in abattoirs

To our knowledge, no studies have been done in Africa on the impact of BTB on weight loss in animals. However, knowing the nature of BTB as being a chronic debilitating disease, it is expected that weight loss would be a major symptom and would therefore lead to substantial economical losses for households and abattoirs, including the flowering export abattoir industry.

The only detailed study on this subject based on field data was done by Meisinger in the 60s in East Germany (Meisinger 1969) who conducted a cohort study on 8000 animals over 5 years. His study showed that BTB caused a loss in meat production between 6 and 12 % in positive herds. These herds were defined as herds having at least 80% reactor animals.

In our study, we investigate if BTB caused by *M. bovis* has an impact on animal weight and carcass weight, therefore leading to economical losses for abattoirs. A further loss would include carcass condemnations (total and partial condemnations) due to BTB lesions.

Since abattoirs in Ethiopia lack entirely equipment such as scales and record keeping, we had to launch a two-step study:

In Luna export abattoir in Modjo (200 km South from Addis Abeba in the Rift Valley), which keeps records on animal information, we collect detailed data on live weight of animals using a scale and the animeter. Breed, sex and carcass weight are also recorded as well as TB-like lesions found by meat inspectors during slaughtering.
This export abattoir is buying animals from remote rural areas, feeds them up for two months before slaughtering them. Main breeds are the Danakil breed form the North, especially Tigray and the Boran breed from the South, particularly Borana region.

Once the use of the animeter is validated in the export abattoir, we will use it in the second step of the study, namely in the regional abattoirs, which lack weighing scales. In the following six abattoirs: Gondar, Jinka, Gimbi, Woldia, Butajira and Addis Abeba, lymph node lesions are also

collected in the frame of the project by Work Package 1 and analyzed for *M. bovis* (culture and spoligotyping), thus enabling the link between isolation of *M. bovis* and animal weight.
The study is however, complicated by the fact that animals may have parasites such as fasciola, which has an effect on weight as well. We therefore record presence of fasciola or any other pathological feature for each animal. We also record the season when the animal is being slaughtered, since seasonal variation may lead to seasonal animal weight changes (dry versus rainy season). The study done in Sellale (cf. above paragraph "productivity") will help estimating the percentage of weight loss due to seasonal variation.

So far 870 animals have been weighed with the animeter in the regional abattoirs and 350 in the export abattoir.
The collection of data will continue for another six months.

15.4. Market analysis

Market data such as the price of live animals, meat, milk and other animal products are being recorded by means of questionnaires since the beginning of the study in September 2005. Sources of information are: farms where cattle have been tested for BTB, household surveys, local markets, farms included in the productivity study, national statistics and probably later from Delphi panels if some information are missing or are incomplete. So far 836 data points have been collected. In addition a macro-economic analysis will be performed in 2009 (e.g. trade barrier costs, import/export, quarantine costs, inflation rate).

Because of the regional and seasonal variation of the Ethiopian market (e.g. dry versus rainy season, religious holidays), information are collected in all different sites of the project over the total duration of the project and at least twice a year. Due to these fluctuations, it will probably not be possible to calculate an average profitability of an intervention but rather a variation of profitability with minimums and maximums.

16. Approach to assess the economical impact of bovine tuberculosis in Ethiopia

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Abstract

Background: 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

Objectives: Create a dynamic transmission model of disease between animal and human, as a prerequisite of economical analysis of the most profitable intervention in Ethiopia to control BTB.

Approach: 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 transsectoral framework that simulates disease transmission. Different intervention scenarios will then be simulated in the model.

Conclusion: The most profitable intervention to control BTB in Ethiopia has to be assessed as well as the cost sharing scheme between public health and agricultural sector. It has been postulated that test and slaughter policy would have a negative economic impact in Ethiopia. Alternatives will be assessed.

Introduction

Tuberculosis is worldwide distributed 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 only in Sub-Saharan Africa, and the role played by cattle-linked *M. bovis* in the raising 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). Although epidemiology of *M. bovis* is well documented in many countries and control and elimination strategies implemented since a long time in the developed world by a policy based on systematic slaughter of infected animals, meat inspection in abattoirs and milk pasteurization, BTB is still widely distributed and largely uncontrolled in developing countries, which are unable to support the costs of test-and slaughter policies and were 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 omits large pastoralist communities in the country. 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 livestock and human health sector. Field

data collection is still ongoing. We present here the approach to estimate the cost of BTB to society and potential benefits of interventions.

Approach

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 for each flow 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. Following data are collected: field prevalence of BTB in cattle (intradermal PPD testing), abattoir prevalence of 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.

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 linked with 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$63 million (4). The socio-economic impact of BTB to the agriculture and health sector in Turkey has been estimated between 15 and 59 million US$ 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 has not been studied at all (9). Since agriculture remains the backbone of many African countries economy, 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 zoonosis 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 intradermal 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, e.g. Holstein *versus* local zebu) as well as between different management systems. The latter would imply that the burden should be estimated on the one hand for urban/periurban farming systems with intensive management and high milk production rate (urban milk market), and on the other hand for extensive farming system in rural areas of Ethiopia characterized by low milk production but important drought power of cattle for crop production.
An other difficulty faced by the current research is the low rate of *M. bovis* detection in human lymphadenitis cases. The reason of this low detection rate is still largely unknown (e.g. low prevalence of *M. 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. 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 transsectoral 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 breed with probably

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 been investigated if the control of BTB in Ethiopia could be linked with those of other zoonosis (e.g. Brucellosis) existing in the country.

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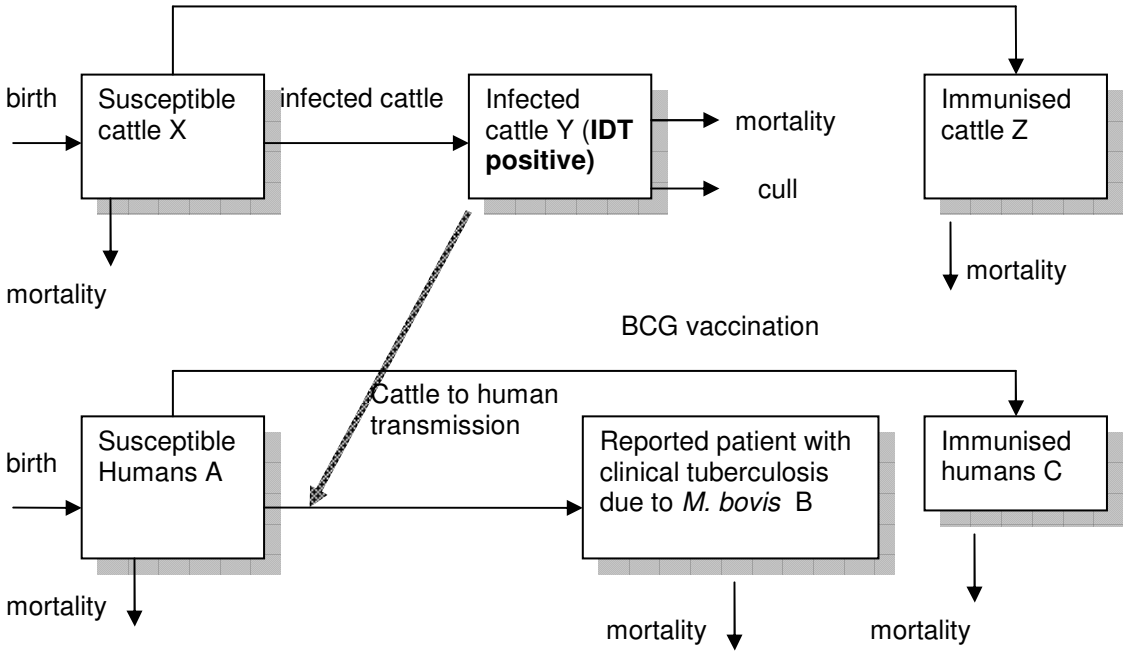

Fig.1. Adapted model framework for joint human-animal BTB transmission in Ethiopia

17. Setting bovine TB in the animal health context in Ethiopia: Animal health and husbandry practices

Working group report
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17.1. Major threats to the health of livestock and wildlife in Ethiopia

Ethiopian livestock is threatened by many infectious diseases, most of them being listed by the OIE (Office International des Epizooties). The list is very exhaustive. To name only some of them: Foot and Mouth disease (FMD), Chronic Bovine Pleuropneumonia (CBPP), Trypanosiomasis, 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; these reports 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 in the lowlands; Highlands.

However, diseases are not the only threat to livestock and wildlife health. 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 than livestock regarding its health risk. 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 lead 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

17.2. 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 in small scale studies. However, the magnitude of the impact of BTB on animal health in Ethiopia is still largely unknown. The disease is known to affect a large host range (cattle, goat, sheep, camel, humans, and wildlife), which makes its assessment difficult. Pathological lesions are seen in abattoirs during meat inspection, and the disease has been described in cattle. 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 dairy 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 of Central Ethiopia (dairy farms located in Shoa region). It is therefore difficult to describe an association of BTB with particular geographical areas. Detailed information is missing and a lot of areas especially the pastoral lowland areas are not covered by adequate studies. The situation of BTB on a nationwide level is therefore 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 settings (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. So far, M. bovis has been successfully isolated from humans mainly in Tanzania, Uganda and Ethiopia. Considering the prevalence described in cattle and the existence of risk factors of disease transmission to humans, the prevalence in humans should be expected to be much higher. The question arises whether there is a problem of detection in the laboratories.

17.3. 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, which 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 on the human health sector 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), and BCG vaccination of people.

