Prevalence, Molecular Characterization, Transmission Dynamics and Cost Analysis of Bovine Tuberculosis in Morocco

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Prof. Dr. Jörg Schibler
The Dean of Faculty

In memory of my beloved father, I know you would have been proud of me

Dedicated to my family and friends who have always loved me unconditionally and who have been constant sources of support and encouragement during the challenges of my studies and my life. I am truly thankful for having you in my life.

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Summary

Bovine tuberculosis is a chronic disease, caused by *M. bovis*, mycobacteria which belongs to the mycobacterium tuberculosis complex; it is notable for having one of the broadest spectrum of hosts. The preferred host of *M. bovis* is cattle, but it has the ability to infect humans and a wide range of domestic animals.

In Morocco, cattle production is one of the most important components of the agricultural economy; a sector which contributes heavily to the development of the Moroccan economy. The development of this sector is faced by many problems, like poor infrastructure, lack of services and climate change, in addition to infectious diseases like bovine tuberculosis

Bovine tuberculosis is a zoonosis which affects the livestock industry, the public health sector and wildlife reservoirs. BTB has also effects like international trade restrictions for countries where BTB is endemic. Tourism and other areas of public and private interest could also be affected indirectly by BTB infection.

The respiratory route is considered to be the primary mode of infection between cattle. In addition, *M. bovis* is largely transmitted to humans through consumption of unpasteurized milk, but there is also the possibility of inhalation of aerosols due to contact with cattle.

Bovine tuberculosis in endemic in Morocco, the prevalence in Moroccan cattle is estimated at 18% (95% CI: 16.5%-20.3%), and 33% (95% CI: 31%-35%) at the individual and the herd level respectively, but the human burden needs further clarification.

A prevalence study have been conducted in Sidi Kacem province in Morocco in 2012, 1201 cattle were screened using single comparative intradermal tuberculin skin test, the apparent prevalence was 20.4% and 57.7% in the individual and herd level respectively. The individual prevalence found in the present study is in line with the last national survey conducted in 2004 in collaboration with the FAO in Morocco. Consequently, Morocco is in an endemic stable state, similarly to other African countries.

The livestock production sector in Morocco is continuously growing, due to the ambitious “plan Maroc Vert” launched in 2008, and also to the increasing demand of animal protein in Morocco. Consequently, livestock production system in Morocco is moving to intensified and irrigation rearing systems. Those factors in Sidi Kacem have been shown to be associated with higher risk of BTB compared to the extensive livestock system.

In order to investigate BTB molecular epidemiology in Morocco. Bovine tuberculosis samples were collected from two slaughterhouses in Morocco, Rabat

and El Jadida, 8658 animals were examined, 3.7% of them showed gross visible lesions suggesting bovine tuberculosis. However this prevalence reflects the prevalence in young bulls and old cows rather than the prevalence in the whole cattle population.

Molecular characterization of the samples collected from the previously reported slaughterhouses has shown grown cultures in 225 isolates, 63.6% (n=143) have been confirmed to be *M. bovis* (absence of the RD4).

From 134 samples analyzed using spoligotyping, 43 different spoligotypes were found; ten of them were new patterns (23%), they were submitted to the *M.bovis* database and they were given new reference numbers. The most prevalent spoligotypes were SB0121, SB0265, and SB0120, which were already reported in many other countries, mainly in Algeria, Spain, Tunisia, and also in the United States and Argentina.

Spoligotypes of African 1 and African 2 clonal complexes were not reported among the characterized isolates. Considering the localization of Af 1 and Af 2 in West Central Africa and East Africa respectively, we could consider Sahara as a potential efficient barrier preventing the introduction of BTB to Morocco from West Central and East Africa.

More molecular characterization is needed to investigate the strains circulating in the south and the north of Morocco. In order to investigate more deeply transmission dynamics of BTB in Africa, an overall study using whole genome sequencing and including several African countries is needed.

The present thesis presents the first cattle to cattle and cattle to human compartmental deterministic mathematic model. Bovine tuberculosis reproductive number was consequently calculated, it was found to be equal to 1.375, in the range of both low and high risk areas.

The sensitivity analysis of the model showed that the birth rate and the sensitivity of the single comparative intradermal tuberculin skin test are the most sensitive parameters of the model for the total cost and the time to elimination respectively. High birth rate values lead to an increased cattle population yielding higher costs for elimination. In the other hand, low test sensitivity cases low detection of infected animals and therefore less culling which leads to a longer time to elimination.

Simulation of test and slaughter interventions led to a decline of BTB prevalence depending on the proportion of testing (p). Using a severe cut off (2mm) for the SICTT, the time of freedom from BTB ranged from 75 years for p=20% to 25 years for p=50%. The cumulated cost was largely stable ranging from 1.47*10^9 (p=100%, time to disease freedom of 12 years) to 1.87*10^9 (p=20%, time to disease freedom of 12 years).

Deterministic and matrix models were used to develop a demographic model of Moroccan cattle population based on real data. The cost of bovine tuberculosis was consequently calculated using the established model.

The productivity losses triggered by BTB (5%) were estimated for 18 years, applying Leslie matrix with and without BTB. Cattle Moroccan population was compared with and without the disease, and the loss in term of animal numbers was then calculated. Considering the productivity loss, the asset value of the living animals lost due to BTB in year 18 is 98 Million Euro.

The present thesis informs Moroccan stakeholders involved in bovine tuberculosis regarding the updated prevalence in Sidi Kacem Area, molecular epidemiology of BTB among slaughtered cattle, the time frame, and range of cost and levels of intervention, in addition to the cost of BTB considering productivity losses.

Further research is needed in Morocco, in one hand, investigations of the molecular epidemiology of BTB in the north and the south of the country will give more insight about the dynamics of BTB in Morocco, a broader investigation using whole genome sequencing including several African countries could be even more efficient. In the other hand a herd based transmission model will provide a more realistic cost estimation of BTB intervention in Morocco.

Elimination of bovine tuberculosis is a costly and long process, the achievement of BTB control of Morocco will need the commitment of the different stakeholders involved. In addition, public-private collaborations could be helpful in order to achieve a sustainable control intervention of BTB in Morocco.

Keywords: Morocco, Bovine tuberculosis, Transmission dynamics, Molecular characterization, Cost analysis.

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List of abbreviations

MTBC	Mycobacterium tuberculosis complex
LPS	Lipopolysaccharide
BTB	Bovine tuberculosis
UK	United Kingdom
USA	United States of America
Se	Sensitivity
Sp	Specificity
SIT	Single intradermal tuberculin test
SCITT	Single comparative intradermal tuberculin test
IFN	Interferon
PPD	Purified protein derivative
ELISA	Enzyme-linked immunosorbent assay
EVELISA	Ethanol vortex ELISA
ZN	Ziehl-Neelsen
PCR	Polymerase Chain Reaction
VNTR	Variable Number Tandem Repeat
DNA	Deoxyribonucleic acid
REA	Restriction endonuclease analysis
PFGE	Pulsed-field Gel Electrophoresis
RFLP	Restriction fragment length polymorphism
ML-PCR	Mixed linked PCR
LM-PCR	Ligation-mediated PCR
SNP	Single-nucleotide polymorphism
RD	Regions of differences
ETR	Exact tandem repeat
MPTR	Major polymorphic tandem repeat
MIRU-VNTR	Mycobacterial interspersed repetitive units
VENoMYC	Veterinary Network of Laboratories Researching into Improved Diagnosis and Epidemiology of Mycobacterial Diseases
OIE	World Organization for animal health
PZA	Pyrazinamide
MDR	Multidrug-resistant
INH	Isoniazid
WHO	World health organization
LDPS2	Livestock demographic model
FAO	Food and agriculture organization of the united nations
OH	One health
TD	Transdisciplinary

I. Introduction: Bovine tuberculosis in animal health in Morocco

A. Etiology, pathogenesis and transmission

1. Etiology

a) Responsible agent

Bovine tuberculosis is caused by *M. bovis*, a member of the mycobacterium tuberculosis complex (MTBC). To date, 175 Mycobacteria species exist (1). In addition to *M. bovis*, the other most important members are *M. tuberculosis, M. canettii, M. africanum, M. caprae, M. microti, M. pinnipedii,* and *M. mungi* (2–5). MTBC members have different cultural characteristics and requirements and vary in their pathogenicity.

MTBC members are characterized by a very complex cell wall envelope which impacts the cell permeability and allows for the differential staining procedure (Ziehl Neelsen), due to the existence of a long chain of α-alkyl and β-hydroxy fatty acids.

Mycobacteria are divided into two groups based on the growth rate. *M. bovis* is part of the slow growing group, together with *M. tuberculosis* and *M. leprae*. However, *M. smegmatis* is a fast-growing mycobacteria (6). The capsular structure of the mycobacteria is thought to be involved in the permeability barrier of the cell envelope and contribute to protect the mycobacteria from the microbicidal activities of host macrophages (7).

b) Morphology

The mycobacterial cell wall has been described as having three layers, an outer layer of lipopolysaccharide (LPS), an intermediate layer of LPS-lipid-protein complex, and an inner layer of LPS muco-peptide (8). The disposition and composition of the mycobacterial cell wall contributes to the intracellular survival of the bacteria and its immune modulating abilities. These are key elements to consider in developing potential new drugs and treatment strategies as they impact drug resistance. In addition, they contribute to initial host responsiveness (9).

c) Hosts

M. bovis is notable for having one of the broadest host spectrum. The preferred host of *M. bovis* is cattle, but it has the ability to infect humans and a wide range of domestic animals. *M. tuberculosis* infects preferably humans, but has also been isolated in many settings from cattle. *M. africanum* and *M. canettii* can also infect humans. *M. caprae* is usually isolated from goats (6), but has been described in cattle and humans (10), while *M. microti* is a rodent pathogen (6).

d) Pathogenicity

Mycobacteria lack toxins, a typical element in other bacterial pathogens. However, MTBC species have several virulence genes, which mostly encode for enzymes of several lipid pathways, cell surface proteins, regulators, or proteins of the signal transduction system. Another group of genes of relevance for mycobacterial pathogenicity are the genes involved in mycobacterial survival inside the aggressive micro environment of the host macrophages. Many of the MTBC virulence genes are also conserved by non-pathologic mycobacteria.

The virulence determinants have been reviewed by Forrelad et al. (6) and categorized into the following groups based on their function, molecular features or cellular localization:

- Lipid and fatty acid metabolism, including catabolism of cholesterol,
- Cell envelope proteins: including cell wall proteins, lipoproteins and secretion systems,
- Proteins inhibiting antimicrobial effectors of the macrophage, including those involved in responses to oxidative and nitrosative stresses, phagosome arrest and inhibition of apoptosis,
- Protein kinases,
- Proteases, including metalloproteases,
- Metal-transporter proteins, divided into importer and exporters,
- Gene expression regulators, including two component systems, sigma factors and other transcriptional regulators,
- Proteins of unknown function, including PE and PE_PGRS(Polymorphic CG-repetitive sequences) families,
- Other virulence proteins.

B. Pathogenesis

MTBC strains infect mammalian hosts primarily in the lungs, where the mycobacteria are engulfed within alveolar macrophages. Inside of the macrophage, the bacteria are contained in endocytic compartments. MTBC species have several mechanisms to circumvent the hostile environment of the macrophage, including by inhibiting the phagosome-lysosome fusion and by escaping the acidic environment inside the phagolysosome (11).

Infection by an MTBC strain is normally contained in the lungs through formation of granulomas due to the activated macrophages and other immune cells which surround the site of infection to limit tissue damage and restrict microbial dissemination. Within the granuloma, the mycobacteria may remain dormant for decades without any clinical disease (latent tuberculosis). Subsequent immune suppression could allow activation of the dormant bacteria, followed by replication and spread into the lungs and other tissue (12,13).

C. Transmission

1. Animal to animal transmission

The respiratory route is considered to be the primary mode of infection between cattle (14). Most lesions in tuberculous animals are found in the upper and lower respiratory tract and the associated lymph nodes (15).

Cattle to cattle transmission plays an important role in introducing infection into negative herds, through purchased infected animals with contiguous spread.

After mycobacteria infect cattle, a variable period of latency occurs before the animal excretes the pathogen, generally from 8 up to 65 days. However, this period could be as long as 7 years. Barlow et al used a period of 6 to 20 months to construct a simulation model for the spread of bovine tuberculosis within New Zealand cattle herds(16). In addition, transmission of *M. bovis* from cows with tuberculous mastitis to calves via milking has been reported (17).

2. Animal to human transmission

M. bovis is largely transmitted to humans through consumption of unpasteurized milk, but there is also the possibility of inhalation of aerosols due to contact with cattle (18). In order to decrease the transmission risk of *M. bovis* from cattle to humans, a strict milk pasteurization policy should be applied, in addition to application of strict safety measures to protect populations with high risk of BTB transmission (livestock keepers, abattoir workers, veterinarians) (19).

3. Human to human transmission

Human to human transmission of *M. bovis* occurs less commonly than animal to human transmission, although it has been described between immune deficient TB patients in the UK and Spain (20,21). In addition, human to human transmission of *M. bovis* between immune-competent patients has been described in the United Kingdom (UK), United States of America(USA) and France (22–24).

D. Diagnostic tools

1. Ante mortem diagnostic tools

Bovine tuberculosis diagnosis is still a challenge, and the available tools have sensitivity (se) and specificity (sp) limitations. Type III hyper sensibility immunological response in BTB infected cattle is cellular immunity based on T-cells (25). Cattle in late stages of infection develop *M. bovis* antibodies (26). Consequently, ante-mortem tests based on cellular immunity can identify cattle infected with BTB earlier than other tests (27). The most used ante-mortem diagnostic tools are the single and comparative intradermal tuberculin tests (SIT and SCITT), in the cervical area in Europe and in the caudal region in Australia, New Zealand and the USA (28).

The intradermal test has some important advantages, including low cost and the technique is well documented. On the other hand, the intradermal test also has significant disadvantages, such as requiring a second visit to read the reaction, as well as difficulties in test administration and interpretation of results (27). The use of the intradermal test requires trained personnel.

The interferon (IFN) gamma assay has been approved by several national control programs in the European Union, Australia, New Zealand and the USA. This test is employed to enhance sensitivity in parallel testing with the intradermal skin test or to enhance specificity when used for serial testing. One of the advantages of the IFN gamma test is that the animals only need to be handled once because no second visit is required (29). The European Food Safety Authority indicated that the IFN gamma assay has a comparable performance to the intradermal skin test; however, under some conditions, the specificity of the IFN gamma test might be lower than that of the tuberculin skin test (30).

The IFN gamma is analogous to the intradermal skin test, but is an in vitro laboratory based assay which detects specific cell mediated immune response from circulating lymphocytes. The assay consists of incubation of heparinized blood with purified protein derivative (PPD) or specific antigens. This antigenic simulation results in the release of IFN gamma by T lymphocytes. Subsequently, the released IFN gamma in the plasma is quantified using a sandwich enzyme-linked immunosorbent assay (ELISA).

The median sensitivity and specificity of the IFN gamma assay are 87.6 % and 96.6 %, respectively. The assay sensitivity has been demonstrated to be higher than that of the intradermal skin test, but the specificity is problematic. Many elements contained in the PPDs are present in non-pathogenic environmental mycobacteria (31) which could explain the decreased specificity. A cocktail composed of peptide derived from the mycobacterial antigens ESAT-6, CFP-10, and Rv3615c has been shown to increase the specificity of the IFN gamma assay. The addition of the antigen Rv3020c improves the diagnostic sensitivity of the assay (32). An “in-tube” or “in-plate” device for rapid stimulation of lymphocytes after blood collection might overcome one of the biggest disadvantages of this diagnostic test, making the IFN gamma assay more practical (33).

Serological tests for BTB diagnosis have been studied in the last 15 years, as new methods have shown that *M. bovis* antibodies are produced soon after infection. The serological antibody based assays have the advantages of requiring little time, with good cost effectiveness and flexibility of use (34). In 2000, a panel of mycobacterial antigens was tested using antigen specific lymphocyte proliferation and cytokine responses. The two antigens PPD-M and ESAT-6 were the only ones to show positive responses throughout the infection period, whereas for the other antigens the response was not constant. Therefore, it was recommended to use a cocktail of antigens for BTB serological diagnostic (35). In addition, ELISA using the

recombinant proteins ESAT-6, MPB70 and MPB83 has shown satisfactory agreement with the SCITT, while also discriminating between BTB positive and negative cattle prior to tuberculin skin test (36). More recently, an ethanol vortex ELISA (EVELISA) was used to detect anti *M.bovis* antibodies in the sera of cattle using MPB83 antigen. EVELISA showed a positive potential for BTB diagnosis but needs to be studied further (37). Serological antibody based assays for BTB diagnostics need more studies prior to recommendation as standalone tests.

2. Post mortem diagnostic tools

Bovine tuberculosis can be diagnosed after the death or slaughtering of cattle by histopathology, bacteriology, and molecular techniques. Animals which have gross visible lesions at slaughter are condemned, and the lesions showing characteristic histological changes (caseous necrosis, mineralization, epithelioid cells, multinucleated giant cells and macrophages) may be sampled for further analysis. A bacteriological examination can first be performed using Ziehl-Neelsen (ZN) coloration and microscopy observation. The observation of acid-fast bacilli provides presumptive confirmation of the presence of mycobacteria. However, microscopic examination does not allow for characterization of the mycobacteria. Isolation of the mycobacteria may be performed after a decontamination of the sample using solid egg based culture media for the primary culture (29). Additionally, pyruvate supplementation has been shown to enhance *M. bovis* growth, while glycerol inhibits *M. bovis* growth (38).