17.4. Building capacity

A massive capacity building is needed in Ethiopia (technology, human resources, education). 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 efficiently problems.

17.5. 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 in order to assess 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 highly productive exotic breeds.

Human TB caused by *M. tuberculosis* is declared as an 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 future BTB funding.

18. General discussion and conclusions

18.1. Epidemiology of BTB in Ethiopia

18.1.1. Multi-disciplinary approach

The strength of this study lies without doubt in its multi-disciplinary approach and integration with other studies carried out by different work packages of the project. This allows connecting various disciplines together, such as veterinary and human medicine, social science, economy, genetics, molecular biology, field and molecular epidemiology, ecology, and finally the involvement in control strategies and policy making.

The advantages are also the ability to share logistics and resources and thus to cover numerous study areas. Study areas were chosen depending on the existence of an abattoir and a hospital in the area so to allow the essential epidemiology linking animal and human results.

18.1.2. Prevalence of BTB in cattle

Previous studies have shown that BTB is endemic in Ethiopian cattle with some prevalence variations depending on cattle breeds (Ameni et al., 2003). However, these studies were carried out in central Ethiopia, where there is a high concentration of dairy animals, intensive farming and exotic breeds (Holstein Friesian). Ameni et al. (2007) found individual prevalences reaching 11.6% in zebus grazing with exotic breeds; in the exotics prevalence was as high as 22.2%.

In order to advise on any future control strategies at a national level, it was important to know the situation regarding BTB in cattle in rural areas that are characterized by extensive management of local zebu breeds. Central Ethiopia is important in terms of milk supply for the big cities; however, over 80% of the Ethiopian population is rural and dependent on a daily basis on their livestock for animal traction, meat, hides, manure and milk. It was therefore an important aspect of this study to assess the prevalence of BTB in cattle from these more remote rural areas.

In contrast to previous studies from Central Ethiopia, we found an apparent BTB reactor prevalence of less than 1%. Our results were similar to those found for instance in Tanzania, where Cleaveland et al (2007) found an overall individual prevalence of 0.9%. This is low compared to other African countries such as Ghana, Uganda and Chad, where reactor prevalence was as high as 13.8% , 6% and 17% respectively (Bonsu et al., 2000, Bernard et al 2005,

Schelling 2002). In common with all these countries however, is the high herd prevalence (Ameni et al., 03, Cleaveland et al., 2007, Bonsu et al., 2000, Bernard et al 2005). It is actually surprising that individual prevalence is not higher considering the fact that cattle are kept together during grazing, around water points, and during the night inside the people's houses. In Ethiopia where grazing land is scarce, overcrowding on pastures is great, but still individual animal prevalence is low. Interestingly was also the fact that when considering neighboring villages, some would be negative whereas one would have many positive animals (a so called hot spot village). Although these animals move between villages and use communal pastures, they do not transmit the disease to cattle from other villages. So infection rates between animals seems to be low in rural settings, even when animals are kept in an overcrowded way; this is the opposite of what is found in intensive dairy farm practice. So what makes an animal become positive in these rural areas? And what is the reason for this very low transmission rate between animals? The study described in chapter 11, showed that no risk factors prevailed in our study sites for BTB positivity in cattle, except for the following factors: purchasing of a positive animal and the presence of livestock other than cattle in the herd.

As mentioned earlier, cattle positivity is much higher in Central Ethiopia. The discrepancy in prevalence with the one we found in our study raised some concern that the skin testing conducted may have been flawed (e.g. sampling bias, bias linked with the testing method or with the quality of PPD reagents). However, abattoir prevalence carried out by WP1 in the same areas found similar figures, with an overall abattoir prevalence of 0.8% (unpublished data), thus providing an independent estimate to support our field testing on live animals. Furthermore, in order to minimize the above mentioned bias, the same person performed all CIDT each year for three years in the same villages. This was done during the same season, with PPD reagents coming from the same manufacturer and we found similar results each time.

Nonetheless we observed variation in prevalence in our various study sites as described in chapters 10 and 11.

What are the possible reasons for such differences in skin positivity between regions?

1) Breed and breed susceptibility: Although less than 1% of the total breeds are exotic and cross-breeds (CSA 2008), they are mostly concentrated in Central Ethiopia around large urban areas (e.g. Addis Abeba, Nazareth, Debre-Zeit, Debre Berhan). Ameni et al (2007) found in the WP3 study a statistical difference in BTB prevalence between exotic breeds, their cross-breeds and traditional zebus. In chapter 10, we also tried to assess the impact of breed on CIDT results. Statistical analysis was not robust enough to allow any definitive conclusion, since we tested only an average of 1.1% cross-breeds. However, results suggested that there might be some variation even within the zebu class when differentiating animals by proper breed. Genetic analysis on breed susceptibility carried out by WP4 (Trinity College, Dublin, Ireland) will hopefully also shed more light on this question. Are exotic breeds and their cross-breeds more at risk from BTB, and if so are they a risk factor for disease transmission to other cattle breeds? Is there also a variation in susceptibility amongst the different zebu types found in specific regions in Ethiopia, e.g. Boran breed in the South, the Fogera breed in Gondar area, the Afar/Danakil breed in the Nort-East, the Arsi breed in Central Ethiopia.

2) Farm management: intensive versus extensive farming

3) Involvement of other Mycobacteria spp. We saw in our study that *M. avium* reaction in cattle showed big variations between regions. How do environmental mycobacteria influence *M. bovis* infection? Presensitization with environmental Mycobacteria was shown to mask *M. bovis* infections and thus lead to false negative skin results (Doherty et al., 1996, Amadori et al., 2002, Walravens et al 2002, Alvarez et al., 2008). Paratuberculosis (Johne's disease), a chronic granulomatous enteritis disease of bovids caused by *M. avium subsp paratuberculosis* is very likely to be prevalent in Ethiopia, unfortunately, no published studies on paratuberculosis are available.

4) Presence of other concomitant diseases highly prevalent in some regions (e.g. trypanosomiasis, contagious bovine pleuropneumonia-CBPP). Diseases, especially the chronic type ones, would impair the animal immunity and affect the skin test reaction. National veterinary statistics on disease prevalence are very scarce especially from remote areas. We know for instance that trypanosomiasis is highly prevalence in some regions of Southern Ethiopia (Miruk et al., 2008), but no studies have been done further South, in South Omo for

instance. However, discussion with local veterinarians and personal observations highlighted the problems of these cattle diseases, which seemed to be highly prevalent in some of our study sites (chapter 13), particularly those areas where veterinary services are poor to non-existent (e.g Hamer or Bako Gazer region).

5) Parasitic load. If access to veterinary services was provided, regular cattle deworming was done by only 1/3 of the farmers (chapter 15). High parasitic load, which in Ethiopia is likely to be aggravated by the fact that livestock are overcrowded on communal pastures, as seen in chapter 15, can lead to severe loss in body weight and is likely to impair immunity in cattle as well. In chapter 11, we observed that cattle that were not regularly dewormed were nearly twice at risk for being a positive reactor, than cattle that were dewormed. Studies on the effect of endoparasites (e.g fasciolosis) on BTB prevalence are lacking. Monagham et al. (1994) stated that malnutrition was leading to false negative skin test results. In contrast, Doherty et al. (1996) showed that malnutrition and severe loss of body weight did not affect sensitivity of the skin test.

6) Different risk factors of disease transmission in different regions were related to culture/tradition, farm management, presence of other livestock than cattle, presence of wildlife, cattle density in the region, and environment (climate, soil type and vegetation).

In conclusion, this thesis suggested that the assessment of BTB prevalence has to be carried out with caution. Many factors are likely to influence CIDT results (e.g. testing methods, bias inherent to the PPD reagents, study regions, concomitant diseases, body condition of animals that can be influenced by seasonal droughts or parasitism, and cattle breeds present in the study areas).

Knowledge of these different regional prevalences is important for future control program implementation. Although final control programs can only be elaborated once the cost of BTB to the traditional livestock sector has been assessed, our study gave some important baseline information for such possible programs. With a cattle population of over 43 million in the Highland alone (CSA 2008), it is evident that no control programs can logistically and financially involve the entire cattle population, regardless of whether the strategy involves test and slaughter, or vaccination of animals. Test and slaughter is the common strategy in most

developed countries, whereas vaccination of cattle has been carried out in Malawi (Ellwood and Waddington 1972). Results gathered during this thesis suggest that two different strategies could be implemented in parallel:

1) Test and slaughter program in rural areas with very low prevalence (< 1%)
2) Vaccination of dairy cattle in urban and peri-urban settings with higher BTB prevalence

In addition, animal movement should be controlled and/or restricted. There are well known cattle routes from rural areas into peri-urban areas (ILRI 2007; Appendix 5). Care should be taken with movement of animals from a known positive region into a naïve or know negative region. Therefore it is also important to know the prevalence of disease in the different regions of Ethiopia.

18.1.3. The case of Boran cattle

The Boran is a zebu breed maintained by pastoralists in Southern Ethiopia and contiguous areas of Kenya and Somalia. They are known to be well adapted to harsh environments and can withstand water stress. In addition, they have good milk and meat productivity compared to other zebu breeds, which gives them a good balance between productivity and general resistance. During this PhD thesis, we noticed an increase in the number of Boran cattle throughout the country. For instance, farmers in Butajira area received Boran cattle from the Productive Safety Net Programme (PSNP), which was initiated by the government and donors (DFID, World Bank) in 2005 in order to secure the livelihood of rural households in the long term and to break the dependency on food aid (Devereux et al., 2006). To our knowledge no published data exist on the BTB status of Boran cattle. A study has been started in the Borana area as part of this project, and will hopefully give more information on BTB in this cattle breed. Are Boran cattle more or less at risk for BTB? Is there a risk of spreading the disease over the country with such programmes? Due to very small numbers of Boran cattle in our study sites (N=3), we could not assess in chapter 10 the effect of this breed on CIDT positivity.

18.1.4. The case of Holstein cattle

Dairy cattle are increasing in urban and peri-urban areas (CSA 2008) in order to supply the growing demand for milk. There is also an increasing flow of high productive Holstein cattle from urban centers into rural areas, as seen for instance already in Sellale and Meskan. This flow shows a radiating pattern from Addis Abeba over the years. It is possible that the prevalence increase we observed in Meskan is due to the increased number of Holstein and cross-breeds coming from Addis Abeba. WP3 showed a high prevalence of BTB in these exotic breeds, and a future challenge will be the control of the dissemination of the disease by such routes as selling them to rural farmers, or via artificial insemination.

18.1.5. Cut off used for skin test result evaluation

More and more debates about which cut-off should be used in the evaluation of skin test results have been raised these last couple of years. The official standard cut-off defined by the Office International des Epizooties (OIE 2004) is 4 mm, meaning that positive reactors are defined as an animal having a reaction at the bovine site of injection 4 mm bigger than the reaction at the avium site. Doubts in the scientific world rose as whether this cut-off was appropriate in the African context. Recent publications (Ameni et al., 2008, Müller et al. 2008) suggested that in Africa a cut-off of 2 mm would give better sensitivity without loss of specificity. Our study supported the idea that different cut-offs should be used depending on the prevalence prevailing in specific areas, and that flexibility should be allowed as to which cut-off should be used. However, this flexibility may also lead to difficulties in comparing BTB prevalence results between different studies and between different countries. More research is warranted in this field in order to find the best testing option for the African context, and to make the method officially recognized by the OIE.

18.1.6. Wildlife-livestock-human interface

Zoonotic diseases are commonly found at the human-livestock-wildlife interface and wildlife was shown to play an important role both as reservoirs and spreaders of many diseases (appendix 2). An important contribution of this PhD study was the assessment of BTB in wildlife, at the human-livestock-wildlife interface. No studies have been done on this topic in Ethiopia. Our preliminary results suggested that BTB is not prevalent in wildlife; this is based on results from culture, which is generally recognized as the gold standard method. However, so far we only have results from a very small population, and more animals have to be sampled.
More samples are also needed to try to validate the rapid test (RT) for serology. So far, the test has been validated for only a few wildlife species (e.g. red deer, wild boar, badger and possum) and none of them are from Africa (Lyashenko et al., 2008). It is intriguing that the serology prevalence in our study was high (23%), but none of the cultures yielded Mycobacteria from the MTC-complex. However, serology and culture has only been done in parallel in a few animals, and it is possible that some of the sero-positive animals in which no culture was performed, did indeed have BTB. So, on the one hand the statement "no BTB in wildlife" has to be confirmed, and on the other hand the rapid test has still to be validated for African wildlife species. From a conservation point of view, our results showed sero-positive wildlife belonging to rare endemic species such as the Mountain Nyala (*Tragelaphus buxtoni*) and the elephant (*Loxodonta africana*), as well as in the grass-rats which are the prey of the Ethiopian wolves (*Canis simensis*).

Such a quick test is valuable to field wildlife veterinarians and conservation authorities to rapidly, easily and cheaply screen animals without having to kill them for tissue culture. Screening of various mammal species may become important in the future when it comes to translocations of wild animals from one area to another, whether to facilitate the survival of endangered species, or whether to remove so called "problem animals" out of the "problem zone" (e.g large carnivores/human conflicts often caused by the encroaching of humans and their livestock into wildlife habitat). In such a scenario, knowledge of the prevailing TB status will be essential to prevent the introduction of the disease into previously-negative territories.

18.1.7. Zoonotic transmission

In the literature, BTB caused by *M. bovis* is described as being a potential zoonotic threat through consumption of raw animal products (Cosivi et al., 1998, Ayele et al., 2004). In the developed countries, human cases have drastically decreased with the implementation of national control programs which eradicated or controlled the disease through test and slaughter of cattle, and pasteurization of milk. However, human cases of BTB are more and more frequently reported in those countries in recent years. Apart from elderly people suffering from a reactivation of latent BTB acquired prior the above mentioned control strategies, the disease is also seen in immune compromised people (e.g. HIV/AIDS, diabetes mellitus), people consuming raw milk and milk products, such as cheese imported from countries where BTB is still prevalent and where there is no pasteurization (Bouvet et al., 1993, Dankner et al., 2000, Hancox 2002, Gibson et al., 2004, de la Rua et al., 2006, Hlavsa et al., 2008).

BTB is widely distributed in sub-Saharan Africa and control of the disease in cattle, as well as pasteurization schemes in rural areas, is poor to non-existent (Ayele et al. 2004). People live in close contact with their livestock and consume raw milk and milk products; it is therefore very likely that they might get infected with the pathogen.
One important aim of this study was to show the transmission of *M. bovis* between animals and humans in close collaboration with Work package 2 of the BTB project. This included the following studies: 1) culturing and typing fine needle aspirates (FNAs) from patients with lymphadenitis, 2) conducting farmer surveys, in which risk factors for disease transmission were assessed on a farm level. These studies were focused in areas where BTB positive cattle had been identified (chapter 11), specifically targeting villages where PPD positive cattle were identified during the thesis work, and taking FNAs from farmers with lymphadenitis directly on-site with a medical research team. The latter study has so far been conducted in Butajira and final results are pending. Out of 750 samples from lymphadenitis patients cultured in AHRI, none yielded *M. bovis* (unpublished data). Furthermore, no *M. bovis* was isolated by the culture of milk from BTB positive cattle from Holetta farm (unpublished data, AHRI). In our study none of the classical risk factors known from the literature for BTB in humans (consumption or raw

animal products, close contact with animals) were significantly associated with TB cases in households.

Are the risks to humans of BTB in Ethiopia really "negligible"? Or is there a problem related to the isolation of *M. bovis* in the laboratory? In Tanzania where similar environmental and farming practices prevail, and where BTB in cattle population is also endemic, *M. bovis* was repeatedly isolated from humans and raw milk, and thus shown as being a risk factor of disease transmission (Kazwala et al., 1998). On the other hand, studies carried out in Chad did not isolate any *M. bovis* from humans, although BTB prevalence in cattle was high and people were consuming raw animal products (Diguimbaye et al., 2004).

What are the possible explanations for failing to isolate *M. bovis* from human patients in Ethiopia?

1) Low BTB prevalence in cattle in rural areas, as shown in this thesis.
2) Sampling technique: is FNA (fine needle aspiration) a good enough method? Would excision biopsies of lymph nodes be a better method to collect more tissue material for culture? This was done in Uganda, for instance, by Oloya et al (2008).
3) Laboratory failure: technical flaws during the culture process, for instance over-decontamination that would kill *M. bovis*.
4) *M. bovis* strain virulence for humans
5) Genetic make up of some Ethiopian ethnic groups which could lead to decreased susceptibility to infection.
6) Other mycobacterium such as environmental Mycobacteria. Our studies carried out in cattle and wildlife suggested a high burden of non-tuberculous Mycobacteria. Not much is known about their composition in Ethiopian patients and how they relate to each other. Environmental Mycobacteria have been isolated from TB and/or adenitis patients (Mfinanga et al., 2004, Diguimbaye et al., 2006, Perez-Martinez et al. 2008) and little is known about their interaction with pathogenic Mycobacteria. Could they have a protective effect against *M. bovis*?

18.1.8. Impact of the disease on animal traction

Due to its chronic debilitating nature BTB causes a direct health impact on cattle, and affects their productivity potential, with reduced milk yield and meat production (Meisinger 1969), which can lead to major economic losses for a country.

Ethiopia has a cattle population of 43 million heads (CSA 08). High productivity exotic (Holstein Friesian) and their cross-breeds only account for 0.07% and 0.58% of the total cattle respectively. The rest (99.3%) is composed of traditional zebu breeds characterized by very low productivity. Dairy farms with exotic and cross-breeds are mainly found around the capital city and other major regional cities, in order to supply the increasing demand for milk in the urban populations. National statistics show that there are around 6 million dairy cows in the country and the milk production for 2007 was 2.63 billion liters. In intensive dairy farms in peri-urban areas, BTB might well have an impact on milk production. This project is planning to investigate the amplitude of this impact in dairy farms around Addis Abeba, and results will be available at the end of 2009.

However, in rural areas, the situation might well be different and the impact of BTB might take another dimension. A peculiarity to this country is its cattle herd structure. The proportion of males in the overall herd structure is very high. Official statistical figures state 44.6% of the total population is made out of males (CSA 08); in our study (chapter 15) we found even a higher percentage, with males accounting for 52% of the total herd. One quarter of the males were oxen (22%). In rural areas of the Highlands, females are therefore kept in order to produce the next male generation rather than for milk production. Males are used for reproduction, and potential oxen.

The reason for keeping so many males is, as explained in chapter 15, intricately linked with the agriculture system in place in the Highlands. All ploughing, harvesting and threshing is done using animal traction. The more animal traction a farmer possesses, the more land he can plough and the more he can harvest. Poverty is therefore also linked with the number of oxen that a farmer has. Over 80% of the land is cropping land (CSA 08) and oxen are mainly used to do all field work and cereal production.