Culture is a reliable diagnosis method, but it is time consuming because mycobacteria grow slowly. In addition, sampling of BTB suspicious lesions and their handling in the laboratory should be performed using the required biosafety level 3 measures (39). PCR (Polymerase chain reaction) based techniques, like RD typing (Region of Deletion) are a good alternative for culture, as they need less time; however, a well-equipped laboratory with trained personnel are necessary, and PCR is expensive compared to the other post mortem diagnostic methods (40).

Variable Number Tandem Repeat (VNTR) typing and spoligotyping, either alone or together, have shown good results in molecular characterization of MTBC, although VNTR provides better discrimination than spoligotyping (41).

E. *M.bovis* in the mycobacterium tuberculosis complex

The DNA (Deoxyribonucleic acid) of the MTB complex is 99.9% similar, in addition to having virtually identical 16S rRNA sequences, cited by (42). The whole genome sequencing for *M. bovis* was performed for the first time in 2003 (43). Smith et al suggested in 2009 (44) that *M. bovis* evolved from *M. tuberculosis*, supporting the hypothesis addressed by Brosch in 2002 (45)

Differential hybridization arrays identified 14 regions of difference in the MTBC, in addition to H37Rv related deletions (RvD 1-5), and the M. tuberculosis specific

deletion TbD1 (46). *M. bovis* is the most recent member of MTBC lineage, presenting the largest number of deletions, as shown in the figure 1. It has been suggested by Brosch that the common ancestor of the tubercle bacilli resembled *M. tuberculosis* or *M. canetti,* and was likely to already be a human pathogen, in contrast to the hypothesis previously presented that *M. tuberculosis* emerged from *M. bovis* (45).

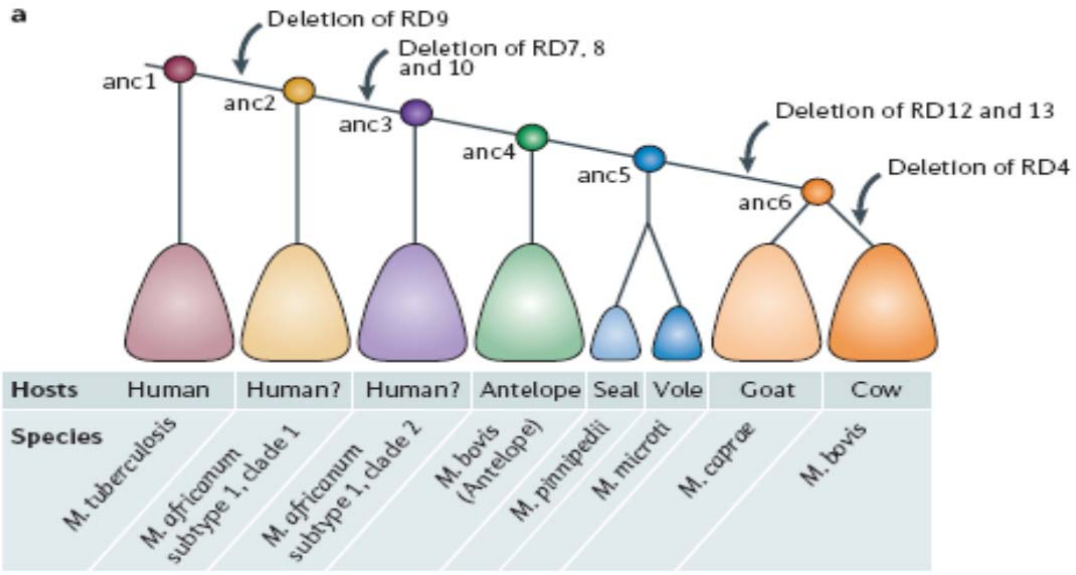

Figure I.1. Phylogeny of MTBC

So far, four clonal complexes of *M. bovis* have been identified. The first one is African 1, which is characterized by the chromosomal deletion RDAf1, in addition to the absence of spacer 30 in the spoligotype pattern. The second one is African 2, which has the RDAf2 deletion and the absence of spacer 3 to 7 in the standard spoligotyping scheme. The third clonal complex is European 1, which is characterized by the RDEu1 deletion. The African 1 clonal complex is geographically localized in Mali, Cameroon, Nigeria and Chad, while the African 2 is localized in East Africa. The European 1 complex is rare or absent in African countries, with the exception of South Africa, being found in European countries (e.g., France, Portugal, Spain), as well as the USA, Canada, Australia, and New Zealand (47–49). The fourth clonal complex of *M. bovis*, European 2 complex, was identified in the Iberian Peninsula and is characterized by the absence of spacer 21 from the spoligotype pattern, in addition to the presence of a specific SNP (Single-nucleotide polymorphism) in gene guaA. This clonal complex was isolated in low frequency in France and Italy (50).

Before 2000, data about the molecular epidemiology of *M. bovis* were scarce, since no well-established molecular typing system was available (51). The first molecular typing technique used for *M. bovis* was restriction endonuclease analysis (REA) (52) in the late 1980s and early 1990s in New Zealand (53) and Ireland (54). However, REA is technically demanding (55).

While REA deals with many small fragments of DNA, PFGE (Pulsed-field Gel Electrophoresis) separates a few large fragments (51). This typing technique was first developed by Shwatz et al for the differentiation of yeast chromosomes (56). The PFGE was then adapted for bacteria and used for *M. tuberculosis* (57), before it was utilized for *M. bovis* typing and differentiation between *M. bovis* and *M. bovis* BCG (57,58).

Both REA and PFGE are whole genome techniques (51). Later, partial genomic techniques were developed, such as restriction fragment length polymorphism (RFLP). This technique initially used radioactive labeled probes, and it was not recommended due to safety concerns until these were replaced by chemi-luminescent systems (59).

Restriction fragment length polymorphism using insertion sequence 6110 (IS6110) is a technique which was widely used, mostly for *M. tuberculosis*, as the genome carries several copies of IS6110, which results in a high discriminatory power. Consequently, IS6110-RFLP was long considered as a gold standard in the molecular epidemiology of human TB (51). This technique has less often been used for *M. bovis* molecular typing, because of the low number of copies which exist in the genome of *M. bovis*. However, some studies showed that the number of copies of the IS610 in *M. bovis* is variable depending on the host and the specificities of the local strains (60,61). In fact, IS6110-RFLP is highly discriminatory for *M. tuberculosis*, as the genome has up to 20 copies of IS6110. However, the discriminatory power decreases when the genome has one or only a few copies of IS6110 (62). It has been recommended to combine IS6110-RFLP with another molecular technique for *M. bovis* molecular typing (59).

Even though RFLP has a high discriminatory power, it has many limitations; most importantly, it is technically demanding and requires a large amount of DNA, which makes its application directly on clinical specimens difficult (63). These limitations were a strong motivation for the development of more rapid and automated techniques, like PCR based molecular techniques (51).

PCR based methods are used in most cases as a confirmatory tool to culture, which is a gold standard, or to differentiate MTBC members. For this purpose, different genes, insertions or deletions are targeted. In order to differentiate between tuberculous and non tuberculous mycobacteria, the duplex MrpoB PCR is usually used (64). On the other hand, PCR amplification and DNA sequencing of 16s rRNA are used for samples confirmed to not be a mycobacteria (65).

In order to differentiate between the members of MTBC, a large number of deletions /insertions are available so far, which are used separately to identify *M. tuberculosis* or *M. bovis*, or in multiplex PCR (66–68). Amongst the most important PCR based techniques are Mixed linked PCR (ML-PCR)(69), Ligation-mediated PCR (LM-PCR) (70), and Fast ligation-mediated PCR (Flip)(71). The discriminatory potential of those three PCR based techniques is good but somewhat lower than

IS6110-RFLP analysis (71,72). Deletion typing using the regions of differences (RD) is also usually used for the discrimination between the MTBC members (45).

Currently, the most used PCR based techniques for the molecular identification of *M. bovis* are spoligotyping (73) and variable number tandem repeat analysis (VNTR) (74).

F. Molecular typing methods used for *M. bovis*

Spoligotyping is based on polymorphism of one specific genomic region, called direct repeat (DR) locus. The DR locus is composed of a series of well-conserved direct repeats (DRs) interspersed with a unique, non-repetitive spacer sequence, identified first by Hermans et al in 1991 (75).

Spoligotyping is a fast, robust and cost effective technique, which can be used to differentiate *M. tuberculosis* and *M. bovis*. However, the discriminatory power of this technique is lower than IS6110-RFLP (73). Spoligotyping targets only a single genetic locus, covering less than 0.1% of MTBC, which is the cause of the lower discriminatory power of the technique. However, spoligotyping remains a useful tool for differentiation of MTBC strains with a low number of copies of IS6110 (76,77).

Two large databases are available online which list all of the previously reported spoligotype patterns, and new patterns can be submitted (SITVIT and mbovis databases) (78,79). *M. tuberculosis* lacks spacers 33 to 36, and *M. bovis* lacks spacers 39 to 43 (80).

From human gene mapping, forensic analysis and paternity testing, genetic loci containing variable numbers of tandem repeats (VNTR loci) were then used for the characterization of bacterial species, including MTBC species (51,63). VNTR consists of the amplification of the DNA containing variable numbers of tandem repeat sequences and the determination of the size of the products by gel electrophoresis (51) or fluorescence (74). In 1998, Frothingham and Meeker-O'Connell identified one major polymorphic tandem repeat (MPTR) loci and six exact tandem repeat (ETR) loci (ETR-A to F) relevant for MTBC typing (81).

First VNTR typing ETR A to F was found to be less discriminatory than spoligotyping used alone or IS6110-RFLP typing (81,82). Consequently, a set of mycobacterial interspersed repetitive units (MIRU-VNTR) has been proposed (74,83,84). A 15 locus system was first proposed as a standard for routine epidemiological discrimination of *M. tuberculosis* isolates, while the 24-locus system was proposed as a high-resolution tool for phylogenetic studies (83). However, for *M. bovis*, six loci (ETR A, ETR B, ETR-D, QUB 3232, QUB 11a, QUB 11b) were shown to result in a high discriminatory power (74,85–87) and were recommended for typing *M. bovis* by the Veterinary Network of Laboratories Researching into Improved Diagnosis and Epidemiology of Mycobacterial Diseases (VENoMYC) Consortium (EU Coordination Action SSPE-CT-2004-501903 (88). It has been recommended that a

definition of an appropriate combination of heterogenic loci for each country and MTBC panel studied is necessary(84), although different sets of the most discriminatory loci for *M. bovis* are available for many settings (84,85,89,90).

G. International situation of bovine tuberculosis: focus on Africa and Morocco

BTB is listed by the OIE (World organization for animal health) as a cattle infection in list B and as having an economic and public health burden, in addition to being a significant element in international trade of animals and animal products (91).

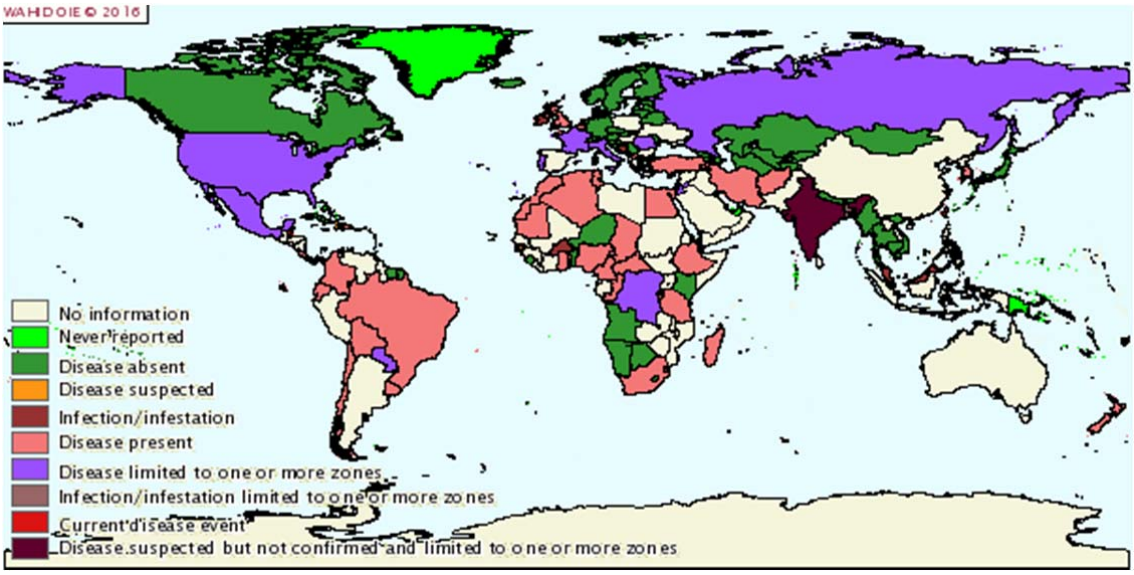

Figure I.2. Bovine tuberculosis distribution map (92)

Bovine tuberculosis is present worldwide. After the introduction of control measures and milk pasteurization in developed countries, the prevalence of BTB dropped drastically. Currently, bovine tuberculosis is mostly present in Africa and in South America. Many countries on these two continents have no data available on this infectious disease. In North America, most of Asia and Europe, BTB is absent or limited to one or few regions.

BTB has been eliminated from several countries (e.g., Switzerland, Australia, New Zealand). The countries where BTB has been eliminated have the absence of a wildlife reservoir in common. In countries like the UK, which have a wildlife reservoir for BTB, elimination of the disease is ongoing, with low prevalence level of BTB in cattle but still a big problem in the wildlife.

Table I.1. Bovine tuberculosis prevalence in some countries using a cut off recommended by the OIE (positive when the difference is >4 mm)

Country	Nb. Tested animals	Diagnostic tool(s) used	Year	Individual prevalence (%)	Reference
Morocco	13021	SCITT	2004	18	(92)
Mozambique	1136	SCITT	2004	39.6	(93)
Tanzania	_	SCITT		2.4	(94)
Eritrea	15354	SCITT	2011	21.5	(95)
Ecuador	1446	CITT	2008	8.4	(96)
Ethiopia	2550	CITT	2015	5.5	(97)
Pakistan	556	SCITT	2015	5.75	(98)
Brazil	22990	SCITT	2015	0.81	(99)
Niger	393	SCITT	2009	3.6	(100)
Zambia	944	SCITT	2004	49.8	(101)
Cameroun	807	SCITT	2010	3.5	(102)

H. Control strategies for bovine tuberculosis in developed countries and lessons learned

Australia was one of the first countries which eliminated BTB. A joint BTB and brucellosis eradication program started in 1970, and Australia was declared free of BTB by 1997. Australia was fortunate as no feral host for *M. bovis* was present (103). However, the wildlife reservoir represents a cause of reemergence of BTB in many countries (104). In Ireland, badgers are a reservoir for *M. bovis*, and prevention of the transmission of BTB from badgers to cattle is one of the challenges faced in order to move forward in the control of BTB (105). In addition, New Zealand used a strategy based on the control of the wild host, which resulted in decrease of the infected cattle herds to less than 100 by 2014 (106).

While the problem of BTB eradication in the developed world is mainly the wildlife reservoir, in Africa, as a developing continent, the control of zoonoses like BTB are affected by political and economic factors, the lack of efficient organization of veterinary services and inadequate communication networks (107). However, wildlife reservoirs are an additional obstacle for BTB elimination for some African countries (feral baboons in Kenya (108) and warthog and buffalo in Uganda (109), and African buffalo (Syncerus caffer) in the Kruger National Park in South Africa (110)). Bovine tuberculosis constitutes a neglected problem in developing countries, where the few human and financial resources are mainly engaged in the control of other acute and fatal diseases and parasitic diseases (107).

Strong industry and government support in funding and policy development are important factors for the success of a zoonosis eradication campaign, as confirmed by the Australian experience. Other factors could affect success of disease control

and eradication programs, like extreme rigor in the application of the selected strategy, in addition to the implication of the process for the owners(111).

Trust between all the stakeholders, especially the industry, the government and the owners, is a very important component which contributed to the success of BTB control program in Australia. In addition, animal traceability and abattoir surveillance were applied from the early years of the control program in Australia (112). While in the US, the application of animal traceability was a challenging element for abattoir surveillance and needed more effort to achieve an effective elimination of BTB (113). Correct application of livestock biosafety, early diagnosis of the disease, application of quarantine, and animal movement control are other parameters which markedly affect the success of a control campaign (114).

I. Current control approaches in developing countries

In Nigeria, reporting of BTB is not mandatory, and there is no active TB surveillance program (115). Like in Nigeria and other African countries, the control of BTB is challenging in Cameroon as well due to the high cost of surveillance and the limited veterinary and public health infrastructure (116).In Burkina Faso, there is no BTB control strategies (117).

In 1967, Iran initiated a compulsory test and slaughter program for BTB in all cattle, and this control program, although having several weaknesses, has been successful, decreasing the prevalence from 28% to less than 0.5% (118).

The control strategy shown to be effective to control and eliminate BTB in developed countries is test and slaughter, but such intervention remains unaffordable for low and middle income countries with large cattle populations (107). A test and slaughter strategy could be applied in developing countries when logistically and financially feasible, but it should be integrated with many other actions, like abattoir surveillance, a national animal identification system, and promotion of milk pasteurization at local and national levels (119). A trans-disciplinary approach is potentially the best way to apply a successful BTB control intervention. Considering that an increased awareness and knowledge of the disease is an important step in the development of control measures, health communication is a tool which has been proven to be cost effective (119). Awareness could contribute to a better acceptance of a potential control intervention by the population; in addition, it is a crucial element for the sustainability and the effective implementation of the control strategy(120).

A preliminary investigation of the important key risk factors which could influence the success of BTB control interventions should be performed, as done in Zambia, where the livestock production system and grazing strategy have been determined as important risk factors to be considered (101).