A disease affecting draft animals will therefore have a major impact not only on the animal itself, but on the agriculture sector as a whole and lead to poverty and famine. This has already been illustrated by the Rinder Pest outbreak in the 19th century, which led to severe famines on the continent because fields could not be ploughed or harvested anymore (Schwabe 1984). The question arises as to what extent oxen are affected by BTB infection, and how the disease affects their daily work. Farmers, who were questioned about the working capacity of their oxen and about any changes noticed over the last year, did reply in terms of animal weakness and fatigue. They commented that they had to exchange the animal more frequently with another ox to pull the plough, and that the animal needed to be pushed more since it was reluctant to do the work after a couple of hours in the field (chapter 10). These are all subjective answers and the true daily "loss of traction" is difficult to measure unless these animals are followed over time as they work in the field, which is logistically impossible. Nonetheless, farmers' answers are a good hint that traction animals are very likely to be affected, if infected with BTB, thus having major consequences on the work they do in the crop fields and finally affecting the crop yields as well. Our results of chapter 10 also showed that oxen are twice at risk for being positive reactors; this result was highly statistically significant.

18.1.9. Increasing awareness of the disease

One achievement of this PhD study was also to promote increased public awareness of BTB on-site at farm and village level, as well as in National Parks. The PPD cattle study included over 2000 farmers in a total of 80 villages in 5 Woredas. The results from Bale and Hamer (South Omo) were not included in chapter 10. The Bale results are shown in chapter 11 and those from Hamer in chapter 13. Interviews, focus group discussions and cattle testing were conducted in the first visit. During the second visit, 72 hrs later, cattle skin reactions were assessed, and deworming tablets (Albendazole) in the Highlands or Veridium ^{TM} (Isometamidium chloride hydrochloride) in South Omo, which is used against trypanosiomiasis, distributed to farmers as compensation/incentive (appendix 6). A final discussion took then place with the farmers in each village where, with the help of local translators, we discussed about the disease, the agent, the symptoms of the disease, and the transmission between animals and humans. Each farmer with positive animals was told so and available options discussed (boiling of the milk, animal separation, slaughtering). We also answered questions from farmers regarding BTB, and advised

on seeking medical treatment when farmers showed signs of TB (pulmonary symptomatic or adenitis). Each year, three times in a row for certain villages, we repeated the same discussion. Changes in awareness were observed in certain villages after each yearly visit, which was especially striking at the third visit. A farmer from Butajira told us proudly that now his family and his neighbors were always boiling the milk before consumption. This experience showed us that awareness can be increased on a house-hold level through repeated discussions

We also trained 50 scouts in three National Parks or sanctuaries (Awash, Senkele and Yagundi Rassa) to recognize BTB lesions in the course of a post-mortem. Power-Point lectures on BTB were given on-site (appendix 1).

However, one drawback of increased awareness of the disease, as personally seen in the field, could well be that farmers who know the positive status of their animals would get rid of them as soon as possible, not by slaughtering but by selling them, which could therefore contribute to the spread of the disease.

18.2. Economical impact of BTB

We want to keep this paragraph brief since only the approach of the economical analysis has been described in this thesis (chapter 16, 17). The studies and final analysis will go beyond this PhD thesis

1) Economical impact of BTB on the public health sector

So far the question of the burden of BTB for the public health sector could not be answered, since *M. bovis* was not isolated from human patients. However, studies will continue for another year.

2) Economical impact of BTB on the livestock sector

Knowledge of prevalences of BTB in cattle is essential to help assessing the economical impact of the disease in the livestock sector. In addition, productivity studies started during this thesis work, and carried out in Sellale and Butajira, will give us baseline productivity parameters for

cattle kept under traditional management. In addition the burden of BTB in abattoirs is being assessed in collaboration with WP1. Pooling of these data will help in assessing the impact of BTB on parameters like milk productivity, and weight loss in cattle. Abattoir studies will also help assess the financial losses due to carcass confiscation and decreased exports of meat. The Horn of Africa has a long history of exporting live animals to the Middle East. However, exports dropped abruptly following a ban imposed by Saudi Arabia and other Middle Eastern countries in 1998 after outbreaks of Rift Valley Fever in The Horn of Africa. The loss caused by the ban was estimated to be as high as US$ 100 million (www.regoverningmarkets.org). The ban was lifted in 2003 and an FAO project assisted in establishing an Export and Certification of Livestock for Export (EXCELEX) in Ethiopia and Somalia (FAO 2005). Live animals, especially small ruminants, are exported via Djibouti for the Gulf market. However, in the meantime the country lost some export markets in the Middle East due to the ban, and had to shift to new markets for meat export such as Egypt and Congo Brazzaville (personal communication from export abattoirs). The animals are slaughtered and packed in export abattoirs based in Nazareth, Debre-Zeit and Modjo.
A disease such as BTB can therefore have a severe effect on exports due to 1) reduced meat production (loss of weight gain in live animals; carcass condemnation due to BTB lesions) and to 2) export bans or restrictions.

In addition, in case of a ban due to disease, the large surplus of animals will lead to overstocking, overgrazing of already scarce grazing land, and further land degradation, which in turn will affect livestock productivity. As seen in chapter 15 of this thesis, Ethiopia is faced with overpopulation of livestock and unsustainable grazing. As described in the chapter, few solutions exist to solve the problem, but increasing export rates of livestock that are not directly involved with cropping and milk production, may help in reducing the pressure of animals on natural resources.

18.3. National intervention strategies to control BTB

Data collected during this PhD thesis will ultimately be fed into the compartments of an animal-human SIR (Susceptible-Infected-Recovered) transmission model. Additional data will flow in from WP2 (data on human BTB) and WP1 (data on abattoir prevalence) to complete this model. Such a model is a basis for assessing different intervention strategies that could apply to Ethiopia. Information gathered from the pending economical analysis, together with the transmission model, should help in assessing the most profitable intervention for the country. Conclusions drawn from this PhD thesis suggest that we will probably have to run two different models, one for the rural setting with low animal prevalence and low risk factors of disease transmission, and one model for the urban setting characterized by high prevalence and probably high economical impact of the disease in the intensive livestock sector.
Such mathematical models have already been used, for instance in the UK, to investigate the effect on cattle BTB by culling badgers as intervention strategy (Cox et al., 2005).

18.4. Messages and recommendations of this thesis

1) This thesis provided baseline epidemiological and economical information to elaborate ultimately an animal-human transmission model and thus to assess most profitable intervention strategies for the control of BTB in Ethiopia.

2) We showed that BTB has a low endemicity in zebu cattle (<1%) in rural areas but with regional variation of prevalences. In addition, prevalence in rural areas was shown to be much lower than in peri-urban and urban areas where exotic breeds had higher BTB prevalence (WP3). Movements of high productive exotic cattle from urban areas into rural areas might therefore be a risk of spreading the disease throughout the country.

3) True prevalence of BTB was in some regions impossible to assess since the apparent prevalence was very low. In addition sensitivity and specificity of the skin test has only

4) been evaluated for Central Ethiopia characterized by high numbers of Holstein and may not be applicable for zebus in rural areas.

5) Results from the comparative skin testing in cattle have to be interpreted with caution since they can be influenced by technical bias linked with the testing method itself, by the quality of the PPD reagents and by factors influencing the immunity of the animals (e.g. parasitism, malnutrition, stress, infectious diseases such as trypanosiomiasis or CBPP) or by Mycobacteria other than from the MTC (e.g. paratuberculosis) that can impair the test results for *M. bovis*. Our study highlighted that animals in good body condition were more at risk for being positive reactors because probably their immune system is not impaired, whereas emaciated animals are more likely to be in an anergy state.

6) *M. avium* reaction was described in all Woredas but with clear regional variation. Woldia had the lowest prevalence whereas Meskan had the highest prevalence.

7) *M. tuberculosis* has been isolated in cattle (WP1). It is therefore possible that in our study also cattle infected with *M. tuberculosis* could have reacted on the skin test.

8) Purchasing cattle, presence of livestock other than cattle and older cattle were a risk factor for having BTB positive cattle in a herd. This suggests that avoiding keeping or buying old animals could help controlling the disease. On the other hand, oxen that are kept for cereal cropping are in general older than 10 years. Males are usually castrated when they are between 5-7 years old.

9) None of the “classical” risk factors were a significant explanatory variable for BTB positivity in cattle (e.g. herd size, keeping cattle inside housings, communal grazing)

10) None of the risk factors associated with possible transmission of disease between BTB positive cattle and humans (e.g. raw milk consumption, close contact with cattle) were a significant explanatory variable for TB occurrence in humans

11) This thesis did the first wildlife survey of BTB in Ethiopian wildlife. *M. bovis* was so far not isolated in any wildlife samples, although the cattle-wildlife interface is intensive. However, serology was as high as 23% and could suggest that BTB exists in some wildlife species. Flagship endangered species (e.g. Mountain Nyalas, and Ethiopian wolves) might be at risk for BTB.

12) A high proportion of non-tuberculous Mycobacteria were isolated from wildlife. The isolation of some of these agents (e.g. *M. terrae*) also from the mediastinal lymph nodes suggests another route of transmission than by environmental contamination (e.g. fodder, water) and suggests a possible emergence of Mycobacteriosis with agents other than *M. bovis* or *M. tuberculosis*.

13) BTB may affect primarily animal traction rather than milking cows in rural areas and therefore have an impact on cropping and cereal yields, which in turn might influence the severity of future famines in the country. Oxen were shown to be twice at risk for being reactors than females.

14) Grazing land was shown to be insufficient to support the high livestock population. This will lead to increased conflicts, and encroaching on wildlife habitat, thus intensifying further the human-livestock-wildlife interface

15) Herd structure analysis highlighted that half of the Ethiopian herd is composed of males (bull and oxen). Oxen account for 22% of the herd. Farmers need to maintain a minimum herd size in order to secure permanently draft animals to work in the fields. Females are therefore mainly kept to produce oxen and breeding program aiming at increasing animal productivity, thus giving the possibility to reduce the number of animal is therefore not an option. Surplus of animals (e.g. males that are not used in the field) can only be eliminated by increased slaughtering/export. Export bans due to diseases will therefore have a long term ecological impact as well due to overstocking, increased land degradation and loss of biodiversity.

The results of this thesis led to new research questions, among them:

- What are other potential risk factors of disease transmission between animals? The role of the environment has to be assessed more in details as well as the role of Holsteins as risk factors for spreading the disease.
- Is there different cattle breed susceptibility amongst the various traditional zebu cattle (e.g. Boran, Fogera, Kola, Arsi)?
- Why do we observe a high inter-regional variation in BTB prevalence in cattle?
- Does altitude not affect the prevalence of BTB, although this is well described for human TB? We have found no differences in a country which large altitude differences between regions (from sea level to over 4000m),
- What are the risk factors of disease transmission to humans? Is BTB a "negligible" zoonosis in Ethiopia?
- What is the role of environmental Mycobacteria? How do they interact with tuberculous Mycobacteria?
- What is the prevalence of Paratuberculosis in the country? How does the disease interact with the skin test?
- What is the role of small ruminants that are herded with cattle and are also kept inside the owner's house at night?
- Is the high human burden of *M. tuberculosis* a risk for their cattle. How does this human-animal transmission occur? How do *M. tuberculosis* infected cattle react to the skin test?

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19. Appendix 1: Photos illustrating the different ecological zones of the study areas and field work performed during this PhD

(Photo credit: R. Tschopp)

Part 1: Study sites

- Bale Mountains (Web Valley)
- Afar
- Meskan
- Lowlands of Woldia
- Highlands of Woldia (Baba Seat)
- Male (Bako-Gazer)

Part 2: Field work

- Skin testing of cattle
- Training of scouts in Awash National Park and Senkele
- Various work with farmers, DVM students and wildlife

Part 1: Study sites

Bale Mountains

Afar

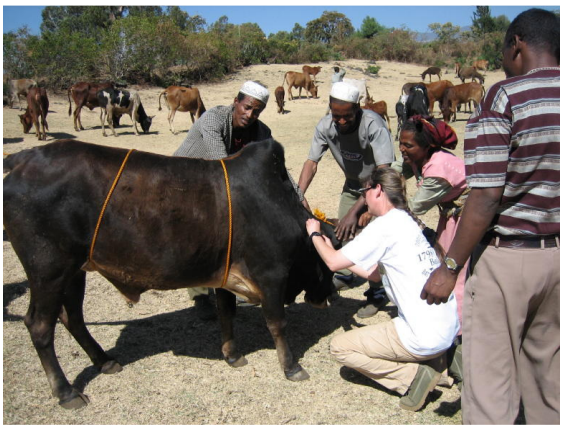
Meskan

Woldia lowlands

Bako Gazer (Male)

Part 2: field work

Skin testing of cattle



Training

Awash National Park

Senkele

Work with farmers, students and wildlife

Wollo

Appendix 2: Photos

Calving Butajira

Babille

Hamer (South Omo)

Dolo Odo

Bale Mountains (field lab work)

20. Appendix 2: Environmental change and the impact of wildlife on diseases

Part of this chapter was a contribution to:

Bonfoh B., Schwabenbauer K., Wallinga D., Hartung J., Schelling E., Zinsstag J., Meslin F-X., **Tschopp R**., Akakpo J.A., and M. Tanner. 2010. *Human health hazards associated with livestock production*. In: Livestock in a changing landscape: drivers, consequences, and responses (Vol I). Eds: Steinfeld H., Mooney H.A., Schneider F., and Neville L.E. Island Press. Pp 197-221.

20.1. Introduction

In the last decades, the importance of the livestock-wildlife-human interface has increasingly been recognized worldwide and its issues tried to be addressed. Wildlife, people and their livestock interact, share the same land and compete for the same natural resources in increasingly intensified interfaces [22]. There are over 77 million cattle in Africa [18] and around 80% of the sub-Saharan population is rural, co-existing with its animals and depending directly on livestock for their livelihood. The steady population and livestock growth puts an ever increasing pressure on natural resources and ecosystems.

Sixty-two percent of human pathogens are known to be of zoonotic origin (i.e. diseases transmitted from animals to humans), including the so-called emerging diseases in humans [20]. Evidence suggests that wildlife is involved in most of these diseases, thus playing a key role at the interface.

Diseases at the interface involving wildlife hosts or reservoirs can have multiple impacts: they present a major health threat for human populations and/or their livestock; they have an important economical impact at household and national level thus exacerbating rural poverty and hampering national economies, but also threatening global economies [14]. From a conservation point of view, these diseases have the potential to increase the risk of extinction of valuable endangered wildlife species and lead to major losses in biodiversity (e.g rabies outbreak in the Ethiopian wolf population) [31].

The following chapter aims at describing the interface concept, the diseases found at the human-livestock-wildlife interface and the role of wildlife as reservoirs for diseases.

20.2. The wildlife-livestock-human interface

20.2.1. Definitions

Wildlife-livestock-human interfaces are not fixed or defined in a strict sense. They can move temporally and spatially depending on various factors and new interfaces are constantly created [18].

The following factors are physically influencing the creation of an interface:

- Livestock production system prevailing in the region or in the country (e.g. extensive ranching system, pastoralism)
- Social factors, such as steady population growth, population movements, population behaviour, conflicts and political instability.
- Environmental factors, such as land degradation, habitat destruction, de-or reforestation, decrease of natural resources and climatic factors (e.g. drought, rainy season)
- Biological factors such as endemic wildlife species, seasonal migration, breeding and formation of bachelor groups

20.2.2. Implications and consequences of an interface

An increase of disease incidence at the wildlife/livestock interface has been reported over the last decades (e.g. bovine tuberculosis, foot and mouth disease, anthrax, Rinder Pest) [19]. This is most probably related to increased human and livestock population encroaching into wildlife ranges. These diseases are of serious concern for both the agriculture sector and wildlife conservation sector. They affect directly the health status of livestock through mortality (e.g. anthrax) or morbidity (e.g. trypanosiomiasis). More subtle, disease will have a medium and long term impact on livestock immunity (e.g. increasing susceptibility to other diseases) and productivity (e.g. decreased milk and/or meat production, reduced fertility), thus exacerbating rural poverty, which is an important constraint to development and environmental conservation in Africa [18].

The interaction between wildlife and livestock is a key issue in livestock economies. The current disease status found at the interface is a major constraint on livestock exports (“Sanitary and Phytosanitary Measures” of the World Trade Organisation, WTO) and thus hampers national and

global economies [19]. FAO declared in 2003, one third of the global meat trade to be embargoed due to livestock disease epidemics, such as avian influenza, foot and mouth disease, bovine spongiform encephalopathy [14]. Forty-four million birds were culled in Vietnam at an estimated cost over US$ 120 million to stop the spread of avian flu [34]. Following an outbreak of Rift Valley fever in East Africa, a ban imposed by Saudi Arabia in 1998, resulted in the loss of exports of live animals worth an estimated US$ 100 million [10]. Later, in 2000, Saudi Arabia embargoed Ethiopian livestock exports due to the outbreak of sleeping sickness in East Africa [30]. Since the 1990s, emerging livestock diseases have cost the world's economy US$ 80 billion [14].

Wildlife plays a key role not only in disease dynamics involving livestock at the interface, but also in the epidemiology of most traditional zoonosis and emerging human diseases as infection source or amplifier (viral diseases: West Nile Virus, SARS: Severe Acute Respiratory Syndrome, Avian Influenza, Hendra/Nipah/Hanta virus, Monkeypox; bacterial diseases, such as Lyme disease (*Borrelia burgdorferi*) and protozoal disease (e.g.Trypanosoma spp) [6, 20].

Interfaces are two-way streets. New emerging diseases have also been described in African wildlife, such as canine distemper in free-ranging lions [28], bovine tuberculosis in free-ranging lions [17], and parafilariosis in South African buffaloes [16].
The introduction of novel diseases at the interface can be a serious threat to free-ranging wild animals and a risk of extinction of endangered rare species.

20.3. Diseases at the interface

20.3.1. Disease transmission

Disease transmission occurs either through direct contact between individuals (e.g rabies), through consumption of contaminated food or water (e.g. bovine tuberculosis), through aerosols (e.g. avian influenza) or through vectors (e.g trypanosiomasis). Therefore, a large spectrum of

transmission modes of diseases are seen at the interface, considering factors influencing direct contact and/or access to contaminated food/water and those influencing vectors (e.g. number, distribution):

Direct contact between wildlife- livestock and humans

Wildlife usually tends to avoid any contact with cattle and humans whenever possible. For example they tend to use water points at different time as cattle do [19]. Therefore, if the ecosystem and ecology allows it, direct contact between wildlife and livestock (and humans) will be superfluous and indirect disease transmission pattern will prevail, such as through contaminated pasture/water/soil and via vectors/intermediate hosts [9].