II. Economics of Bovine Tuberculosis: A One Health Issue

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This chapter is focused in the economics of bovine tuberculosis (TB), taking into consideration the burden of this disease for livestock and also for human health, with a stong emphasis in One Health (OH) as a control approach. The current chapter starts with an overview of One Health, followed by a review of the economics of bovine TB as an OH issue, through a summary of One Health and its added value for bovine TB and human TB control.

A. One Health

OH can be defined as the added value of closer cooperation between human and animal health in terms of better health of humans and animals, financial savings and improved ecosystem services (121). OH is part of the broader consideration of ecology and health. It contributes to improve health by engaging different institutions and disciplines in a closer way by improved communication, closer collaboration and better information sharing based on the recognition that human and animal health are mutually dependent.

Obstacles of the broad acceptance of the benefits gained from an OH approach are mostly economic. In fact, it is critical for the establishment of an OH approach to demonstrate that public and private stakeholders may save money from a closer cooperation (122).

Veterinary attention should be drawn to many sectors related directly or indirectly to the animal health, such as international trade and travel, global climate changes, habitat destruction, overpopulation, ecotourism and food safety, and all those sectors should be aware of the positive impact of the collaboration with other disciplines. However, the establishment of an OH initiative and setup of its principles should be performed at the academic level; in addition to the creation of specialized Masters' in OH (123), the academic training of OH should be adapted to different countries and contexts in order to be most efficient. Still, the OH approach should be embraced also by several institutions and organizations outside academia, such as industrial firms, especially those that will benefit from addressing the challenges posed by bovine TB using an OH approach (e.g. the milk and meat industries).

Public health schools remain among the biggest institutions that deploy considerable efforts to educate global health experts and prepare them to confront the global burden diseases. One of the strengths of public health schools is their multidisciplinary orientation and their aspiration to develop, test and validate new approaches, technologies and systems in order to reach the global health needs, especially in developing countries (124). Moreover, OH courses are available in many universities, non-governmental organizations and government agencies, for example, the University of Edinburgh, London School of Hygiene and Tropical Medicine, Swiss Tropical and Public Health Institute, and many other universities and institutes.

Examples of OH approaches include a vaccination campaign in Chad for both pastoralists (vaccination against diphtheria, whooping cough, tetanus and against

polio) and their livestock (vaccination against anthrax, pasteurellosis, blackleg and contagious bovine pleuropneumonia), in addition to the delivery of health care. This was a successful intervention integrating human and animal health workers, where this joint action allowed to reduce costs by 15% compared to a separate campaign (125,126).

Moreover, it has been validated in a prevalence study performed in Chad for brucellosis and Q-fever that using an OH approach in prevalence investigations of a zoonosis could decrease the detection time when sampling humans and animals in parallel (127). However, this joint investigation should be justified with a higher incremental knowledge, and more importantly, no concessions should be made in the quality of the methods (128).

Zinsstag et al. (2007) demonstrated, using brucellosis, rabies and avian influenza examples, that interventions against zoonoses become cost saving when considered from a societal perspective. An intervention may become highly cost effective when costs are shared between different sectors in proportion to their benefits (130). In contrast to developed countries, many zoonoses are still endemic in many developing countries, as financial and organizational resources cannot be focused on the animal reservoir (131).

B. Human Tuberculosis: The International Epidemiological Situation and Control Strategy

According to the World Health Organization (WHO), in 2015, TB caused 1.8 million deaths worldwide, which puts human TB as a leading cause of death. In addition, 12% of all TB cases are co-infected with HIV. The estimated number of new cases of human TB in the world for 2015 is 10.4 million. The incidence of TB is variable from one region to another; Southeast Asia and the Western Pacific accounts for 58% of all TB cases. Africa has 28% of worldwide TB cases, but has the most severe burden relative to population (132).

On the other hand, Western Europe and North America showed a low incidence of human TB compared to the most populous countries of Asia, where human TB is very prevalent (e.g. Bangladesh, India, China, Indonesia, and Pakistan) (133). In addition, in some developing countries, an increase in new TB cases has been observed within the last 20 years, and this could be explained, among other reasons, by better data management and diagnostic rates (132).

In May 2014, the End TB strategy was established with the goals of reducing the number of TB deaths by 90% by 2030 (compared to 2015 rates) and reducing the number of new TB cases by 80% (132).

C. The Economic and Public Health Burden of bovine Tuberculosis

Bovine TB affects the national economy of the countries where this disease is endemic by causing a decrease in productivity, condemnation of meat in the abattoirs and an influence on the international trade of animal products (134) Ongoing bovine TB transmission also has important effects on ecosystems by affecting wildlife (135). Bovine TB is more difficult to eliminate from wildlife than from cattle. This is currently an obstacle for the eradication of bovine TB in some developed countries, for example, badgers in the United Kingdom (UK) and Ireland (136,137), the brushtail possum in New Zealand (138), wild boar (*Sus scrofa*) in the Iberian Peninsula (139) and white-tailed deer (*Odocoileus virginianus*) in Michigan, USA (140).

The public health burden of zoonotic TB in industrialized countries is low because of the pasteurization of milk and/or its effective elimination in cattle. Rare cases are contracted abroad (141). For example, in Australia *Mycobacterium bovis* represented 0.2% of all human TB cases in 2010, and *M. bovis* infection is linked with employment in the livestock industry and immigration from countries in which bovine TB is endemic (142). In the United States (US), between 1995 and 2005, the majority of human *M. bovis* patients was born outside of the US and could have contracted zoonotic TB abroad. In addition, the consumption of fresh cheese ('queso fresco') produced from unpasteurized milk in Mexico has been described to be a potential source of *M. bovis* in the US (143). Mexico is a country where bovine TB has a high prevalence in cattle, and studies have found high prevalence in humans (144–146). A recent study described a prevalence of 26.2% of *M.* bovis among human TB patients (*n* = 1165) in Mexico. However, this high proportion of *M. bovis* among human TB patients has been explained by the authors as potentially linked to immunosuppressed patients; in addition, isolates obtained from HIV-infected patients accounted for 19.2% of the local samples in the same study (147).

Bovine TB has been previously classified by the WHO to be a neglected zoonosis in developing countries, where the public health burden of bovine TB is high and many risk factors linked to the transmission and persistence of *M. bovis* are present, for example, consumption of unpasteurized milk (107). Moreover, neglected zoonoses such as bovine TB in developing countries are associated with poverty (148). The quantified burden of zoonotic TB on public health is still not well known in developing countries. A recent review of *M. bovis* among humans in Africa reported a mean prevalence of 2.8% of *M. bovis* among human TB patients. Considering an incidence rate of 264/100 000 population/year, this review resulted in a crude estimate of 7 zoonotic TB cases/100 000 population per year (149); however, more studies are needed to better investigate the public health burden of bovine TB in developing countries. In June 2016, the WHO included zoonotic TB as a priority and it is now endorsed by the Strategic and Technical Advisory Group (STAG). In order to follow this development, OH approaches are needed to continue improve the situation.

1. Livestock

Bovine TB causes economic losses to the livestock industry, as it increases mortality and reduces milk and meat production. It also results in condemnation of organs and carcasses in the slaughterhouses when animals show gross visible lesions suggestive of bovine TB infection (150). To date no study has been performed in Africa to estimate the productivity losses in terms of meat and milk caused by bovine TB.

In Ireland, a study showed that bovine TB infection caused a decrease of milk production by 0.5% to 14.6%; however, decreased milk production has been shown to be a risk factor for bovine TB (151). These findings are in line with earlier estimates of milk production losses of 10% among tuberculin-positive animals in the former East Germany (152). In Bangladesh, a study showed bovine TB to be responsible for 18% of milk losses (153). In addition, annual calving rates are reduced by 5% among bovine TB-positive animals, thus affecting the fertility and demographic composition of the herd (154). Overall, the cost of bovine TB to the Ethiopian livestock production systems was estimated at 1% of the net present value in the rural and 4% to 6% in urban areas (155).

2. Human health

The emergence of drug-resistant *M. bovis* is an important public health problem that affects the success of TB control programs in many developing countries, for instance in Mexico (156). Consequently, it causes an increased illness burden and financial losses due to relapses considering the resistance of *M. bovis* to a first-line drug (pyrazinamide) used in human TB treatment (157–159)

In most developing countries, no microbiological identification of TB causative agents is made before the administration of treatment. Considering the natural resistance of *M. bovis* to pyrazinamide, in addition to the re-emerging mutations in *M. bovis* genome, which cause resistance to other TB drugs (158), a human infection with *M. bovis* could be considered as one the causes contributing to the relapse of TB patients. Consequently, there is an urgent need to quantify the exact burden of zoonotic TB among human TB patients in developing countries and, more importantly, among the groups that are the most at risk to contact *M. bovis* from cattle.

D. Bovine Tuberculosis: Transmission and Risk Factors for Cattle and Humans

Bovine TB is a zoonosis caused by *M. bovis*, a Gram-positive bacteria belonging to the *Mycobacterium tuberculosis* complex (MTBC). The most important host for *M. bovis* is cattle (160); however, this species infects a wide range of domestic and wild animals as well as humans (139,161).

A brief description of factors impacting transmission of *M. bovis* among cattle and to and between humans is presented in this section to highlight the need of an OH approach to control bovine and zoonotic TB. Several risk factors are linked with bovine TB infection in cattle. The risk of bovine TB infection has been described to increase with age (162), while local breeds have been linked with lower prevalence of bovine TB (163). The risk of bovine TB infection regarding gender has been observed to be linked to livestock management practices and cultural behavioral habits related to each country (164). In developing African countries, imported cattle are usually kept under intensive conditions, a factor that has been previously described as a risk factor for bovine TB infection (165). In addition, intensive breeding is usually practiced in larger herds, a factor that has been shown to increase the risk of bovine TB infection (164). The type of production could also be a risk factor for bovine TB, as described in a cohort study in New Zealand from 1980 to 2004, where dairy herds were observed to have a higher risk of infection compared to fattening schemes (166).

Two routes of transmission have been described in humans: for adult and older patients, airborne transmission is the most common route causing pulmonary TB, while in younger patients, foodborne transmission occurs more often, which may lead to extra-pulmonary tuberculosis (143). Consumption of unpasteurized milk has been recognized as to be a major risk factor (167). However, the transmission of *M. bovis* to humans can be enhanced by other factors, such as HIV co-infection (143,168). Person-to-person transmission of *M. bovis* has been previously reported in immune-deficient patients (169,170), as well as in immune-competent patients as described in France in 2009 (Sunder *et al*., 2009). The transmission of *M. bovis* between animals and humans depends on many risk factors, which vary from one epidemiological context to another. In developing countries, the livestock management system is a very important risk factor for bovine TB transmission. As the economy of a country grows, the livestock keepers tend to move from more extensive pastoral systems to more intensive livestock management for dairy production. In such systems, animals are closer together in less ventilated spaces and with less sunlight. Such intensified production systems provide a more favorable environment for the persistence of the disease, as *M. bovis* is more easily transmitted (165,171).

Moreover, human TB due to *M. bovis* has been suggested as an occupational hazard after the isolation of *M. bovis* from 5 abattoir workers among 3000 abattoir workers during a 2-year period in Australia (172). In Pakistan, human TB caused by *M. bovis* was found in livestock keepers and abattoir workers. Almost all of these workers do not work safely and they do not protect themselves (173). These facts suggest that biosafety measures should be applied for workers in direct contact with *M. bovis* hazards from livestock to abattoirs, and strict routine surveillance for bovine TB gross visible lesions should be applied in order to protect the consumer from *M. bovis* exposure.

E. The Cost of Bovine Tuberculosis

The economics of bovine TB have been summarized by Zinsstag *et al*. (2006). The authors emphasized the multifaceted and multi-sector nature of bovine TB with costs to livestock production and animal health, in addition to wildlife and human health. However most of the time economic analyses of bovine TB focused only in one sector: the cost to livestock production. In areas where cattle are the only reservoir host, the control of bovine TB is possible with a test and slaughter policy, whereas in countries with wildlife reservoirs it is more difficult and increases the cost of efforts to control bovine TB. The cost for the control of bovine TB in the UK decreased from an average of GB£92 million annually from 2003 to 2005 (174)) to GB£74 million in 2006 (136) and increased again to GB£99 million pounds in 2013 (175). A total of GB£66 million has been directed for operational, policy and lab work performed by the animal health services and the veterinary laboratories agency; in addition to the payment for private veterinarians for TB testing, GB£23.5 million of the total amount is for cattle compensation costs (176). In Turkey, the annual socio-economic impact of bovine TB to the agriculture and health sectors is estimated to range from US$15 to US$59 million (177), while in Argentina, the losses due to bovine TB has been estimated to be US$63 million as reported by Cosivi *et al*. (167).

Very few cost estimates are available for bovine TB in developing countries. As one of the first, in Ethiopia the cost of this disease was estimated using a livestock demographic model (LDPS2, Food and Agriculture Organization) with some modifications to allow the stochastic simulation of parameters. It was shown that the cost of bovine TB in the peri-urban dairy production system in areas of Addis Ababa (where the disease has a higher prevalence, and the present value of livestock products is US$13.9 million) was found to range from US$0.5 to US$4.9 million in 2005 and in 2011, respectively, whereas in the rural areas, where bovine TB has a lower prevalence, with a present value of livestock products of US$7.5 billion, the cost of bovine TB ranged from US$75.2 million in 2005 to US$358 million in 2011 (155). This cost analysis in Ethiopia concluded that the intervention to control bovine TB in the country would not be cost effective and was not possible within the current economic situation of Ethiopia (155).

In addition, a recent review in Ethiopia identified the test and slaughter control strategy to be financially and logistically unfeasible for bovine TB. This review also highlighted the need to explore alternative control options such as milk pasteurization, meat condemnation in the abattoirs and animal movement control (179).

The above analysis was not multi-sectorial in the sense that it considered that the estimation of the full societal cost of a bovine TB should take into account the social and private sectors, direct and indirect losses to livestock production, and animal and human health.

Table II.1 summarizes the different losses triggered by bovine TB in humans and animal sectors. The human health sector losses could be estimated considering the burden of *M. bovis* on human TB cases.

Table II.1. Direct and indirect economic losses associated with BTB in human and animal sectors.

	Animal	Human
Direct	• Condemned meat in slaughterhouses • Diminution of the animal value	• Diagnostic and hospitalization (ministry of health) • Out-of-pocket expenses for health care (contribution of the patient)
Indirect	• Diminution of milk production • Diminution of fertility • Change of herd demographic composition	• DALY's lost • Transport costs (travel expenses) • Expenses related to the patient visitors and accompanying person. • Jobs lost (change in the household income)

Bovine TB is not a disease that receives the most attention in developing countries, as many other infectious diseases in animal health given higher prioritization (e.g. foot and mouth disease and peste des petits ruminants). In addition, as the burden of bovine TB has not yet been estimated in most of the countries, the stakeholders are not aware of the real burden of this disease, especially for human health. In many developing countries, physicians are not convinced of the added value of working closer with the animal health sector in order to control this disease, as they assume the proportion of *M. bovis* among human TB patients to be very low, although this is not yet estimated in many developing countries (e.g. Morocco), and that *M. bovis* has not been officially considered or investigated as the causal agent of human TB. In countries where bovine TB has a high prevalence in cattle, and where no prevention control measures are applied (e.g. mandatory milk pasteurization), the proportion of *M. bovis* infections among human patients could potentially be higher than expected.

F. One Health Economics of Bovine Tuberculosis

OH approaches to control zoonoses have been applied in developing countries, mainly in epidemiological investigations. Examples include human and animal seroprevalence studies performed in Kyrkyzstan (180,181) and in Mangolia (182). Moreover, OH showed a great potential in the contribution to rabies elimination in Africa as explained by (183).

To apply an OH approach to bovine TB, the first step to be undertaken is to investigate the burden of *M. bovis* among human TB patients. This information could be used in order to start a dialogue between the human and animal health sectors.

Before an integrated approach to control human TB and bovine TB can be developed, an economic study assessing the cost of the control of human TB using a OH approach should be performed, in addition to the evaluation of the added value of this approach. Potential savings achievable through the implementation of OH concept in 139 World Bank client countries have been estimated to range from 0% to 40% depending on the task considered (184).

The adequate resources needed to achieve this collaboration should be available; in addition, the persons involved in OH interventions should be trained in order to have the necessary skills needed for a better management of the intervention (186).

Decision makers are a key stakeholder in bovine TB control strategy, and they should be involved in the process from the beginning. The economic and societal impact of each approach suggested must be communicated to the decision makers, in addition to a time line for intervention and a cost-effectiveness analysis (186). Interventions to control a zoonosis should be performed in parallel with a health education campaign, as this will contribute to a better acceptance of the control program by the local population and its sustainability (187).

G. Bovine Tuberculosis as a One Health Issue

The burden of *M. bovis* for public health is very low for developed countries, where the disease in cattle has a low prevalence or has been eradicated. In developing countries, the burden of *M. bovis* in humans is known for only a few countries. In a systematic review and meta-analysis, (149) reported an average prevalence of *M. bovis* among humans of about 2.8% (7 zoonotic TB cases/100,000 population per year) in Africa, based on information from African countries for which data is available. However, no data are available for quantifying the number of human TB patients infected with *M. bovis* in many other developing countries in Africa and elsewhere. This could be due to a weak communication between human and veterinary health systems, which calls for an OH approach. In addition to a lack of awareness of a potential high burden of *M. bovis* among humans, in settings where bovine TB has a high prevalence among cattle and several risk factors for transmission of bovine TB to humans are present (e.g. consumption of unpasteurized milk, close contact between human and cattle), the burden of *M. bovis* should be urgently considered.

Morocco is a developing country where the prevalence of bovine TB among cattle is 18% (188), and 40% of the population live in rural areas (189) in close contact with cattle. The burden of *M. bovis* among human TB cases should be investigated, as the risk factors for zoonotic TB transmission from animals to humans are present. In this context, an OH approach should be introduced where the human health and veterinary efforts are integrated in order to investigate the real burden of *M. bovis* in humans in such settings, but such collaboration needs an improved communication between both sectors.

A recent transmission model (Figure. II.1) of bovine TB in Morocco showed that the disease could be controlled within 20 years, if 60% of Moroccan cattle were tested annually and infected animals were slaughtered. This 20-year campaign is projected to cost €1.53 billion (190). Further analyses on the profitability and cost effectiveness are ongoing. The transmission model used to estimate the cost of bovine TB elimination in Morocco considered three categories for cattle (susceptible, exposed with latent TB, and infected with active TB). The human population was divided into four categories (susceptible, exposed with latent zoonotic TB, infected with active TB, and recovered from TB). In order to represent the human burden of bovine TB, a transmission from infected cattle to exposed humans was considered in the model (190).

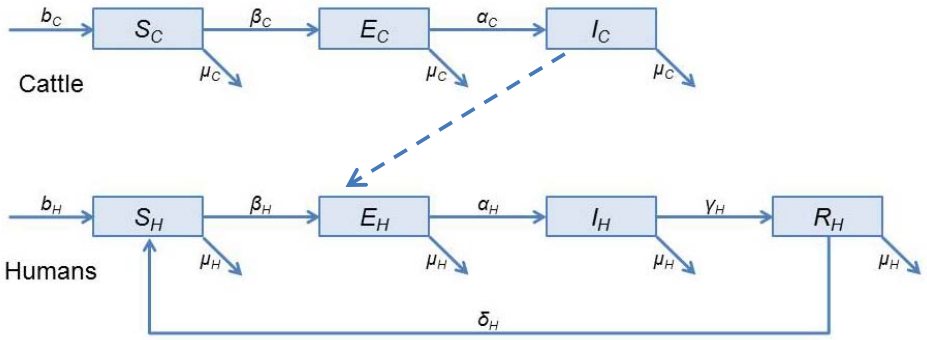

Figure II.1. Schematic diagram of the bovine TB cattle-human transmission model for Morocco (Abakar et al., 2016)

H. Towards the Control and Elimination of Bovine Tuberculosis in Developing Countries

In Japan, the tuberculin skin test was introduced in 1948, and the test and slaughter strategy was applied, followed by an annual examination. Consequently, bovine TB prevalence dropped quickly and the disease was nearly eliminated from cattle in Japan (191). Several developed countries were able to eliminate bovine TB using the test and slaughter strategy, and the success of this strategy was supported by the absence of a wildlife reservoir (192). Switzerland is one of the success stories of bovine TB eradication, where the test and slaughter strategy was applied for 10 years followed by 20 years of surveillance campaign (193). Australia also successfully controlled bovine TB using a mandatory test and slaughter strategy (103). Test and slaughter is the only control strategy that shows success for the control and elimination of bovine TB; however, this strategy remains unaffordable for developing countries, primarily because of the compensation needed for the slaughtered cattle (107).

In order to control a particular disease, a clear understanding of the biology and epidemiology of the causal agent is an important starting point that allows for the identification of all the realistic intervention points and the design of control strategies that are in line with the economic situation of the country. The implementation of a

control strategy should be done in a way that allows progressive adjustments. Epidemiological surveillance procedures and tools should be used to monitor the progress of the control strategy and adjust it if necessary (194). According to Morris (2015), the most problematic point in dealing with the control of a disease is the fact that the previously explained points are marginalized. Disease control could be achieved in a more efficient way by integrating suitable management tools by the appropriate stakeholders (195). Bovine TB control should be motivated by both the public health implications of *M. bovis* and the economic losses triggered (160). Transdisciplinary research using participatory stakeholder involvement could be used in order to contribute to the control of bovine TB from developing countries, like Morocco, where there is very little or almost no dialogue between the different stakeholders (veterinarians, medical doctors, decision makers and farmers).

The importance of an OH-integrated approach including livestock, wildlife and public health sectors was identified as a key element in bovine TB control in Ethiopia (179). In developing countries, the control of bovine TB must begin with many transdisciplinary workshops in order to set a dialogue between the different stakeholders, as well as create a trust environment between these sectors. In this process, farmers and decision makers will be informed by the scientists about the economic losses caused by bovine TB and about the different ways or actions that could be undertaken in order to control this disease. The needs of all the stakeholders involved in controlling bovine TB should be considered, as this will ensure their engagement in the application of control strategies and contribute to its sustainability.

In developing countries, bovine TB control strategy should be focused on many levels. Good management of the resources that will be involved in the campaign is required, in addition to training the teams that will participate in the intervention. In parallel, an awareness campaign should be launched in order to make the local population aware of the effectiveness of the control strategy and its positive effect on the long term. Sustainability of the control strategy is essential for the success in controlling of bovine TB and its elimination in the long term. The integration of all the stakeholders in all the processes from the formulation to the application of the control strategy and including the monitoring is crucial to ensure the achievement of the interventions.

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III. Research rationale

1. Goals

One health approach has been increasingly popular in the past years, and showed positive results regarding zoonosis control. In Morocco, OH has been introduced first in 2009 with the launching of the European Union project ICONZ (Integrated Control Of Neglected Zoonosis). ICONZ project was dealing with dog transmitted zoonosis and bacterial zoonosis. One of the bacterial zoonosis is Bovine tuberculosis; this chronic disease is endemic in Morocco.

M. bovis, the causative agent of BTB could be transmitted to humans, as reported so far, *M. bovis* is responsible for a respectively small proportion of human TB cases; Muller et al reported a proportion of 3.1 % of *M. bovis* among human TB cases in Africa. However, in many developing countries, where BTB is highly prevalent, there is no estimation yet of the proportion of *M. bovis* among human TB patients.

Actually, in Morocco the human burden of *M. bovis* is not yet known, in the absence of any collaboration between the human and animal health services, this data is difficult to investigate.

The application of OH approach for bovine tuberculosis is a long term process, as BTB is currently not a priority for veterinary services in Morocco. In addition the burden of *M. bovis* on human health is not fully recognized by the human TB experts in Morocco. Trans-disciplinary process could be an efficient tool in bringing all the stakeholders together in order to discuss and design adapted solutions BTB problem in Morocco.

The goal of the present thesis is to contribute on the understanding of bovine tuberculosis problem in Morocco, to identify the key points to consider for a future BTB control strategy. In addition to provide advocacy elements, this could be used to set up stakeholders meetings between human and animal health sectors.

2. Objectives

- Investigation of the prevalence of bovine tuberculosis in Sidi Kacem area in Morocco, and analyze the respective risk factors
- Molecular characterization the strains responsible of bovine tuberculosis in two Moroccan slaughterhouses.
- Realization a mathematical model of BTB transmission from cattle to cattle to human in Morocco
- Realization of a demographic model for cattle population in population
- Assessment of the societal cost of BTB for the human and the animal health sectors.

IV. The prevalence of ruminant brucellosis and bovine tuberculosis in Sidi Kacem area in Morocco

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Abstract

Bovine tuberculosis (BTB) and brucellosis are major endemic zoonoses in ruminants in Morocco that impact on both human and human health. This study presents an assessment of the epidemiological and socioeconomic burden of bacterial zoonoses in Sidi Kacem Province in Northern Morocco from a cross- sectional survey of 125 cattle and/or small ruminant-owning households. In total, 1082 sheep and goats were examined from 81 households. The single intradermal comparative cervical test to screen for bovine tuberculosis was undertaken on 1194 cattle from 123 households and all cattle were blood sampled. Cattle and small ruminant blood samples were tested for brucellosis using the standard Rose Bengal Test (sRBT). Bacteriology was performed on 21 milk samples obtained from cattle that were seropositive for brucellosis for isolation and phenotyping of circulating *Brucella* strains.

Individual and herd prevalence for BTB in cattle of 20.4% (95% CI [18%-23%]) and 57.7% ([95% CI [48%-66%]) respectively were observed in this study. The prevalence of Brucellosis in cattle, at individual and herd level in cattle was 1.9% (95% CI [1.2%-2.8%]) and 9% (95% CI [4.5%-1.5%]) respectively. *Brucella* pathogens were isolated from three cattle milk samples and were identified as *Brucella abortus* using Bruceladder ® multiplex PCR and *Brucella abortus* biovar 1 from application of classical phenotyping. All small ruminants were seronegative to sRBT, two were positive to mRBT..

A higher risk of BTB and brucellosis was observed in cattle in intensive livestock systems, in imported and crossed breeds and in animals from larger herds (>15). The three risk factors were usually present in the same herds, leading to higher transmission risk and persistence of both zoonoses.

These results highlight the importance of implementing control strategies for both BTB and brucellosis to reduce productivity losses in ruminants and reduce the risk of transmission to humans. Prioritising control for BTB and brucellosis in intensive livestock production systems is essential for human and animal health.

Keywords: Morocco, Bovine tuberculosis, Brucellosis, Ruminants, Prevalence, Risk factors.

Introduction

Bovine tuberculosis (BTB) and brucellosis are bacterial zoonoses that are endemic in ruminant cattle and small ruminants (for brucellosis) populations in Morocco. These diseases are prioritized in Moroccan veterinary legislation (1,2), but remain poorly controlled. Infectious diseases impose a heavy financial burden on the livestock sector (3) and zoonoses have a dual impact through human disease burden and productivity losses of livestock, on which rural families depend for their livelihoods (4). While Brucellosis and BTB have been controlled and/or eliminated in many developed countries (5,6), in developing nations, these diseases are neglected (7) with WHO considering control of zoonotic TB to be a major priority (8). Rapid growth and intensification of livestock systems is expected to result in an increase in prevalence of both brucellosis and BTB (19).

The infectious agent of Bovine tuberculosis (BTB) is *Mycobacterium bovis*, a member of the *Mycobacterium tuberculosis* complex. Despite a host preference for cattle (9), *M. bovis* can infect a wide range of domestic and wild animals (10,11). Cattle to cattle transmission occurs via direct contact (aerosols) and depends on a number of factors including the number of bacilli excreted and herd density (12). Transmission of BTB to humans is mainly through consumption of infected raw milk, although direct transmission can occur (13). In Morocco BTB is still highly prevalent. A national tuberculosis survey in cattle in 2004 using the single intradermal tuberculin test showed individual and herd prevalence of 18% (n= 13,021) and 33% (n=2263) respectively (14). BTB is responsible for meat losses due to carcass condemnation and causes a decrease in herd productivity and milk yields (15).

In Morocco, Government BTB control initiatives include tuberculin testing and the slaughtering of any positive animals (reactors). At national level, the mandatory BTB control strategy is for test and slaughter but legislation is poorly enforced and no programme of systematic BTB screening of cattle is in place (2,16).

Brucellosis is caused by gram-negative bacteria of the genus *Brucella* which includes eleven species (17). *B. melitensis* and *B. abortus* are the most economically important for Morocco and cause disease in small ruminants and cattle respectively (18). In West Africa *B. abortus* infection in small ruminants is noted to occur in areas where the animals are in contact with cattle and where *B. melitensis* is absent (19).

Brucellosis is spread through contact with abortion products and vaginal fluids, from milk feeding or as a venereal disease. Contact with infected animals and consumption of raw dairy products is the most common source of transmission (20). Brucellosis causes economic losses in livestock due to abortions and prolonged calving to conception intervals.

National epidemiological surveys for brucellosis in Morocco in 1996 and 2010 showed that bovine brucellosis is more prevalent in the north-west coastal and central zones where the cattle density is the highest, with a mean individual and herd prevalence of 2.1 % (n=8991) and 4.9 % (n=1168) respectively (Government survey, 2010). Herd seroprevalences have remained at a similar level to those reported in 1977 (4.6%) and in 1988 (4.9%) (19).

Initiatives to control brucellosis in Morocco have had varied success. A national vaccination campaign using S19 vaccine that ran from 1989 to 1994 showed little impact on herd prevalence (21)

By contrast, a public-private initiative (2007) to roll out RB51 and/or S19 vaccination to farms that are members of professional associations or cooperatives reduced brucellosis prevalence from 40% to 0.4% in member farms (22). In total 81230 cattle were serologically tested for brucellosis, 55869 were vaccinated and 2901 were culled at a cost of $US 2.6 million. A large bacterio(23–25)logical study comprising 500 samples from 357 cattle isolated *B. abortus* biovar 1 and 3 in the 1980s (26).

Brucellosis in small ruminants is a recognized problem across the northern Mediterranean zone of Morocco and in inland mountainous areas where sheep and goat populations dominate. In the mid-1990s, small ruminant brucellosis emerged in in the Oriental region where Morocco shares a border with Algeria and prompted a program of mass vaccination with Rev 1 (FULL NAME) via the conjunctival route of small ruminants in the zone between 1997 and 2003. A premature closure of the vaccination campaign resulted in disease re-emergence and between 2009 and 2013, 48 individual case reports of small ruminant brucellosis were reported in the Northeastern region. A National survey in 2006 testing 11,609 small ruminants, yielded 8 sero-positives, 5 of which from the Oriental region (19). Bacteriological evidence of brucellosis in small ruminants in Morocco is limited to only three studies reporting isolation of *B. melitensis* biovar 3 (23–25).

This study aimed to estimate the prevalence of bovine tuberculosis in cattle and the seroprevalence of brucellosis in cattle, sheep and goats; to identify *Brucella* strains circulating in Sidi Kacem province and to assess BTB and brucellosis risk factors in this epidemiological context.

Material and methods

Study area

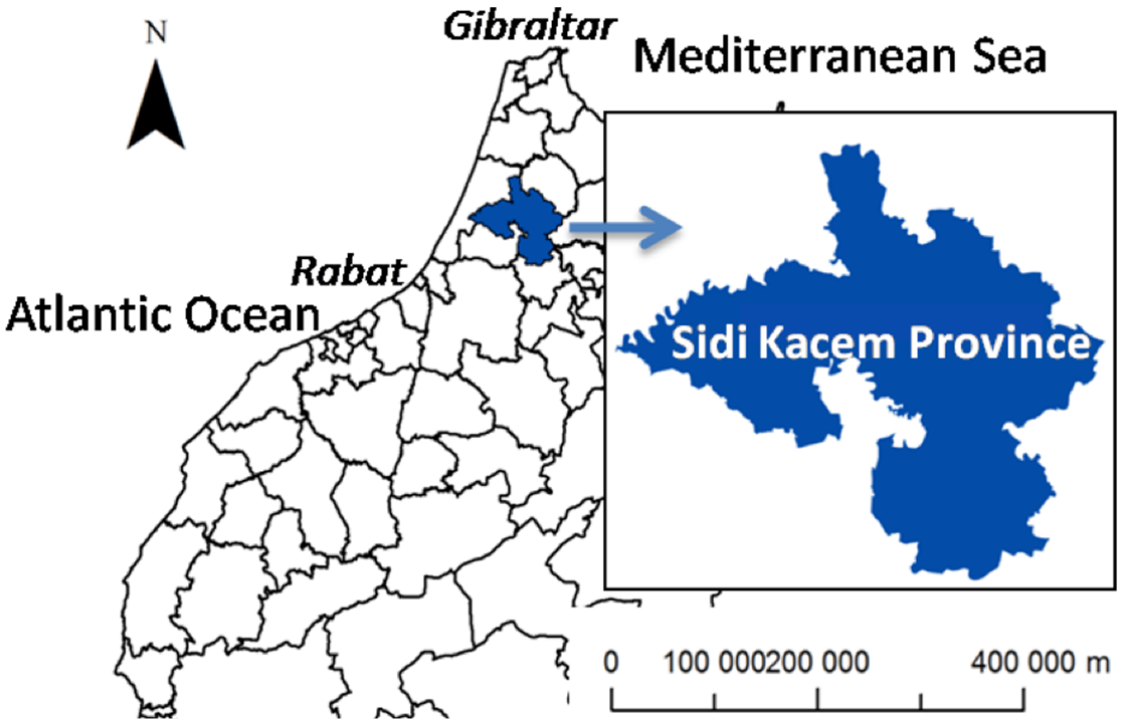

Figure IV.1. Location of Sidi Kacem, North West Morocco (27)

Sidi Kacem province (figure 4.1) comprises 29 communes, and is sub-divided into two agro-hydrologic zones: “rain-fed” (bour) in the northeast and “irrigated” in the southwest (figure 4.2). The irrigated zone is characterized by low-lying plains, in contrast to the rain-fed zone, which is mountainous (altitude from 150 to 500 m). The number of cattle in the rain-fed zone is 40% of the total number in the province with an average herd size of 5 cattle; by contrast, average herd size in the irrigated region is 15 cattle (28). The irrigated zone is dominated by an intensive mode of livestock rearing characterized by a larger herd size and European dairy breeds (Holstein-Friesian, Montbeliarde, Tarentaise) or cross-bred cattle. The livestock production

system in the rainfed zone is more extensive, with dominance of local cattle breeds and smaller herd size.

Sampling

Sample size was determined using standard formulae for cluster surveys(29). assuming the following parameters, i) a BTB prevalence of 18% (national survey 2004 (14)), ii) a mean number of cattle per household of 9, iii) an average number of 2 households selected per cluster and iv) an intra-class correlation coefficient of 0.2 (30). An average of 62 villages (douars) and 124 households are required to estimate the prevalence with a precision (defined as one half-length of the 95% CI) of 5%-points. Clusters were randomly selected based on the official village lists available for Sidi Kacem province. Two households per village were randomly selected based on livestock-owning households provided by the chief of the commune upon arrival in each village; all cattle in cattle owning households were sampled. As small ruminant flock numbers were high, not all sheep and goats were sampled in small-ruminant owning households; a minimum of 20 animals were sampled for households that owned more than 20 sheep or goats.