However, nowadays this situation is modified due to rapid environmental changes. These changes have natural as well as anthropogenic origins: habitat change (deforestation, land erosion, land over-use), demographic factors (population growth, population movements or sedentarisation of nomadic people), climatic changes (recurrent drought) [8]. For instance, major wildlife/livestock disease epidemics have been observed during drought periods [19]. A possible explanation being that wildlife is forced to share scarce water points and pastures with livestock and thus the rate of direct contact between animals is increased.

The consequences of theses changes are an ever increasing encroachment of human population and their livestock into wildlife ranges, thus intensifying interfaces and making direct contact between free ranging wild animals, livestock and humans much easier and sometimes unavoidable. The yellow fever outbreak in Ethiopia between 1960 and 1962 illustrates this concept: Yellow fever, an arthropod borne virus (flavivirus) is transmitted by mosquitoes of the genus Aedes (*A. aegypti*) and is characterized by a mosquito-monkey-mosquito sylvatic cycle. The outbreak in Ethiopia caused 300 000 human cases and was the largest outbreak ever reported in Sub-Saharan Africa. It was the result of humans entering the sylvatic setting and becoming part of the yellow fever cycle [1]

Indirect transmission: vectors and access to contaminated food/water

In addition, the above mentioned climatic and ecological factors influence the host/vector and pathogen dynamics in terms of distribution and number [7]. For example, Rift Valley Fever outbreaks are seen after heavy rainfall, which promotes the multiplication of mosquitoes [5].

Reforestation favours transmission of tick-borne diseases (e.g. Lyme disease in humans) through increased tick population and wildlife hosts on whom ticks can feed [7].

Acquiring a disease through contaminated food is best illustrated by bovine Tuberculosis (BTB). BTB caused by *M. bovis* is transmitted either by aerosols/contaminated droplets from the respiratory tract or by ingestion of contaminated animal products (BTB infected preys are a source of infection for wild carnivores; consumption of raw milk or raw meat from infected cattle can lead to the development of extra pulmonary TB lesions in humans (e.g. lymphadenitis); herbivores acquire the disease while grazing on contaminated pastures) [23].

Population immunity

Population immunity at the interface is an important aspect in disease epidemiology and dynamics. Usually, local livestock breeds and endemic wildlife develop a certain degree of immunity against endemic pathogens circulating between wildlife and livestock in a specific region [31]. There is a co-evolution between host and parasites, which will remain in balance in a stable bio-ecological system [19]. Disruption of this stable system such as the introduction of new immunological naive hosts (e.g. import of domestic cattle from Europe and Asia into Sub-Saharan Africa) or of alien pathogens can trigger the onset of an epidemic (e.g. Rinder Pest pandemic on the African continent in the last century) [34].

It is also important to keep in mind that pathogens (especially virus and bacteria) are in constant transformation (evolution, changes, and adaptations), for example through mutations, genetic variation such as shift, genetic drift, and recombination [20]. These transformations impose constant new challenges in disease dynamics.

20.3.2. Wildlife and livestock diseases

Three types of diseases are found at the interface: indigenous, exotic/alien and emerging/re-emerging or truly novel diseases [3]. They have been described and reviewed in detail by many authors [3, 4, 19, 31]. The most important diseases found at the interface are classified under List A by the Office International des Epizooties (OIE); List A groups diseases with potential rapid spread within countries and across borders, with serious socio-economic and/or public health

impact and with major constrains to international trade [24]. In Africa these diseases are: Rinder Pest and Peste des Petits Ruminants (morbilivirus), Rift Valley fever (phlebovirus), foot and mouth disease (aphtovirus), African swine fever (ASV virus), and contagious bovine pleuropneumonia CBPP (*Mycoplasma mycoides mycoides S.c*). Especially highly contagious viral diseases with epizootic potential have devastating impacts on livestock and wildlife economy. Other diseases (trypanosiomias, cowdriosis, theileiriosis.) commonly have an more pronounced impact at household and community levels, thus exacerbating rural poverty through reduced livestock production.

Ungulates and especially the Bovidae family are often involved in outbreaks at wildlife/livestock interfaces. In Africa, wildebeest (*Connochaetes taurinus*) are carrier of malignant catarrhal fever virus, which can be lethal for cattle [13].

The buffalo population (*Syncerus caffer*) is proved to act as reservoir for BTB and as source of infection for other species (domestic cattle and wildlife) [15]. Buffaloes are also involved in the transmission of the virulent form of theileriosis (corridor disease) and are carrier of the SAT (South African Territories) Foot and Mouth Disease virus [31]. However, despite a few key wildlife species involved in major disease outbreaks at the interface, and the fact that free-ranging wild animals play a key role in the epidemiology of most diseases (multi-species and exotic diseases) at the interface, wildlife is usually a poor maintenance host for pathogens in a given endemic area.

Table 1 shows some of the more important wildlife-maintained diseases that are commonly transmitted to domestic livestock in Africa

Disease	**Causative pathogen**	**Transmission pattern**	**Maintenance host**	**Affected domestic animal**
Foot and Mouth Disease	Aphtovirus	Direct	African buffalo, cattle	Cattle, pigs, sheep and goats
African swine fever	Asfarvirus	Direct *Ornithodorus spp*	Argasid ticks, warthogs	Domestic pigs
African horse sickness	Orbivirus	Culicoides midges	zebra	Horses and donkeys
Rift valley fever	Phlebovirus	Aedes and culex spp.	Aedes mosquitoes	Sheep, goats, cattle, wild bovidae
Bluetongue	Orbivirus	Culicoides midges	Artiodactyls (uncertain)	Sheep and cattle
Malignant catarrhal fever	Alcelaphine herpesvirus-1	uncertain	Blue and black wildebeest	cattle
Newcastle disease	Paramyxovirus	direct	Wild and exotic pet birds	poultry

20.3.3. Wildlife and classical and emerging zoonoses

Wildlife is involved in the epidemiology of most zoonoses and thus constitutes a major threat for public health throughout the world [20] (table 2).

Table 2: Involvement of wildlife in some zoonoses

Disease	**Causative pathogen**	**Transmission**	**Main Wildlife reservoir**
Lyme borreliosis	*Borrelia burgdorferi*	Ixodes spp	White tailed deer, wild rodents
BTB	*Mycobacteria bovis*	aerosols, ingestion	Wild and domestic herbivores
Echinococcosis	*Echinococcus multiloculares*	ingestion	Canidae (e.g. foxes)
Yellow Fever	Flavivirus	Aedes spp	Monkeys
Hanta	Hantavirus	aerosols	Wild rodents
Rabies	Lyssa virus	bites	Wild and domestic canidae
monkeypox	Pox virus	Direct contact	Monkeys, rodents
SARS	coronavirus	aerosol	Wildlife, unknown
Ebola	Filovirus	Direct contact	Unknown (wildlife probable)
Hendra	Hendra virus	Direct contact	Fruit bats
Menangle	Menangle virus	Direct contact	Fruit bats
Nipah	Nipah virus	Direct contact	Fruit bats
Influenza A	Influenza A virus	Aerosols	Wild waterfowl

Thirty-five new infectious diseases have emerged in humans in the last 25 years [14], most of them having a wildlife component.

As for the diseases at the wildlife/livestock interface, zoonoses with wildlife reservoir show a large spectrum of transmission patterns, including direct contact (e.g. tularemia caused by *Francisella tularensis*) [25], consumption of contaminated animal products (e.g. bovine tuberculosis caused by *M. bovis* acquired through consumption of raw milk/meat [33] and insect vectors (e.g. West Nile Virus, Rift valley fever, yellow fever) [32].

20.4. Wildlife reservoir and control strategies

Seventy-seven percent of pathogens found in livestock are shared by other species [14]. This explains why wildlife is often considered by livestock producers as a danger (e.g. competition for grazing land, transmission of diseases), thus fuelling conflicts between communities and wildlife. Considering the existence and role played by wildlife reservoirs, control strategy and disease management at the interface is crucial (from a health as well as conservation point of view) however, it remains technically, financially and ethically challenging. Vaccination, quarantine, stamping-out, test-and-slaughter strategies, abattoir surveys, and vector control can be achieved to a certain extent in livestock populations, but these approaches are impossible in free ranging wildlife. Oral vaccination of wildlife has been a success in some countries, for example in Switzerland, where rabies has been eradicated since 2000 thanks to oral vaccination of the fox population. However, this approach is not feasible, especially in African countries due to the abundance of different wildlife species, size of the territory and cost-effectiveness of the procedure.

Culling of wildlife is sometimes promoted to stop the spread of diseases (e.g. culling of badgers in the UK and possums in New Zealand to control BTB [7, 12]. However, this method remains controversial and is a source of discussions concerning its successes and ethics. Containment, consisting in physical separation of livestock from wildlife (via fences, cordons, and animal movement control) and zoning seems to be a more appropriate approach [3]. However, these methods will not prevent vector borne diseases and winged hosts such as migratory birds [3]. Some authors state that livestock may better cope with pathogens in the presence of wildlife [2] and the question arises whether actually more harm is done in trying to completely remove a wildlife host from the bio-ecological system.

Although diseases at the interface are known and described, detailed and thorough epidemiological studies are still lacking for most of them, thus weakening control and prevention strategies. Furthermore, the exact role played by wildlife and the species involved in the maintenance and transmission dynamics of diseases are largely unknown especially in new emerging diseases, due to lack of data and wildlife studies. With exceptions, disease surveillance and monitoring is usually poor or non-existent in wildlife populations [7]. There is a crucial lack of knowledge as to which wildlife species act as reservoir hosts. As discussed above, the epidemiology at the wildlife-livestock-human interface is extremely complex and multisectoral (demographic, ecological and anthropogenic factors, factors taking into account wildlife species and their behaviour and finally the pathogen itself and all factors influencing the virulence and distribution of pathogen and /or vectors).