Herd and animal level data

At herd level, GPS coordinates (Figure 4.2), livestock production system, grazing system and herd size were recorded.

Most sampled households owned cattle, in contrast to small ruminants. Table 4.1 shows the number of households and the species present within the household. For every animal, age, gender, breed and body condition score (BCS) was recorded.

Table IV.1. Number of sampled households in terms of present species

Species owned by household	**Number of households**
Cattle only	44
Sheep only	2
Goats only	0
Cattle sheep and goats	4
Cattle and sheep	75
Cattle and goats	0
Sheep and goats	0
TOTAL	125

Diagnosis of bovine tuberculosis.

The single intradermal comparative cervical skin test (SICCT) was used following OIE terrestrial manual standards for BTB screening. Briefly, injection sites were clipped and cleaned; a fold of skin within each clipped area was measured with calipers and 0.2 ml each of bovine and avian PPD with 25'000 IU/ml was injected in the left neck region. Injections were performed using a separate intradermal gun for each PPD in two spots separated by approximately 15 cm. The skin-fold thickness at each injection site was re-measured 72 hours after injection by the same individual. Any exudate, edema and pain observed in the injection sites were recorded. The SICTT was interpreted using the OIE recommended cut off and an animal was considered positive if the increase in skin thickness in the Bovine PPD injection site was superior to the skin thickness in the Avian PPD injection site by at a minimum of 4 mm. The reaction was considered inconclusive if the difference was between 1 and 4 mm and if the increase in the skin thickness was the same in the bovine PPD and avian PPD injection sites, the reaction was considered negative (31).

Brucellosis sero-diagnosis and bacteriology

Blood from 1194 cattle and 1082 small ruminants across 125 households was collected (Figure 2) and all sera were stored in cool boxes before processing. After 24h storage at 4°C to allow serum separation, the tubes were centrifuged and sera extracted and aliquoted. One aliquot was used for immediate screening of both cattle and small ruminant sera using the modified Rose Bengal Test (mRBT), 75 µl serum and 25 µl antigen.

The initial screening aimed to collect milk samples from seropositive lactating females for bacteriological investigations, and using mRBT increased the diagnostic sensitivity thereby maximising the number of milk samples collected. A second aliquot was stored at -18 C and sent to the Instituto de Salud Tropical y Depto. Microbiología y Parasitología, Universidad de Navarra (UNAV) for screening. Cattle samples were screened using sRBT. Small ruminant samples were screened using sRBT and mRBT in parallel.

The antigen used in RBT was a suspension of fully smooth *B. abortus* 1119 standardized according to international guidelines (31) and controlled for quality using a panel of brucellosis positive and negative serum samples (32).

Bacteriology was undertaken on milk samples obtained from 21 seropositive cattle (three CITA and three Farrell's selective media plates for each sample). After incubation for 4-7 days 37ºC (5-10% CO2 atmosphere), suspicious colonies were re-plated on the same media for preliminary identification. Isolates were considered presumptive for *brucella* by means of the staphylococcus co-agglutination, oxidase and urease tests (33). Colonies found to be suspicious based on morphological evaluation were stored at -20ºC in vials of Triptic Soy Broth (TSB) with 5%DMSO for further typing at the WHO and OIE collaborating centres for the diagnosis of animal and human brucellosis at the University of Navarra (UNAV) and Centro de Investigación y Tecnología Agroalimentaria de Aragón (CITA) in Spain.

Isolates were confirmed as Brucella species and typed at species level using Bruceladder ® multiplex PCR (34) and, at biovar level, using classical phenotyping methods (33), i.e. oxidase and urease tests, CO2 requirement, agglutination with monospecific anti-A / anti-M sera, lysis with phages (Tb, Wb, Iz1 and R/C), sensitivity to dyes (fucsine, thionin and saphranine) and Crystal Violet exclusion test (to assess absence of dissociation).

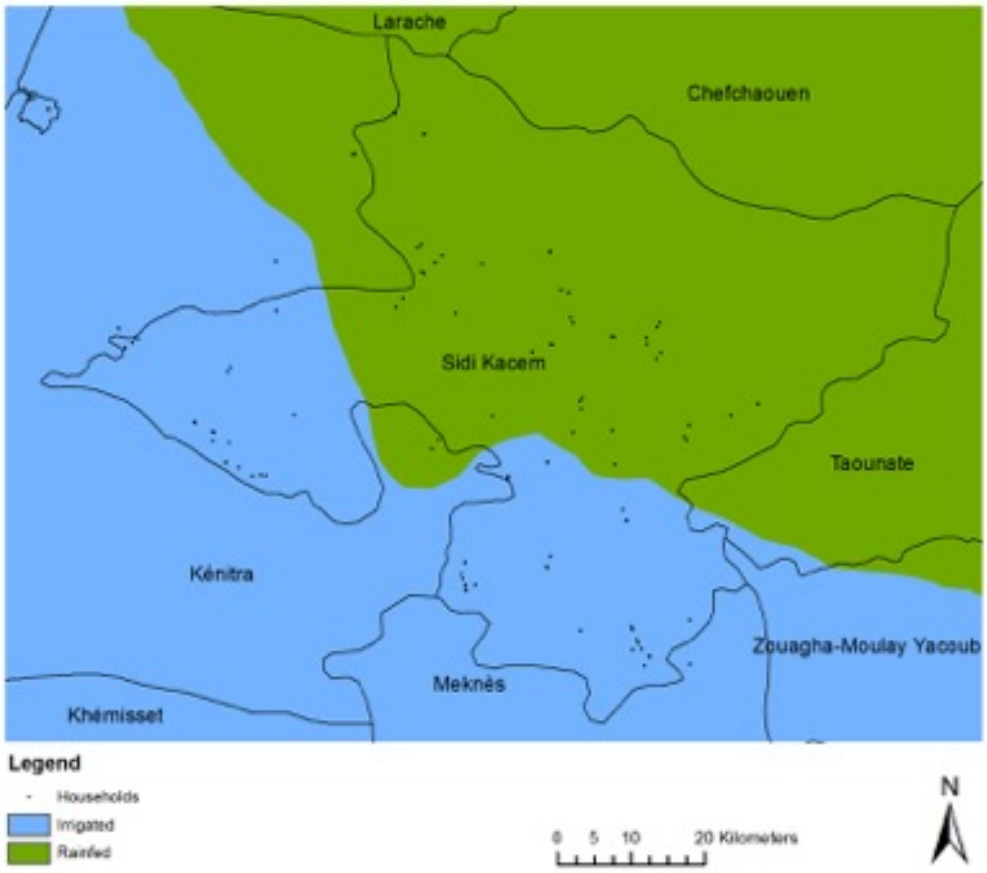

Figure IV.2. Sidi Kacem rainfed and the irrigated regions and geo-localisation of the households screened

Statistical analyses

Data was entered in Access 2012 and analysed using Generalized Estimating Equation models in Stata 12.1, to account for clustering at household level. Prevalence and individual and herd risk factors for BTB and brucellosis were investigated.

Using diagnostic sensitivity (51,1%) and specificity (98.9%) values (previously reported by Müller et al. from Chadian cattle using SICTT (42)) and apparent prevalence estimated in our study, the true prevalence of BTB was calculated using the formula proposed by Morgan et al. (35): {True prevalence= (Apparent prevalence+sp-1)/(sp+se-1)}. Given the high sensitivity and specificity of sRBT apparent prevalence is close to true prevalence (24, 28).

Analysis showed that most of the within-cluster correlation occurred at household rather than at village level and so household was selected as the cluster level for analysis. Sensitivity analysis used villages as clusters.

Results

Cattle characteristics

In total, 1201 bovines from 125 farms in 62 villages were examined of which 78.4% (n = 938) were female, 85% were crossbreeds (n = 1016). Almost all of the cattle (96%, n = 1150) had a BCS equal to or less than 3. Most cattle (78%) (n = 936) were over 12 months. Cattle were almost equally distributed between the rain fed and the irrigated area, most from semi intensive herds (82.8%, n = 994) and only a few (n = 20) from extensive farming systems. Most herd sizes were below 15 (Table 4.2).

Table IV.2. Basic characteristics of 1201 cattle sampled in Sidi Kacem, Morroco.

Characteristics	Classes	N (%)
Herd level		
Livestock production system	Intensive	15 (12.2)
	Semi-intensive	105 (85.4)
	Extensive	3 (2.4)
Grazing system	Irrigated	67 (53.6)
	Rainfed	58 (46.4)
Herd size	1-15	92 (74.8)
	>15	31 (25.2)
Animal level		
Livestock Production System	Intensive	187 (15.6)
	Semi-intensive	994 (82.8)
	Extensive	20 (1.6)
Grazing system	Rainfed	596 (49.6)
	Irrigated	605 (50.4)
Age	0-12	265 (22.1)
	13-36	397 (33.1)
	>36	539 (44.8)
Sex	Male	258 (21.6)
	Female	938 (78.4)
Breed	Crossed	1016 (84.9)
	Imported	100 (8.4)
	Local	81 (6.7)
Body Condition score	1-2 (including 2)	366 (30.5)
	2-3 (including 3)	784 (65.3)
	3-4	15 (1.3)

Prevalence for BTB

Tuberculin skin test results were derived for 1194 cattle, 107 animals were found inconclusive for BTB using the OIE interpretation criteria of the SICCT and were not included in the analysis. An individual apparent BTB prevalence of 20.4% (95% CI 18%-23%) and a herd prevalence of 57.7% (95% CI 48%-67%) were found. Considering a sensitivity of 51% and specificity of 99% as reported in Müller B et al (37), a true individual BTB prevalence of 38.2% was estimated.

Brucellosis results using sRBT diagnostic protocols were available for 1179 cattle. Prevalence of bovine brucellosis was 1.9% (95% CI 1%-3%) at individual level and 9% (95% CI 5%-15%) at herd level. None of the 1044 sheep or 51 goats screened was sRBT positive and only 2/1044 sheep were mRBT positive.

Risk factors analysis

a. Bovine tuberculosis

The univariate and multivariate analysis of BTB risk factors are shown in Table 4.3. Local breeds were observed to have a lower risk of BTB compared to crossbreds (9% vs 21%, OR: 0.5, 95% CI 0.2-1.3). Male animals showed a significantly higher risk of BTB compared to female animals (12% vs 22.7%, OR: 0.3, 95% CI 0.4-4.8). Age of 12-36 months and higher than 36 months was associated with a significantly higher risk of BTB compared to a lower age (14.4% and 28.2% vs 13.7% OR:1.4, 95% CI 0.8-2.5, and 3.9, 95% CI 2.3-6.6). BCS higher than 2 showed a significantly lower risk of BTB compared to lower BCS score (16.7% and 15.4% vs 29.5%, OR: 0.6, 95% CI 0.4-0.8).

At herd level, semi-Intensive and extensive livestock production system showed a lower risk of BTB than intensive rearing (18.1% and 5.3% vs 34.7% OR: 0.4, 95% CI 0.1-0.9). Irrigated grazing systems showed a significantly higher risk of BTB compared with rain fed systems (30.1% vs 11%, OR: 3.5, 95% CI 1.8-6.5). Large herd size showed a higher risk of BTB than small to medium herd size (26.2% vs 15.7%, OR: 2, 95% CI 0.9-4.1).

Table IV.3. Individual and herd risk factors of BTB in 1087 cattle

	Risk factor	N. Screened	% (N. Pos)	OR (95% CI)	P value	mOR (95% CI)
Individual risk factors						
Breed	**Crossed**	918	21.7 (199)	Ref	--	--
	Imported	96	16.7 (16)	0.3 (0.1-0.7)	0.005	0.4 (0.1-0.8)
	Local	73	9.6 (7)	0.5 (0.2-1.3)	0.14	0.5 (0.2-1.4)
Age	**0-12**	241	13.7 (33)	Ref	--	--
	13-36	367	14.4 (33)	1.4 (0.8-2.5)	0.3	1.2 (0.7-2.3)
	>36	475	28.2 (134)	3.9 (2.3-6.6)	<0.0001	2.6 (1.4-4.8)
Sexe	**F**	845	22.7 (192)	Ref	--	--
	M	241	12.0 (29)	0.3 (0.2-0.6)	<0.0001	0.8 (0.4-1.4)
BCS	**1-2 (including 2)**	325	29.5 (96)	Ref	--	--
	2-4	732	16.7 (122)	0.6 (0.4-0.8)	0.003	0. 8 (0.5-1.3)
Herd risk factors						
Livestock production	**Intensive**	170	34.7 (59)	Ref	--	--
	Semi-intensive & extensive	922	17.8 (164)	0.4 (0.1-0.9)	0.023	0.5 (0.2-1.4)
Grazing system	**Rain fed**	550	11.0 (60)	Ref	--	--
	Irrigated	544	30.1 (164)	3.5 (1.8-6.5)	<0.0001	3.1 (1.6-6.2)
Herd size	**0-15 animals**	600	15.7 (94)	Ref	--	--
	>15 animals	492	26.2 (129)	2.0 (0.9-4.1)	0.06	2.1 (1.0-4.3)

mOR: multivariate analysis OR
ref: Reference category

b. Brucellosis

Univariate and multivariate analysis of risk factors for brucellosis are shown in Table 4.4. Imported and indigenous breed animals, in addition to male sex categories, were negative for RBT leading to perfect prediction. A higher risk of brucellosis was associated with an age greater than 36 months compared with ages lower than 36 months (3.8% vs 0.4%, OR: 6.9, 95%CI 0.8-60.4). Having a low body condition score of 2 to 3 showed a significant lower risk of BTB compared to BCS lower than 2 (0.7% vs 4.7%, OR: 0.2 (95% CI 0.05-0.7).

Semi-intensive production systems showed a lower risk for bovine brucellosis compared with intensive rearing (1% vs 6.9%, OR: 0.3, 95% CI 0.05-2.02). Irrigated grazing showed a significantly higher risk of bovine brucellosis than rain fed grazing systems (3.2% vs 0.5%, OR: 4.7, 95% CI 0.8-26.7). Large herd sizes showed a higher risk of BTB than small to medium herds (3.16% vs 0.77%, OR: 2.8, 95% CI 0.5-15.1) (Table 4).

Table IV.4. Individual and herd risk factors of bovine brucellosis in 1177 cattle

	Risk factor	N. Screened	%Pos (n)	OR (95% CI)	P value	mOR (95% CI)
Individual risk factors						
Breed	**Crossed**	997	2.2 (22)	Ref	--	--
	Imported	100	0.0 (0)	Nd[a]	--	--
	Local	80	0.0 (0)	Nd[a]	--	--
Age	**0-12**	257	0.4 (1)	Ref	--	--
	13-36	388	0.3 (1)	0.5 (0.02-8.5)	0.6	0.4 (0.02-7.41)
	>36	527	3.8 (20)	6.9 (0.8-60.4)	0.08	5.1 (0.6-45.6)
Sexe	**F**	928	2.4 (22)	Ref	--	--
	M	248	0.0 (0)	Nd[a]	--	--
BCS	**1-2 (including 2)**	363	4.7 (17)	Ref	--	--
	2-4	783	0.6 (5)	0.2 (0.05-0.7)	0.01	0.5 (0.1-1.8)
Herd risk factors						
Livestock production system	**Intensive**	187	6.9 (13)	Ref	--	--
	Semi intensive & extensive	994	1 (9)	0.3 (0.05-2.02)	0.2	0.6 (0.1-4.3)
Grazing system	**Rain fed**	595	0.5 (3)	Ref	--	--
	Irrigated	586	3.2 (19)	4.7 (0.8-26.7)	0.083	3.4 (0.6-21.0)
Herd size	**Small to medium 0-15**	643	0.77 (5)	Ref	--	--
	Large (>15)	538	3.16 (17)	2.8 (0.5-15.1)	0.2	2.3 (0.5-11.9)

[a]: Not determined because of perfect prediction
mOR: multivariate analysis OR

Brucellosis molecular characterization

Three *Brucella* strains isolated from female cattle were confirmed as *Brucella abortus* biovar 1 through classical typing. Isolates were from cows belonging to the same herd, from the irrigated zone. The cows were over 72 months old; two had a history of abortions and BCS of less than 2. The BTB herd prevalence was 57% (12/29).

Discussion

The overall BTB individual apparent prevalence in this study was 20.4%, similar to that reported in 2004 (18%) during national tuberculin skin testing. However, given the reported low sensitivity of BTB testing these estimates are likely to be underestimates. The BTB herd prevalence in this study of 57.7% is higher than that previously reported at 33% in 2004 at national level (14). The 2004 survey applied the single intradermal tuberculin skin test (SITT), which is less specific than the SICTT used in the present study.

The BTB prevalence in the present study is far higher than that reported in Uganda (6%) (38), Niger (3.6%) (39), rural Ethiopia (5.5%) (40), and Tanzania (2.4%) (41). The high prevelance in Morocco may be explained by more intensive dairy cattle farming practices. BTB prevalence in Morocco (20.4%) was lower than that reported in Zambia (49.8%) and Mozambique (39.6%) (42,43).

The tuberculin skin test is the recommended OIE test for BTB diagnosis. While the single tuberculin skin test (SITT) may result in a high number of false positives, the comparative tuberculin skin test (SICTT) was shown to reduce false positives and cross reactions (i.e. the single test has a higher diagnostic sensitivity and the comparative test is more specific) (31). In Morocco, legislation stipulates SITT as the recommended tuberculin skin test; the SICTT is used as a confirmatory test when the SITT is positive (16).

Individual apparent brucellosis prevalence found in this work (1.9%) is near the 2.1% reported for the national survey of 2010-2011. National statistics from 1982 to 1992 registered a mean herd prevalence ranging from 2.1% to 4.9%, which is lower than brucellosis herd prevalence (9%) found in the present study (23). Individual brucellosis prevalence found in Sidi Kacem was also lower than those found in Egypt (23.8%) (44), Uganda (5%) (45), Nigeria in 2011 (4.04%) (46) and in Ethiopia in 2006 (2.9%) (47).

In this study only two sheep from two different herds in the irrigated area showed a positive mRBT result, and regrettably slaughter for collection of necropsy samples for bacteriological confirmation could not be performed. The mRBT positive sheep were from a herd found that had had several cases of brucellosis in cattle. Livestock from this village graze on shared pasture and the sheep may have developed

seropositivity due to contact with *B. abortus* infected cattle (as previously reported in Nigeria (12)). These sheep could have developed antibodies but cleared the infection and hence be of negative infection status or alternatively be infected and a source of contagion to other ruminants or humans.

Meta-analysis of diagnostic tests for bovine brucellosis (48) shows that the standard RBT (sRBT) performed with diagnostic sensitivity and specificity values of 98.1% (96.8-99.1%, 95% CI) and 99.8% (99.7-99.8%, 95% CI), respectively, where vaccination is not practiced (as in this study). The modified RBT protocol was used for serological analysis of small ruminant sera in order to optimise sensitivity whilst maintaining specificity in the absence of vaccination (34, 54). In addition, mRBT has been shown to be useful for reducing the cost and the time of brucellosis screening in *B. melitensis* eradication campaigns implemented in Portugal (50). As Morocco is a country where no brucellosis vaccination is currently practiced (thus no interference due to vaccine-induced antibodies), RBT can be recommended for serological surveys in cattle and small ruminants respectively.

Indirect and competitive ELISA tests both show similar sensitivity and specificity to RBT in absence of vaccination (31), but are more expensive (48) and need to be validated in the target population since their diagnostic performance depends on the manufacturer and the selection of an adequate cut-off to discriminate positive and negative samples (51).

The gold standard for brucellosis diagnosis is isolation and identification of *Brucella spp*. Bacteriological culture is cumbersome, expensive and requires skill and facilities rarely available in resource-poor countries and consequently, indirect testing of anti-*brucella* antibodies in serum is commonly applied for brucellosis screening. There is no single serological test which can be applied in all settings (52). Tests most often used for sero-epidemiological surveys or to monitor disease once vaccination has been implemented include the rose Bengal test (RBT), serum agglutination test (SAT; not recommended by OIE for brucellosis testing for the purpose of international trade due to its low sensitivity), and the complement fixation test (CFT - used as a confirmatory test when vaccination is practiced but technically cumbersome). Precipitation tests with Native Hapten and related polysaccharides show good diagnostic specificity even in the context of vaccination, making them useful for eradication programs and situations when infected and vaccinated animals coexist

(53); they are not commercially available making their use outside of research projects difficult.

This study shows that intensive livestock production systems carry a significantly higher risk of BTB and bovine brucellosis. Intensive systems have previously been shown to be linked to elevated prevalence of BTB and bovine brucellosis (47,48,49). Intensive dairy farming conditions within a confined environment with less access to sun, air flow and high humidity conditions may enhance transmission of *Brucella* and *Mycobacteria* between animals. The higher stocking density, larger herd sizes, and propensity to buy in animals together with higher calving frequency in intensive systems may also increase the risk of disease transmission.

Extensive systems, where animals are grazed in lower density on communal pasture, may have a lower risk of transmission because of the effect on the sun and heat on *Brucella and Mycobacteria* environmental contaminants.

Brucellosis and BTB persist in both extensive and intensive livestock farming systems. In pastoralist rangelands, while the proportion of infected flocks or herds may be high, brucellosis may persist unidentified and/or unreported. Livestock intensification generates abortion storms and large productivity losses. The shift to a more intensive livestock production observed in rapidly urbanizing developing countries such as Morocco, could lead to an increase of zoonotic diseases transmission in the absence of animal recording systems, movements control and strong veterinary services management.

Indigenous animals showed a lower risk of BTB, as has been previously reported (43), compared with imported and crossed breed animals. This may be explained by their non-adaptation to local conditions. Local indigenous breeds may not be a protective factor, however, because in Morocco extensive herds are dominated by local and cross bred cattle, while intensive herds are dominated by imported breeds. Consequently, the breed could be a confounding factor for the production system (18).

Older animals showed a higher risk of BTB and bovine brucellosis as described in other studies (57). All positive brucellosis cases were found to be females but the

sample was composed predominantly of female cattle. In Nigeria, females were described to be more susceptible to *B. abortus* infection (58).

In the present study, a low BCS showed a significantly higher risk of BTB. BCS has previously been linked with BTB infection (59,60), although recent studies in Ireland and Tanzania showed no evidence of the association of low BCS and high BTB prevalence (61,62). Low BCS could be linked to clinically advanced BTB (63) but in this study the initial status of the sampled animals was unknown.

The *Brucella* strains isolated from the three Friesian/Holstein cows belonging to the same herd in the irrigated zone were found to be *B. abortus* biovar 1. Phylogenetic studies (not described here) showed these strains have some homogeneity with Spanish strains. This is in line with a study conducted in the mid-1980s yielding 8 *B. abortus* biovar 1 and 28 *B. abortus* biovar 3 strains from 500 samples (26,64). A subsample of these Moroccan strains was examined as part of a study characterising *B. abortus* strains of African origin and found the 12 Moroccan strains to be identical to *B. abortus* biotypes isolated in Europe.

The present study demonstrates that there is added value in investigating multiple zoonoses simultaneously, especially for zoonoses with a reservoir overlap. Undertaking brucellosis and BTB screening in parallel and in multiple hosts is logistically and technically feasible. The added value of an integrated approach to epidemiological investigations on zoonoses has been demonstrated in Chad (65). Morocco could consider a parallel elimination campaign for BTB and brucellosis that optimizes use of human and economic resources.

The present study concluded that bovine tuberculosis and brucellosis are prevalent in Sidi Kacem. Considering the economic losses caused by BTB and brucellosis, in addition to their public health impact, additional efforts should be deployed to design an integrated control strategy. Test and slaughter has been shown to be the most efficient elimination strategy for BTB in several countries. Many factors contribute to the success of a control and elimination campaign. Trust between all the stakeholders especially the industry, the governmental structures and the farmers is a very important component which contributed to the success of brucellosis and BTB control program. Correct application of livestock biosafety measures, early diagnosis of the disease, and application of movement restrictions also affect the success of a

control campaign (66). In Australia, animal movement controls and abattoir surveillance were applied from early years of the control program (67).

In Morocco, relationships between livestock keepers, local authorities and veterinary services are characterized by mistrust. Solid and sustainable control cannot be achieved without the conviction and participation of all stakeholders. Sensitisation and education campaigns of all stakeholders are required to improve adherence to and acceptance of control programs by local populations and decision makers. Strong industry and government support in funding and policy development are important factors for the success of a zoonosis eradication campaign as shown in Australia. A rigorous application of the decided strategy and involvement of the animal owners are pivotal for the success of control and elimination programs (68).

Wildlife reservoirs can complicate control operations. The presence of a wildlife reservoir (e.g. badgers in Ireland) has caused reemergence of BTB in developed countries (69). Wildlife reservoirs have been shown to exist in Africa (feral baboons in Kenya (70) and warthog and buffalo in Uganda (71)). The role of a wildlife reservoir in BTB transmission in Morocco is unknown, and other barriers to control are considered critical including: lack of efficient organization of veterinary services; prioritization of other highly infectious viral and parasitic diseases; limited technical capacity and financial constraints (72).

The best strategy for controlling brucellosis in Morocco would be conjunctival mass vaccination (*B. abortus* S19 in female cattle and *B. melitensis* Rev 1 in male and female small ruminants) every two years just before mating or immediately after calving/lambing/kidding. This is the best option as prevalence is high and the veterinary services are not able to apply individual tagging allowing vaccination of young replacements only (73). However, vaccination needs to be sustained over time to be effective. Premature removal of S19 or Rev1, or replacement with a less effective vaccine e.g. RB51, has led to failure of previous attempts for control in Morocco (19).

Mass vaccination has been effective for brucellosis control in other countries e,g, Spain, which has been able to decrease the prevalence of brucellosis in ruminants. However, vaccination should be associated with adequate organisation of veterinary services, strict control of animal movements and adequate economic compensation

to affected farmers (29). Veterinary services could fall back on private veterinarians to perform BTB testing, as for the vaccination campaigns. Stakeholder engagement is key to success (74).

A BTB transition model for Morocco indicated that BTB could be controlled within 25 years, if 50% of cattle were tested annually, and infected animals were slaughtered at an estimated cost of 1.55 billion Euros (75). Taking into consideration the current infectious diseases prioritized by Morocco (e.g: foot and mouth disease, sheep pox virus and Peste des Petits Ruminants (PPR)), such a control strategy is currently deemed unaffordable for a middle income country.

As for all NTZs, evidence and advocacy is necessary to convince policy-makers and communities of the benefits of disease control (76,77). The evaluation of the cost of BTB should take into account the cost of the human disease, and for this purpose an investigation of the prevalence of *M. bovis* in humans is required, as well as a calculation of the direct and indirect cost of a human TB case. Cross-sectorial socio-economic analysis of the cost of both diseases is needed. In addition, support from private industry (e.g. the milk industry) could sustain BTB and brucellosis control campaigns, as they will also benefit from the control measures. Novel methods for innovative financing should be examined to mobilize investment for interventions that contribute towards elimination of NZDs (78,79) and benefit human and animal health.

Ethical clearance

The methodology of the study including the household questionnaire was reviewed and validated by international and national research expert partners within the ICONZ project. In addition, the Moroccan veterinary services (ONSSA) approved and granted authorization for the study. The purpose of the study was explained to the local authorities and to the farmers. The tests used for the screening of BTB and brucellosis are routinely used by the veterinary services in Morocco.

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Author contributions

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V. Molecular characterization of bovine tuberculosis strains in two slaughterhouses in Morocco

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Abstract

Background

Bovine tuberculosis (BTB) is caused by *Mycobacterium bovis*, which belongs to the *Mycobacterium tuberculosis* complex. *Mycobacterium bovis* have been described to be responsible of most cases of bovine tuberculosis. Although *M. tuberculosis*, *M. africanum* and non-complex mycobacteria were isolated from cattle.

In Morocco, so far, no molecular studies were conducted to characterize the strains responsible of BTB. The present study aims to characterize *M. bovis* in Morocco.

The present study was conducted in slaughterhouses in Rabat and El Jadida. Samples were collected from 327 slaughtered animals with visible lesions suggesting BTB.

Results

A total of 225 isolates yielded cultures, 95% (n= 215) of them were acid-fast (AF). Sixty eight per cent of the AF positive samples were confirmed as tuberculous mycobacteria (n=147), 99% of these (n=146) having RD9 and among the latter, 98% (n=143) positive while 2% (n=3) negative for RD4

A total of 134 samples were analyzed by spoligotyping of which 14 were in cluster and with 41 different spoligotypes, ten of them were new patterns (23%). The most prevalent spoligotypes were SB0121, SB0265, and SB0120, and were already identified in many other countries, such as Algeria, Spain, Tunisia, the United States and Argentina.

Conclusion

The shared borders between Algeria and Morocco, in addition to the previous importation of cattle from Europe and the US could explain the similarities found in M. bovis spoligotypes. On the other hand, the desert of Morocco could be considered as an efficient barrier preventing the introduction of BTB to Morocco from West Central and East Africa. Our findings suggest a low level endemic transmission of BTB similar to other African countries. However, more research is needed for further knowledge about the transmission patterns of BTB in Morocco.

Keywords: *Mycobacterium bovis*, Bovine tuberculosis, Morocco, Cattle, Slaughterhouse, Spoligotype, PCR.

Introduction

Bovine tuberculosis (BTB) is a chronic granulomatous caseous-necrotising inflammatory disease that mainly affects the lungs and their draining lymph nodes, it can also affect other organs, depending on the route of infection (1–3). BTB is caused by *Mycobacterium bovis*, which belongs to the *Mycobacterium tuberculosis* complex (MTBC). *M. bovis* has the particular ability to infect a wide range of host species other than cattle (livestock, wildlife and pets) and humans, (3,4), however, cattle remain the most important reservoir for *M. bovis* (5). There exist important wildlife reservoirs like the badger (*Meles meles*), the Possum (*Trichosurus vulpecula*) (4).

M. bovis can be transmitted between animals by inhalation of aerosols and the ingestion of contaminated food. The transmission is enhanced by many risk factors, mostly related to intensive livestock system (6). The transmission of *M. bovis* to humans occurs by consumption of unpasteurized infected raw milk and by the contact with infected cattle (7).

While BTB is still occurring in some developed countries in low prevalence (8–10) because of a wildlife reservoir (11) i.e. badgers in the UK (12), this zoonosis is endemic in many of the developing countries, who lack the financial resources to control this disease. Bovine tuberculosis is highly prevalent in many African countries (13,14), where it causes economic losses by its effects on animal health and productivity and by international trade restrictions. (15). Bovine tuberculosis is also a zoonosis and, consequently, considered as a public health issue (1,16,17).

In Morocco, the agriculture sector is of key importance to the economy, representing approximately 14% of total gross domestic product (GDP) (75bn MAD/ €6.6bn) and approximately 7% of exports (2009). Livestock represents 38% of the total agriculture sector GDP (18). Both extensive and intensive livestock production systems exist in Morocco, with local, crossbred and imported breeds, mostly Holstein. Local breeds have been shown to be more resistant to the disease (14,19). Bovine tuberculosis is an endemic zoonosis in Morocco. The last national survey based on skin tuberculin test was conducted in 2004 and showed an individual prevalence of 18% and a herd prevalence of 33% (20). Furthermore, a cross-sectional tuberculin study was conducted in the Sidi Kacem area in Morocco in 2012 showing an individual prevalence of 20.4% and a herd prevalence of 57.7% (19).

Officially, skin test and slaughter is the current control strategy applied in Morocco; however this strategy is not fully applied as it is not respected; in addition, there is no systematic BTB screening of cattle at a national level (21).

In a review published by Muller et al in 2013, the proportion of zoonotic human tuberculosis (TB) among all TB cases was estimated at 1.4 % for the non-African countries, and at 2.8 % in Africa (22). The highest prevalence were found to be 13.8 % and 7 % respectively in Mexico (23) and in Uganda (24). However, the World

Health Organization states a worldwide median prevalence of 3.1 % of *M. bovis* among human TB patients (25). Even if those prevalence values are mostly low, Muller et al, Pérez-Lago et al and Navarro, and García-de-Viedma highlight the major consequences of TB due to *M. bovis* on certain groups of the population, and report a potential underestimation of the prevalence of zoonotic human TB (22,25).

The phylogeny of MTBC showed recently that the strains found in animals belong to a single lineage which showed the deletion of the “Region of Difference” 9 (RD9) (22,26). Indeed, *M. bovis* is the most recent strain in his lineage showing the deletion of RD4 (27).

Molecular deletion typing had been found to be an important tool to differentiate *M. bovis* from the other strains of the MTBC (28). Pattern of presence or absence of these RDs would allow a discrimination among MTBC strains (28–30).

Currently there is no data available in Morocco about the molecular characterization of BTB and the prevalence of MTBC among slaughtered cattle. The aim of the present study was to characterize the strains of MTBC which are responsible for BTB among the slaughtered cattle in Morocco.

Materials and methods

Study area and sample collection

The study was conducted in two slaughterhouses in Morocco, one in Rabat and another in EL Jadida, which are two coastal cities separated by 200 km. The cattle slaughtered in these two slaughterhouses come from the rural areas surrounding the two cities and also from many other areas of the country (Figure 5.1). Individual information of every animal, such as gender, age, breed, and the possible origin were recorded in our database, as well as the date of sampling. The sample collection was performed in Rabat from March to July 2015 and in El Jadida from June 2014 to April 2015.

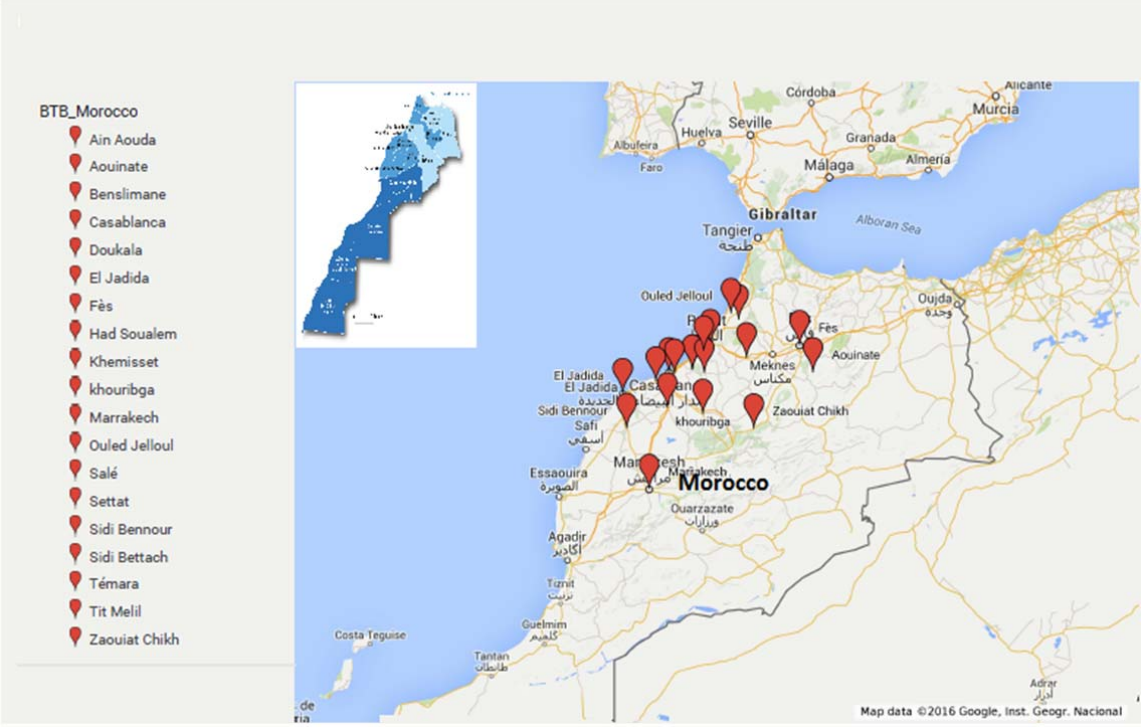

Figure V.1. Geographic distribution of the origin of the sampled animals

The samples were conveyed to the Veterinary and Agriculture Institute (IAV) in Rabat and stored in -20 °C until their treatment.