Due to this close interrelation, a holistic approach between human health, animal health and ecosystem health (the latter including all species living within these systems) is essential to address issues at the interface [20]. Efforts are often put into monitoring diseases in the public health and livestock sectors (e.g. post mortem surveys in abattoirs, blood sampling, record of mortalities and morbidities, education etc…) but the other sectors (e.g. ecosystems, wildlife) are only insufficiently considered in control programs. Ideally they should involve experts from public health sectors, veterinarians, ecologists, biologists, and wildlife professionals and wildlife authorities.

Wildlife can act as disease sentinel, i.e. as early warning system for a coming disease outbreak in livestock and/or humans. In Gabon and Republic of Congo, outbreaks of Ebola in wildlife preceded each of the five human Ebola outbreaks between 2001 and 2003 and handling of infected wildlife carcasses was the sources of all human outbreaks [29]. Wildlife Conservation Society (WCS), Centre for Disease Control (CDC) and the local authorities in Gabon and Republic of Congo created an Animal Mortality Monitoring Network for the surveillance of outbreaks in wildlife [29]. Using wildlife (especially key wildlife species) as sentinel is also a tactical approach in the surveillance of Rinder Pest outbreaks in East Africa or as early warning for West Nile Fever outbreaks in the United States for example [11, 6].

20.5. Conclusion

In a world characterized by rapid change, wildlife-livestock-human interfaces are increasingly intensified. Wildlife plays a central role in the epidemiology and dynamic of most diseases at the interface since the sharing of land and other natural resources becomes inevitable. It is essential to understand the role and dynamics of free-ranging wildlife within the ecosystem in order to set efficient disease control programs at the interface. Diseases at the interface including wildlife hosts will have an impact on public and animal health (livestock and wildlife) but also an economic impact, which goes far beyond the loss of productivity and trade constraints. Finally, it is worth highlighting the socio-economic benefits from wildlife: in Africa the wildlife sector is worth US$ 7 billion, with an annual growth rate of 5% [31], thus playing a crucial role in national economies. Increasing livestock production is one of the key strategies to alleviate poverty in Africa and target the UN Millennium Development Goals [35]. However, as stated by Kock (2003), if wildlife, natural resources and land use options are not taken into account in the equation, in the long-term “one form of poverty will just be replaced by another”.

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21. Appendix 3: Ethiopian wildlife species listed in the IUCN-Red List of Threatened species.

Appendix 3: Table showing some of the wildlife species in Ethiopia listed in the IUCN-Red List of threatened species in 2008 (Source: www.iucnredlist.org)

Species	Scientific name	Category	
Walia Ibex	*Capra walie*	CR C2b	Critically endangered
African wild ass	*Equus africanus*	CR A1b	Critically endangered
Mountain nyala	*Tragelaphus buxtoni*	EN C1	Endangered
Ethiopian wolf	*Canis simensis*	EN C2a(i);D	Endangered
Africa wild dog	*Lycaon pictus*	EN C2a(i)	Endangered
Swayne's hartebeest	*Alcelaphus buselaphus swayeni*	ENA1a, C1	Endangered
Gelada baboon	*Theropithecus gelada*	LR/nt	Low risk, nearly threatened
Bohor Reedbuck	*Redunca redunca*	LR/cd	Low risk, conservation dependent
Tiang/Tsessebe	*Damaliscus lunatus*	LR/cd	Low risk, conservation dependent
Menelik Bushbuck	*Tragelaphus scriptus menelikii*	LR/lc	Low risk, least concern

22. Appendix 4: Worldwide *M. bovis* isolation in free-ranging wildlife

Table 1: Table illustrating the free-ranging wildlife species and location in which *M. bovis* was isolated, worldwide (incomplete list)

Continent	Species	Country	References
Europe	Lynx (*Lynx pardina*)	Spain	Briones et al.2000
	Red deer (*Cervus elaphus*)	Spain	Aranaz et al., 2004, Gortazar et al., 2005, Vicente et al, 2006
		Hungary	Pavlik 2006
		France	Zanella et al., 2008
		Ireland	Dodd 1984
		UK	Delahay et al., 2002, Delahay et al., 2006
	Hare (*Lepus spp*)	Spain	Aranaz et al., 2004
	Fallow deer (*Dama dama*)	Spain	Aranaz et al., 2004
		UK	Delahay et al., 2002, Delahay et al., 2006
	Wild boar (*Sus scrofus*)	Spain	Parra et al., 2003, Aranaz et al., 2004, Gortazar et al., 2005, Vicente et al., 2006
		Hungary	Pavlik 2006
		Italy	Serraino et al., 1999
		France	Zanella et al., 2008
	European bison (*Bison bonasus*)	Poland	Pavlik 2006
	Badger (*Meles meles*)	Ireland; UK	Delahay et al., 2002, O'Boyle et al., 2003
	Roe deer (*Capreolus capreolus*)	UK	Delahay et al., 2002, Delahay et al., 2006
	Sika deer (*Cervus nippon*)	UK	Delahay et al., 2002
	Muntjac (*Muntjacus spp*)	UK	delahay et al., 2007
	Red fox (*Vulpes vulpes*)	UK	Delahay et al., 2002, Delahay et al., 2006
	Mink (*Mustela vison*)	UK	Delahay et al., 2002
	Mole (*Talpa europaea*)	UK	Delahay et al., 2002
	Brown rat (*Rattus norvegicus*)	UK	Delahay et al., 2002
	Ferret (*Mustela furo*)	UK	Delahay et al., 2002
	Stoat (*Mustela erminea*)	UK	Delahay et al., 2007
	Polecat (*Mustela putorius*)	UK	Delahay et al., 2007
	Common shrew (*Sorex araneus*)	UK	Delahay et al., 2007

	Yellow-necked mouse (*Apodemus flavicollis*)	UK	Delahay et al., 2007
	Wood mouse (*Apodemus sylvaticus*)	UK	Delahay et al., 2007
	Field vole (*Microtus agrestis*)	UK	Delahay et al., 2007
	Grey squirrel (*Sciurus carolinensis*)	UK	Delahay et al., 2007
	Bank vole (*Clethriones glaredus*)	UK	Mathews et al., 2006
America	Coyote (*Canis latrans*)	United-States	Bruning-Fann et al. 2001, Payeur et al., 2002
	Racoon (*Procyon lotor*)	United-States	Bruning-Fann et al. 2001, Payeur et al., 2002
	Red fox (*Vulpes vulpes*)	United-States	Bruning-Fann et al. 2001, Payeur et al., 2002
	Black bear (*Ursus americanus*)	United-States	Bruning-Fann et al. 2001, Payeur et al., 2002
	Bobcat (*Lynx rufus*)	United-States	Payeur et al., 2002
	White-tailed deer (*Odocoileus virginianus*)	United-States	Payeur et al., 2002, O'Brien et al., 2002, Jacques et al., 2003
	Elk (*Cervus canadensis*)	United-States	Payeur et al., 2002, Jacques et al., 2003
	Wapiti (*Cervus elaphus manitobensis*)	Canada	Nishi et al., 2006
	Wood bison (*Bison bison athabascae*)	Canada	Nishi et al., 2006
Australasia	Stoats (*Mustela erminaea*)	New-Zealand	DeLisle et al., 2008
	Ferret (*Mustela furo*)	New-Zealand	DeLisle et al., 2008, Coleman et al., 2001, Calley & Hone 2004
	Brushtail possum (*Trichosurus vulpecula*)	New-Zealand	Calley & Hone 2004
Africa	Wildebeest (*Connochaetes taurinus*)	Tanzania	Cleaveland et al., 2005
	Topi (*Damaliscus lunatus*)	Tanzania	Cleaveland et al., 2005
	Buffalo (*Syncerus caffer*)	Uganda South Africa	Woodford 1982 Keet et al. 1996, Bengis et al., 1996, de Vos et al., 2001, Rodwell et al 2001, Grobler et al., 2002, Michel et al., 2008
	Warthog (*Phacochoerus aethiopicus*)	Uganda South Africa	Woodford 1982 Renwick et al., 2006
	Baboon (*Papio spp*)	Kenya South Africa	Tarara et al., 1985, Sapolsky & Else 1987 Keet et al 2000, Michel et al., 2008

Appendix 4: Worldwide M.bovis isolation in free-ranging wildlife

Lechwe (*Kobus leche kafuensis*)	Zambia	Clancey 1977, Zieger et al., 1998
Bushbuck (*Tragelaphus scriptus*)	Zambia	Zieger et al., 1998
Kudu (*Tragelaphus strepsiceros*)	South Africa	Keet et al., 2001, Bengis et al.,2001, Michel et al., 2008
Lion (*Panthera leo*)	South Africa	Renwick et al., 2006, Michel et al., 2008
Leopard (*Panthera pardus*)	South Africa	Renwick et al., 2006, Michel et al., 2008
Cheetah (*Acinonyx jubatus*)	South Africa	Renwick et al., 2006, Michel et al., 2008
Hyena (*Crocuta crocuta*)	South Africa	Renwick et al., 2006, Michel et al., 2008

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by Molecular Epidemiology Techniques. Journal of Clinical Microbiology. 37(9):2766-2771.