Tissue preparation, culture

Prior to treatment, samples were thawed overnight at 4°C. Subsequently, samples were desiccated to remove adipose tissue, and 5g of the desiccated lesions were mixed with sterilized sand and 10 ml of phenol red. The solution (7.5 ml) was placed in a 15 ml conic tube, 2.5 ml of NaOH 1N was added at room temperature for 10 minutes, and then HCl 6N was added for sample neutralization. As a final step, the tube was centrifuged for 25 min at 3500 rpm.

The supernatant was discarded and pellet was distributed in two of the four pre-tested culture media: Lowenstein-Jensen (LJ), LJ with glycerol (LJG) or pyruvate (LJP) and Herrold according to the availability in the laboratory. Cultures were incubated for 12 weeks at 37°C and observed daily for growing colonies during the first week then weekly from the second week onwards.

All the grown cultures were deactivated by adding a loopful of mycobacterium colonies to 1 ml of sterilized water contained in small tubes. The samples were then inactivated for 1h at 90°C.

Determination of MTBC and deletion typing

Mycobacterium molecular characterization was performed using Multiplex polymerase chain reaction (PCR). The PCR was performed in the TB laboratory at Swiss TPH. We performed first MrpoB PCR in order to differentiate MTC and NTM as

described earlier (35). Deletion analysis by PCR was used to differentiate *M. bovis* and *M. tuberculosis* from other species of the MTBC by assessing the presence or absence of Regions of Difference 9 and 4 (RD). The analysis was carried out as previously described (13,28).

Spoligotyping

Spoligotyping was performed as previously described (36). Spoligotyping patterns were defined according to the SITVIT WEB database (37) and to *Mycobacterium bovis* molecular typing database (38). All the patterns which were not found in the two databases were submitted as new patterns; new spoligotype numbers were assigned to them.

Results

Cattle information

In the present study, a total number of 8658 animals were examined. Three hundred and twenty seven animal presented gross visible lesions (3.7%) and were cultured, 66% (n=215) of the total sampled animals were analyzed by Ziehl-Neelsen(ZN), 68% (n=147) of the latter were ZN positive and heat-killed for further molecular typing.

Figure 1 represents the geographic distribution of the samples (figure 1). While the age and gender distribution, in addition to the localization of the lesions sampled are shown in the table 5.1 and 5.2. The majority of the lesions were localized in the lymph nodes and the lungs (Table 5.2).

Table V.1. Age and gender distribution of the sampled animals according to positive and negative cultures

	Male			**Female**			**Total**
	<1 year	**1-3 years**	**>3 years**	**<1 year**	**1-3 years**	**>3 years**	
Culture +	6	153	20	1	3	66	249
Culture -	4	40	6	2	3	23	78
Total	10	193	26	3	6	89	327

The majority of the sampled animals were males between 1 and 3 years and females more than 3 years (Table 5.1).

Table V.2. Localisation of the collected lesions, in addition to the specifications of the animals sampled

Lesion localisation	n	Gender		Breed		
		F	M	I	C	L
Liver	5	1	4	0	5	0
Lungs	4	2	2	0	4	0
Lungs and diaphragm	1	0	1	0	0	0
LN	185	45	140	67	117	8
LN and diaphragm	4	1	3	1	3	0
LN and liver	4	1	3	1	3	0
LN and lungs	70	30	40	35	35	2
LN, lungs, and diaphragm	6	1	5	4	2	0
LN, lungs, and liver	18	7	11	4	14	0
LN, lungs, liver, and diaphragm	24	4	20	21	2	1
LN, lungs, liver, and kidney	2	2	0	0	2	0
LN, lungs, pleura, and pericardium	2	2	0	0	2	0
Miliary tuberculosis	2	2	0	0	2	0
Total	327	88	229	133	191	11

LN: Lymph nodes; I: Imported; C: Crossed; L: Local.

MrpoB PCR

A total of 68% (n=147) of the 215 samples which were AF positive were confirmed as MTBC strains using MrpoB PCR. Thirty two per cent (n=69) of animals were negative for MrpoB.

Deletion typing

The samples confirmed as MTBC strains were all positive to RD9 deletion typing and then confirmed to be not *M. tuberculosis* strains. A total of 144 (1.7%) of the samples were confirmed to be *M. bovis* as they were positive to the RD4 pcr. Three samples were negative for RD4.

Out of the total of the confirmed *M. bovis* samples, 30% were female while 70% were male. The predominant breed in the positive animals was Crossbreed prime Holstein.

Spoligotyping

A total of 136 analyzed samples were lacking spacers 39 to 43. Forty one different spoligopatterns were found. The most frequent patterns were SB0121, SB0265, SB0120, with frequencies of 24.3% (n=33). 16.9% (n=23), 9.6% (n=13) respectively. They were shown to belong to BOV-1 family. Two other spoligotypes were found in 9 and 6 samples respectively, SB0125 and SB0869. Three spoligotype patterns had no SIT reference on the SITVIT database and were designated as orphan. Ten isolates presented nine undescribed spoligotypes, which were submitted to *M. bovis* website (www.mbovis.org) (Table 5.3). The discrimination power of spoligotyping, calculated using Hunter and Gaston's formula was D=0.9057(39).

Table V.3. The different spoligotype patterns of the analysed samples and their relative frequencies

Spol	Nb.	Frequency	SIT	Family	SB Nb.
■■□■■■■■□■■■■■■□■■■■□■■■■■■■■■■■■■■■■■□□□□□	33	24.3	481	BOV_1	SB0121
■■□■■□■■□■■■■■■□■■■■□■■■■■■■■■■■■■■■■■□□□□□	23	16.9	684	BOV_1	SB0265
■■□■■■■■■□■■■■■■□■■■■■■■■■■■■■■■■■■■■■□□□□□	13	9.6	482	BOV_1	SB0120
■■□■■■■■■□■■■■■■□■■■■□■■■■■■■■■■□□■■■■■□□□□□	9	6.6	Orphan	BOV_1	SB0125
■■□■■■■■■□■■■■■■□■■■■□■■■■■■■■■■■■□□■■□□□□□	6	4.4	1026	BOV_1	SB0869
■■□■■■■■■□■■■■■■□■■■■■■■■■■■■■□□□■■■■■■□□□□□	4	2.9			SB1025
■■□■■■■■■□■■■■■■□■■■■□■■■■□□□□■■■■■■■■■□□□□□	4	2.9	696	BOV_1	SB0339
■■□□□■■□□□■■■■■■□■■■■■■■■■■■■■■■■■■■■■■□□□□□	3	2.2	1673	BOV	SB0136
■□□□■■■■■□■■■■■■□■■■■□■■■■■□■■■■□■■■■■■□□□□□	3	2.2			SB2225
■■□■■■■■■□□■■■□□□■■■■□■■■■■■■■■■■■■■■■■□□□□□	2	1.5			SB1269
■■□■■■■■■□■■■■■■□■■■■□■■■■■■■■■■■■■■■□■□□□□□	2	1.5	698	BOV_1	SB0295
□■□□□□□□□□□□□□□□■■■■■■■■■■■□■□□□□■■■■■□□□□□	2	1.5	646	BOV_4-CAPRAE	
■■□□□□□■□■■■■■■□■■■■□■■■■□□□□■■■■■■■■■□□□□□	2	1.5	Orphan	BOV_1	SB1145
■■□□□■■■□■■■■■■□■■■■■■■■■■■■■■■■■■■■■■□□□□□	2	1.5	665	BOV_1	SB0134
■■□■■■■■■□■■■■■□□■■■■■■■■■■■■■■■■■■■■■■□□□□□	1	0.7	820	BOV	SB0856
■■□■■■■■■□■■■■■□□■■■■□■■■■■■■■■■■■■■■■■□□□□□	1	0.7	695	BOV	SB0119
■■□□□■■■□■■■■■■□■■■■■□□□■■■■■■■■■■■■■■■■□□□□□	1	0.7			SB1888
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■■□■□□□■□■■■■■■□■■■■□■■■■■■■■■■■■■■■■■□□□□□	1	0.7	Orphan	BOV_1	SB2271
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■■□■■■□■□■■■■■■□■■■■□■■■■■■■■■■■■■■■■■□□□□□	1	0.7			SB1361
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■■□■■■■■■□■■■■■■□□□■■□■■■■■■■■■■■■■■■■■□□□□□	1	0.7			SB1993
■□□□■■■■□■■■■■■□■■■■□■■■■■■■■■■■■□□■■□□□□□	1	0.7	650	BOV	SB0341
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Discussion

To our knowledge, this is the first study investigating the molecular characterization of bovine tuberculosis among cattle in Morocco. The prevalence of the gross visible lesions suggesting BTB in Rabat and El Jadida slaughterhouses (3.7%) was low compared to the prevalence found previously using tuberculin skin test in the last national survey performed in 2004 (20). This prevalence was close to those observed in Mali (1.8 %) and in Algeria (3.6 %).

Surprisingly, no non-tuberculous mycobacteria was found, while in the African context, among slaughterhouse gross visible lesions, 3.3 % in Mali (40), and 9 % in

Burkina Fasso (41) were confirmed as non-tuberculous mycobacteria. This was also observed in Chadian slaughtered cattle (33,34).

Almost 52.4% of the overall sample size was confirmed to be *M. bovis*, three samples were negative to RD4. While two spoligotypes were typical for the caprae family, the third sample was a new spoligotype. Studies in other Countries (e.g. Nigeria, Ethiopia) found *M. tuberculosis* in cattle (42), whereas in Morocco, remarkably, we didn't find this MTBC species in our samples.

The most predominant spoligotype pattern found in Morocco is SB0121, belonging to the family BOV 1, this spoligopattern was already reported in Algeria (43), in Tunisia (44) and in Spain (45). The second most frequent spoligotype was SB0265 which was reported as the second most frequent in the United States from a set of strains collected between 1989 and 2013 (46), in addition, this spoligotype was isolated in Tunisia from a strain coming from Morocco (37), and was also reported in many European countries (47,48), as well as in Taiwan (49). The spoligopattern SB0120 had the frequency of 11.7 % in our sample size and was reported previously in France from a sample originating from Morocco (SITVIT database), it was also reported in many African countries like Algeria (43), Tunisia (44) and Zambia (50) as well as in Argentina and Spain (45,51). Our study shows no similar spoligotype pattern with Mali, indicating that the Sahara desert and the long distance between cattle rearing areas (cattle are only kept until a Latitude of 12 degrees in Mali) seem to be an effective barrier for the transmission of *M. bovis* and/or that there is probably little trade of cattle between Morocco and Mali (40).

Two spoligotypes of three samples belonged to the *M. caprae* family, of which one was found as well in *M.bovis* database as SB0866, this spoligotype was already reported in Spain from one goat, one cattle and one pig in 2011 (52).

The similarities found in some spoligopatterns between Morocco and Algeria could be explained by the shared borders between the two countries, in addition some patterns found in Morocco were previously reported in the United States and in Argentina, two countries from where Morocco have previously imported cattle.

Spoligotypes of African 1 and African 2 clonal complexes were not found among our characterized isolates (53,54). African 1 is localized in West and Central Africa, and African 2 is localized in East Africa, consequently, the desert of Morocco could be considered as a potential efficient barrier preventing the introduction of BTB to Morocco from West, Central and East Africa.

The relatively low prevalence of proven *M. bovis* infection in two Moroccan abattoirs has to be interpreted with caution. Firstly, the abattoir prevalence reflects more the prevalence in young bulls and old cows rather than the whole population. Secondly, not all granuloma yielded a bacteriological isolate, hence the true prevalence may be much higher than the one observed. Overall, our findings reflect rather the epidemiological situation of a low level endemic transmission similar to other African countries rather than the one of a peri-urban intensive system (55,56). More research

is needed to further characterize the ongoing transmission patterns of bovine tuberculosis in view of the development of a locally adapted elimination strategy of bovine tuberculosis in Morocco (57).

Conclusion

This study presents the first molecular characterization of BTB isolates from Moroccan cattle. *M. bovis* represented a high amount of granulomatous lesions detected in the abattoirs of Rabat and El Jadida. Spoligotype suggests a link of Moroccan BTB to Europe, rather than to Africa, highlighting then the potential of the Moroccan desert barrier for BTB introduction to Morocco from sub-Saharan Africa. Further investigations of BTB strains using new molecular techniques such as whole genome sequencing are needed to clarify more the potential links between Moroccan BTB strains and those of Europe and other African countries.

List of abbreviations

BTB	Bovine tuberculosis
MTBC	Mycobacterium tuberculosis complex
TB	Tuberculosis
RD	Region of difference
NTM	Non-tuberculous mycobacteria
LJ	Lowenstein Jensen
LJG	Lowenstein Jensen with glycerol
LJP	Lowenstein Jensen with pyruvate
PCR	Polymerase chain reaction
ZN	Ziehl-Neelsen

Ethical consideration

No ethical consideration was necessary, as the sampling work was part of routine work of meat inspection in the slaughterhouses.

Consent for publication

Not applicable

Availability of data and materials

The dataset supporting the conclusions of this article is included within the article, and its additional file(s). The data provided shows the original sample name, individual information, in addition to Deletion typing results for all 215 MTBC strains isolated from Morocco, and spoligotype pattern and number for 136 samples which were analyzed by spoligotyping.

Competing interests

The authors declare not having any conflict interest to disclose.

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Author's contribution

HYA: Participated to the acquisition of a part of the funds (IFS grant), participated in the conception and design of the study, culture of Mycobacteria, molecular analysis, statistical analysis, writing of the manuscript. HA: Participated in sample collection. MB: Participated in the conception and design of the study, acquisition of funds, principle supervision in Morocco and intellectual contributions. JB: Participated in the conception and design of the study, Principle supervision of the laboratory work in Morocco (culture of mycobacteria). SR: Participated to the laboratory work in Morocco (culture of mycobacteria). MR: supervision of the molecular analysis in the tuberculosis laboratory in the Swiss TPH. SG: Supervision of the laboratory work in the Swiss TPH, important intellectual contribution. JF: Supervision of the molecular analysis in the tuberculosis laboratory in the Swiss TPH. SB: Supervision of the molecular analysis, and participated to the conception and the design of the study. JZ: Principal supervision of the project, Principle acquisition of the funds, important intellectual contribution. All the authors have read and approved the final version of the manuscript.

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VI. Transmission dynamics and elimination potential of zoonotic tuberculosis in Morocco

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Abstract:

Bovine tuberculosis (BTB) is an endemic zoonosis in Morocco caused by *Mycobacterium bovis*, which infects many domestic animals and is transmitted to humans through consumption of raw milk or from contact with infected animals. The prevalence of BTB in Moroccan cattle is estimated at 18 %, and 33 % at the individual and the herd level respectively, but the human *M. bovis* burden needs further clarification. The current control strategy based on test and slaughter should be improved through local context adaptation taking into account a suitable compensation in order to reduce BTB prevalence in Morocco and decrease the disease burden in humans and animals.

A compartmental deterministic mathematical model for BTB transmission in cattle and humans was established to provide a broader understanding of BTB, in particular regarding transmission to humans. Differential equations were used to establish the different pathways between the compartments for cattle and humans. Scenarios of test and slaughter were simulated for the effects on BTB prevalence in cattle and humans. Simulation of test and slaughter interventions led to a decline of BTB prevalence depending on the proportion of testing (p). The time to freedom from disease (individual animal prevalence of less than one in a thousand) ranged from 75 years for p = 20% to 12 years for p = 100%. For p > 60% the time to elimination was less than 20 years. The cumulated cost was largely stable: for p values higher than 40 %, cost ranged from 1.47 to 1.87 billion Euros with a time frame of 12 to 44 years to reach freedom from disease. This analysis informs Moroccan bovine tuberculosis control policy regarding time frame, range of cost and levels of intervention. Further research is needed to clarify the national human-bovine tuberculosis ratio in Morocco.