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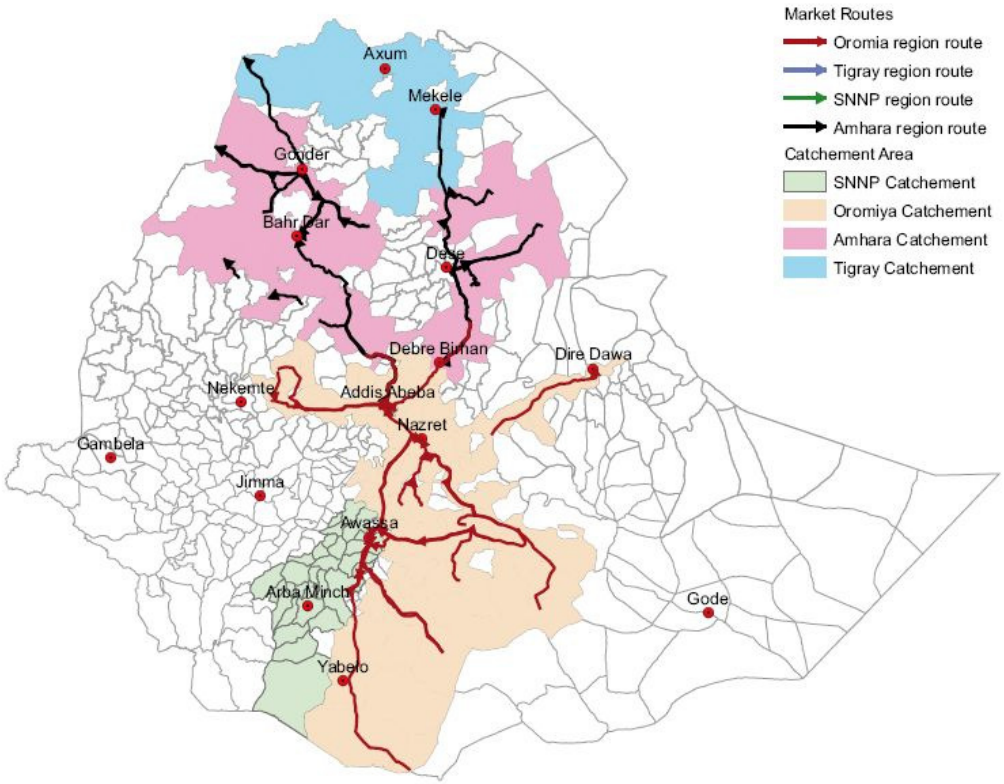
23. Appendix 5: Domestic livestock market routes in Ethiopia

Map illustrating the domestic livestock market routes in Ethiopia (source: ILRI 2007)



24. Appendix 6: Drugs used during the various field works

1. Cattle deworming

Albendazole Bolus 2500 mg, Ashialben 2500, Ashish Life Science PVT.LTD. 213, Laxmi Plaza, New Link Road, Andheri (W), Mumbai- 400053. India

Tetraclozan, 2400mg, Oxyclozanide & Levamisole Hydrochloride, Choong Ang Biotech CO.,LTD. 477, Mokrae-dong, Ansan-city, Kyungki-do, Korea

2. Anti-trypanosomiasis agent

Veridium ^{TM} 1 g (Isometamidium chloride hydrochloride) prevention & treatment of trypanosomiasis. CEVA Santé animale, 33501 Libourne- France

3. PPD

Bovine tuberculin PPD, PL 3326/4006
Avian tuberculin PPD, PL 3326/4007
Veterinary Laboratories Agency (Weybridge), New Haw, Addlestone, Surrey KT15 3NB, UK

25. Curriculum vitae

Surname/Name: Tschopp Rea
Date of birth: 11.01.1974
Nationality: Swiss
Languages: Fluent in German, French, English, basic knowledge of Spanish and Amharic
Adress: Im Moos, 7437 Nufenen, Switzerland
Phone number: +41 81 664 14 14
E-Mail: rea.tschopp@unibas.ch

Education

2005-2008: PhD in Epidemiology
Swiss Tropical Institute (STI)
Department of Public Health and Epidemiology

PhD thesis: Bovine tuberculosis in Ethiopian local cattle and wildlife: epidemiology, economics and ecosystems as part of the Wellcome Trust project on BTB in Ethiopia, in collaboration with Imperial College, London; VLA, Weybridge; AHRI/ALERT Addis Abeba Ethiopia; ILRI Nairobi, and Trinity College, Dublin.

Supervisors: Prof. Marcel Tanner, PD. Dr. Jakob Zinsstag

2002-2003: MSc Wild Animal Health
The Royal Veterinary College, the University of London and the Institute of Zoology

MSc thesis: Infectious keratoconjunctivitis in chamois (*Rupicapra rupricapra*) in Switzerland between 2001-03.

Supervisors: Dr. Marco Giacometti, Dr. Mark Fox, Tony Sainsbury

2000-2001: Veterinary doctorate thesis
Veterinary faculty of Bern, Department of Bacteriology

Doctorate thesis: Epidemiological study of risk factors associated with *Mycoplasma bovis* infections in fattening calves

Supervisor: Prof. J. Nicolet

1998: Diploma of the veterinary medicine faculty of Bern (Med. Vet)
1993-98: Veterinary Faculty of Bern (Switzerland)
1993: Swiss Matura (Chur- Switzerland)
1992: Baccalauréat D (Brazzaville- Congo)

Meetings and Seminars attended

NCCR meeting, Awash, Ethiopia. December 2007. Oral presentation on BTB epidemiology and economics in Ethiopia

Stakeholder meeting "Bovine tuberculosis in Ethiopia", June 2007, Addis Abeba, Ethiopia. Oral presentation on the epidemiology of BTB in Ethiopia

Workshop on epidemiology, June 2007, Addis Abeba, Ethiopia

4th Africa and Middle East Wildlife Disease Association Conference, Sept 2006, Kenya. Poster: BTB at the wildlife-livestock interface in Ethiopia.

NCCR meeting Bahar Dar, Ethiopia, 2005. Oral presentation on BTB in Ethiopia

International *M. bovis* conference, August 2005, Dublin, Ireland

3rd Africa and Middle East Wildlife Disease Association Conference, 11th-13th December 2004, Abu Dhabi- United Arab Emirates: Urinalysis in Falconidae (oral presentation).

Annual Research Conference, 29th October 2003, Institute of Zoology, ZSL, London: Infectious keratoconjunctivitis in chamois in Switzerland between 2001-03 (oral presentation).

Work experience

2004-05 Switzerland: Field-expertise on wolf-sheep interactions on alpine pastures.
Research in infectious keratoconjunctivitis in chamois and alpine ibex
Locum in private veterinary practices (large and small animals)

2004 Belize (Central America): Veterinary work for "Lifeline": rehabilitation and rescue centre for Central American free-ranging wild felidae (puma, jaguar, ocelot, margay); In charge of medical treatment, conservation, education and rehabilitation of wild cats.

2003-04 Nepal: King Mahendra Trust for Nature and Conservation (KMTNC): Veterinary work at the Central Zoo in Kathmandu (medical treatment and education), in
Royal Chitwan National Park and Royal Bardia National Park (rhino translocation, tiger camera trapping, community development work in buffer zones, treatment of domestic elephants.

2003 Dubai Falcon hospital (United Arab Emirates): 3 months internship (Oct- Jan). Clinical work with birds of prey and zoo animals. Translocation of Arabian Oryx and sand gazelles.

2002 Veterinary employee, private mixed animal vet practice of Dr. J.P. Gschwind, Mormont, Switzerland.

1999- 01 Veterinary employee, private mixed animal vet practice of Dr. P. Bonnemain, Porrentruy, Switzerland.

1996 Volunteer work (4 months) in South Africa:

- Dr. G. DeCort, Ogies: Large and small animal practice.
- Dr. G.P.Staley, Howick, Kwazulu-Natal: Large animal practice.
- Dr. Dave Cooper, Umfolozi-Hluluwe National Park, Kwazulu-Natal.

Professional societies

Member of the World Association of Wildlife Veterinarians (WAWV)
Member of WDA (Wildlife Disease Association), Africa and Middle East section

Publications

Tschopp R., Bonnemain P., Nicolet J., and A. Burnens (2001). Epidemiological study of risk factors associated with *Mycoplasma bovis* infections in fattening calves (in German). *Schweiz Arch Tierheilk* band 143, Heft 9.

Tschopp R., Zimmermann L., Frey J., and M. Giacometti (2005). Infectious keratoconjunctivitis outbreaks in free-living Caprinae in Switzerland from 2001 to 2003. *Vet Rec* 157(1): 8-13

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Tschopp R., Getu M., Aseffa A., and J. Zinsstag (2008). Approach to assess the economic impact of bovine tuberculosis in Ethiopia. *Ethiop J Health Dev* 22 (Special Issue)

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Tschopp R., Aseffa A., Schelling E., Berg S., Hailu E., Gadisa E., Habtamu M., Argaw K., and J. Zinsstag (2010). Bovine tuberculosis at the wildlife-livestock-human interface in Hamer Woreda, South Omo, Southern Ethiopia. *PloSOne* 5(8): e12205. doi:10.1371/journal.pone.0012205

Tschopp R., Aseffa A., Schelling E., and J. Zinsstag (2010). Perception of farmers towards agriculture, livestock and natural resources in Ethiopia. *Journal of Mountain Research and Development* 30(4):381-390

Zinsstag Z., **Tschopp R.**, and E. Schelling. *L'interface faune sauvage – élevage – homme de la tuberculose bovine en Afrique*. Book chapter in "Ecologie de la santé et Conservation". In Gauthier-Clerc M. and Thomas F. (Eds) Ecologie de la santé et biodiversité. Group de Boeck SA, Bruxelles. 2010, Partie 2, Chapitre 7. pp 259-270