Key words: Bovine tuberculosis, *Mycobacterium bovis,* transmission modelling, Morocco, disease elimination, cattle

Author summary

Tuberculosis is a disease of humans and animals which mainly affects the lungs but can also manifest in other organs. A variety of tuberculosis bacteria cause the disease and are usually transmitted through air, i.e. inhalation of aerosols. Bovine tuberculosis (BTB) occurs predominantly among domestic cattle, and wild animals are an important reservoir for transmission. Humans are usually infected with BTB through contaminated dairy products or close contact with cattle. While BTB has been eliminated in cattle and human populations of most high-income countries, it is still a major health threat in low- and middle-income countries. In Morocco, the disease frequently occurs in cattle and poses a health risk for humans. An effective intervention to reduce BTB among domestic cattle and reduce the risk to humans is slaughter of cattle which test positive for the disease. We simulated BTB in the Moroccan cattle and human populations using a disease transmission model. We assessed effects of test and slaughter in regard to elimination of disease in cattle and humans and estimated the associated costs with the model. The time to elimination of disease depended on the number of cattle tested. The disease could be eliminated in cattle within 32 years if 40% of Moroccan cattle are tested annually and infected individuals are slaughtered or within 13 years if at least 90% of the cattle population is tested. The total costs for the time periods until elimination ranged from 1.55 billion Euros for testing and slaughter at 50% to 1.48 billion Euros at 90%. These results can be used as an estimate for planning BTB control policy in Morocco with regard to time frames and associated costs.

Introduction

Bovine tuberculosis (BTB) is a zoonotic bacterial infection caused by *Mycobacterium bovis.* It belongs to a group of well-known and newer mycobacteria, together with *Mycobacterium tuberculosis,* all of which derive from a common ancestor forming the *Mycobacterium tuberculosis* complex (MTBC) (1-4). *M. bovis* is capable of infecting a broad range of hosts, including ruminants (predominantly domestic cattle), humans and other primates (5-10). The wide host range makes BTB highly relevant to conservation projects and difficult to eliminate where wildlife reservoirs are involved, for instance, badgers (*Meles meles*) in the United Kingdom (11, 12).

Bovine tuberculosis infection in cattle is a chronic disease which affects first the lymph nodes and then the lungs from weeks to decades later because transmission between cattle occurs predominantly through aerosol inhalation (13-16). The transmission rate is increased by risk factors such as high herd density and intensive breeding (17). The disease can also be manifested in other organs, for example, mammary tissue, and the gastrointestinal or urinary tract (18). Pseudo-vertical transmission from cows to suckling calves through infected milk has been described (19). Factors like a long survival period for the microbes in manure and soil and close contact between animals, for example around water sources, also contribute to increase risk of infection (20, 21). In humans, contaminated dairy products are considered to be the main source of BTB infection, usually resulting in extra-pulmonary infection such as lymphadenitis (22-24). These patients are missed by thoracic radiographic screening and the resulting diagnostic cascade (25). Aerosol cattle-to-human transmission can occur during close contact with infected animals, posing an occupational risk, especially for pastoralists and farmers (2, 26). Infection risks linked to local cultural practices, for instance consumption of fresh blood, are reviewed by Daborn (27). There is evidence that human patients can transmit BTB to animals, and human to human transmission occurs (28, 29).

There is a bottleneck in detecting human BTB cases because the routine diagnostic protocols were developed for patients with pulmonary tuberculosis, as caused by *M. tuberculosis*. Tuberculosis (TB) and BTB cannot be distinguished on the basis of clinical symptoms, radiography or histopathology (30). Glycerol-containing Löwenstein-Jensen medium, the long-time gold standard for TB culture, inhibits the growth of *M. bovis*, thereby increasing the number of undetected cases (31). New molecular diagnostic tools, for example spoligotyping, and even whole genome sequencing have been developed for *M. bovis* detection (32, 33). Although they require enhanced laboratory infrastructure and personnel training which are not currently available in some developing countries, these new techniques offer promise for epidemiological research, control and adequate treatment, particularly since *M. bovis* is resistant to pyrazinamide, one of the first-line antibiotics for TB treatment (34).

Morocco is transitioning from extensive pastoralist livestock and dairy production to more intensified production due to increasing demands for dietary protein by a

growing human population (35). The shift in agricultural practice and increased use of high-producing Holstein cattle in place of local breeds may have an impact on BTB epidemiology and contribute to a higher prevalence (36). The official national control program in Morocco is currently based on a test and slaughter scheme. However, large-scale application remains challenging because testing is not mandatory, and the proposed compensation, ranging from 470 Euro for local breeds to 980 Euro for improved breeds, is considered lower than market value.

In Morocco, BTB is an endemic zoonosis in livestock. Even though the predominant livestock species in Morocco are sheep and goats, cattle remain of major importance. The most recent national survey, conducted in 2004, showed an individual cattle prevalence of 18% and a herd prevalence of 33% (37). This prevalence remained similar in the individual level (18%), while the herd prevalence increased (56%) in a 2012 pilot study of 1'200 cattle using the tuberculin skin test (38). Since 2000, the health risk of tuberculosis in Morocco has been addressed through a national TB program funded by the Ministry of Health in collaboration with the World Health Organization (WHO). In 2014, TB caused 2'800 deaths in Morocco (39), and human tuberculosis had a relatively high incidence, with 36'000 new cases (106 cases per 100'000 inhabitants) (40). These data do not appear to differentiate between *M. tuberculosis* and *M. bovis* infection. A recent meta-analysis estimated the median proportion of human BTB among all TB cases in 13 African countries at 2.8%, with a range of 0-37.7% (41). National prevalence data from a range of countries worldwide were summarized in a 2014 review; in Mexico up to 13% of all TB cases are reportedly due to BTB, while in the United States it is only 1.4% (42). In Morocco, Bendadda et al. reported *M. bovis* prevalence of 17.8% among drug resistant TB isolates from 200 human sputum samples (43).

In the early 20th century, the prevalence in German cattle herds was 90% (44), with 25-80% in other European states and only 2-10% in the US (45). In most industrialized countries, the health risk and economic loss from *M. bovis* were considerably reduced or eliminated through strict test-and-slaughter and meat inspection protocols for cattle, along with the implementation of milk pasteurization and financial compensation of farmers (45). Using a similar control strategy, Switzerland eradicated BTB in 1960 (46). In most developing countries where the disease is endemic, such measures are not feasible due to financial constraints, particularly for farmer compensation, and inadequate veterinary services (47). Alternatives for BTB endemic countries to reduce the health and economic risk related to the disease must be sought.

Although cost estimation is difficult due to immense local variability in production parameters and prices (48), the global economic loss due to BTB is thought to be about 3 billion USD annually (49). In cattle, the disease has a significant economic impact, through an increased death rate and decreased milk and meat production, draft power and fertility (50). Modelling approaches have been used, mainly for developing countries, to estimate different parameters and factors related to BTB. Previous publications on the economics of BTB focus mainly on the cost of disease

and control efforts. Analyses of the profitability of control efforts are very scarce (51). A study on the economics of BTB in Africa showed higher losses in intensive dairy systems in peri-urban areas of Ethiopia when compared to extensive pastoral production systems in rural areas but did not include the cost to public health (48). Zinsstag et al presented a simplified framework for a model of animal to human transmission in which transmission between cattle and from cattle to humans is considered (51). This model allows the simulation of different scenarios over 5-10 years, with and without intervention, where the measurable outcome is prevalence in humans and in cattle. The economic analysis further delineates broader issues such as inter-sectorial contributions from agricultural and public health or private households affected by BTB.

Interventions to reduce health and economic risks, such as those related to BTB, are non-linear processes. Although, statistical versions of compartmental models have been used for many years to analyze different types of health interventions, mathematical modeling represents an alternative approach which provides a broader understanding, especially regarding disease transmission to humans. This paper presents a mathematical model of BTB transmission from cattle to humans in order to estimate the disease cost and simulate potential interventions in Moroccan cattle.

Materials and methods

Epidemiological data collection

Annual data on cattle numbers are estimated using data routinely collected by the Ministry of Agriculture and reported to veterinary services. Our model considered these data from 1995 to 2013. In the transmission model, the cattle population was not stratified by age and sex. We used a tuberculin prevalence of 18% for cattle, as reported in the most recent national survey (2003) [33]. A similar prevalence was noted in a smaller study performed in 2012 in Sidi Kacem, Morocco (38).

Model

A schematic diagram of the model is depicted in Figure 6.1 and the variables and parameters are described in Table 6.1 and Table 6.2, respectively.

The cattle population is divided into three mutually exclusive compartments consisting of susceptible cattle (S_C), exposed cattle with latent BTB which are positive to the tuberculin test, without showing gross visible lesions (E_C) and infected cattle with active BTB showing tuberculosis lesions (I_C). Those parameters were estimated based on Ngandolo et al 2009 (52). The total cattle population (N_C) at time t is:

$$N_C(t) = S_C(t) + E_C(t) + I_C(t) \quad (1)$$

The human population consists of four mutually exclusive compartments: susceptible humans (S_H), exposed humans with latent BTB reacting to the Mantoux test (E_H),

infected humans with active BTB (I_H) and humans recovered from BTB with temporary immunity (R_H). The total human population (N_H) at time *t* is:

$N_H(t) = S_H(t) + E_H(t) + I_H(t) + R_H(t)$ (2)

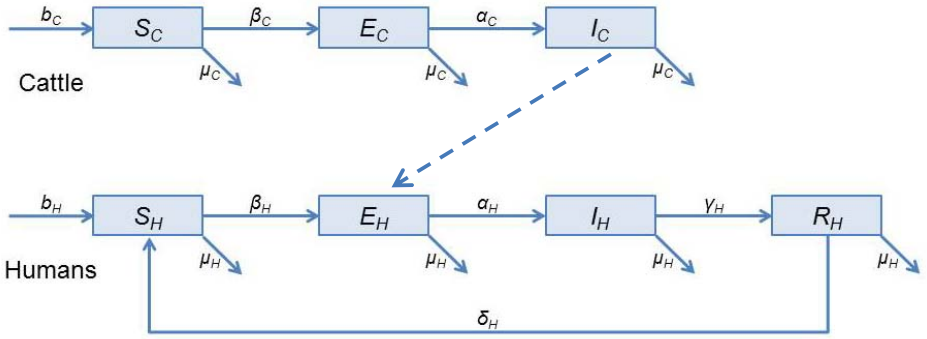

Figure VI.1. Schematic diagram of the BTB cattle-human transmission model for Morocco

The susceptible cattle population ($S_C(t)$) increases through birth (at a rate b_C) and decreases through exposure to BTB (at a rate $(Ic/Nc)\beta_C$) and mortality (at a rate μ_C). Exposure to BTB is assumed to be frequency dependent. According to Bernues (53), the rate b_C decreases by 5% for exposed cattle with latent BTB ($E_C(t)$) and for infected cattle with active BTB ($I_C(t)$), such that:

$$\frac{dS_C}{dt} = b_C S_C + \left(0.95 \times b_C(E_C + I_C)\right) - \beta_C \frac{I_C S_C}{N_C} - \mu_C S_C \quad (3)$$

The population of exposed cattle with latent BTB ($E_C(t)$) is generated through infection of susceptible cattle with BTB (at a rate β_C) and decreased through the development of active BTB (at a rate α_C) and through mortality (at a rate μ_C). Consequently:

$$\frac{dE_C}{dt} = \beta_C \frac{I_C S_C}{N_C} - \alpha_C E_C - \mu_C E_C \quad (4)$$

Similarly, the infected cattle population with active BTB ($I_C(t)$) is generated through the development of active BTB among exposed cattle with latent BTB (at a rate α_C) and decreased through mortality (at a rate μ_C):

$$\frac{dI_C}{dt} = \alpha_C E_C - \mu_C I_C \quad (5)$$

For simplicity, no additional mortality rate due to BTB is assumed for the cattle and human populations in the model.

Table VI.1. Description of the variables of the BTB model for Morocco

Variable	Interpretation
S_C	Population of susceptible cattle
E_C	Population of exposed cattle with latent BTB
I_C	Population of infected cattle with active BTB
S_H	Population of susceptible humans
E_H	Population of exposed humans with latent BTB
I_H	Population of infected humans with active BTB
R_H	Population of humans recovered from BTB

The susceptible human population ($S_H(t)$) increases through birth (at a rate b_H) and through recovered humans becoming susceptible again (at a rate δ_C). The population decreases through exposure to BTB from cattle with active BTB (at a rate β_H) and through natural mortality (at a rate μ_C). For the susceptible human population, the exposure (both direct (aerosol) and indirect (milk) transmission) to BTB from cattle with active BTB is assumed to be frequency dependent and proportional to the number of infected cattle (I_C):

$$\frac{dS_H}{dt} = b_H N_H + \delta_H R_H - \beta_H \frac{I_C S_H}{N_C} - \mu_H S_H \quad (6)$$

Human to human transmission is assumed to be negligible. The population of exposed humans with latent BTB ($E_H(t)$) is generated through infection of susceptible humans with BTB (at a rate β_H) and decreased through the development of active BTB (at a rate α_H) and through natural mortality (at a rate μ_H):

$$\frac{dE_H}{dt} = \beta_C \frac{I_C S_H}{N_C} - \alpha_H E_H - \mu_H E_H \quad (7)$$

The infected human population with active BTB ($I_H(t)$) is generated by the development of active BTB among exposed humans with latent BTB (at a rate α_H) and decreased by recovery of humans with active BTB (at a rate γ_H) and by natural mortality (at a rate μ_H):

$$\frac{dI_H}{dt} = \alpha_H E_H - \gamma_H I_H - \mu_H I_H \quad (8)$$

The population of humans recovered from BTB is generated through the recovery of humans with active BTB (at a rate γ_H) and decreased through humans becoming susceptible to BTB again (at a rate δ_H) and through natural mortality (at a rate μ_H), so that:

$$\frac{dR_H}{dt} = \gamma_H I_H - \delta_H R_H - \mu_H R_H \quad (9)$$

Table VI.2. Description of the parameters of the BTB model for Morocco

Parameter	Interpretation
b_C	Birth rate of cattle
β_C	Cattle to cattle transmission rate
μ_C	Mortality rate of cattle
α_C	Inverse of cattle incubation period
b_H	Birth rate of humans
β_H	Cattle to human transmission rate
μ_H	Natural mortality rate of humans
α_H	Inverse of human incubation period
δ_H	Loss of immunity in humans

Values for variables and parameters of the model

Variable starting values

The most recent estimate (2013) for the cattle population in Morocco is 3'173'000 (N_C), of which 18% [95% CI: 16.5%-20.3%] are tuberculin skin test positive. The initial values of the three compartments I_C, E_C and S_C were calculated such that the pre-intervention endemic equilibrium of the model equals the prevalence of 18% (Supporting information 1). This yield:

$$I_C = \frac{\alpha_c \varphi_C}{\alpha_C + b_C - (b_C - r_b b_C)\varphi} N_C \quad (10)$$

$$E_C = \left(\varphi_C - \frac{I_C}{N_C}\right) N_C \quad (11)$$

$$S_C = N_C - E_C - I_C \quad (12)$$

The human population of Morocco in 2013 is estimated to be 33'008'150 individuals (54). The estimated number of people with active TB is 43'000 (prevalent cases) (40). In Africa, a median of 2.8% of all human TB cases are caused by BTB (41). Between 5 and 10% (mean 7.5%) of TB-exposed people will develop active TB during their lifetime (55). Based on these estimates the starting values of I_H, E_H, R_H *and* S_H are calculated as (Table 6.3):

$$I_H = \frac{\alpha_H \varphi_H}{\alpha_H + \delta_H + b_H} N_H \quad (13)$$

$$E_H = \left(\varphi - \frac{I_H}{N_H}\right) N_H \quad (14)$$

$$S_H = N_H - E_H - I_H \quad (15)$$

Table VI.3. Initial values for the BTB cattle-human transmission model

Variable	Starting value
S_C	2'601'860
E_C	79'610
I_C	491'529
S_H	33'006'946
E_H	784
I_H	420

Parameter values

The average lifespan of the Moroccan cattle is 6 years which yields a death rate of $\mu_C = 0.167$. Form data on cattle populations (Yahyaoui H., unpublished data, Dec. 2015) using least squares the birth rate was estimated as $b_C = 0.177$.

From the UN World Population Prospects we calculated the birth rate b_H and death rate μ_H in humans (54). Although BTB bacteria are not completely eliminated from treated humans (56, 57) it is nevertheless assumed, for the sake of simplicity, that all successfully treated humans are recovered and become susceptible again within a period of 6 months.

The cattle to cattle transmission rate, β_C, and the cattle to human transmission rate, β_H, were estimated from the pre-intervention endemic equilibrium (Supporting Information 2). The model was implemented using MATLAB® (MathWorks, Natick, MA) (Table 6.4).

Table VI.4. Parameters of the BTB cattle-human transmission model assuming a stable prevalence (endemic stability)

Parameter	Baseline value (year^{-1})		Source
b_C	0.177	[0.121 - 0.274]	Yahayoui H., unpublished data, Dec. 2015
β_C	0.249	[10.244 - 0.255]	Estimated from the endemic prevalence in cattle
μ_C	0.167	[0.111 - 0.333]	(58)
α_C	1.083	[0.5 – 1.667]	(1)
Se	0.438	[0.057 – 0.819]	(52)
Sp	0.894	[0.842 – 0.946]	(52)
b_H	0.0229		(54)
β_H	0.00015		Estimated from the endemic prevalence in humans
μ_H	0.0063		(54)
α_H	1.083		
δ_H	2		(59)

The reproductive number of the transmission between cattle R_0 was computed as:

$$R_0 = \frac{\alpha * \beta}{(\alpha + \mu)\mu} \quad (18)$$

Sensitivity analysis of model

A sensitivity analysis of the model recalculated the change of prevalence if individual parameters varied from baseline over 30 years.

Simulation and cost of interventions

Although Morocco has a test and slaughter policy for the control of BTB, it is currently not effectively implemented. The BTB transmission model was used to estimate the effect of the proportion of tested and slaughtered tuberculin positive animals on the duration to reach freedom from disease, achieving an individual animal prevalence of less than one in a thousand tested animals (<1/1000) according to the standards of the World Organization for Animal Health (OIE) (60). The proportion of tested and slaughtered animals was simulated by removing 10-100 % of the exposed (E_c) and infectious cattle (I_c) per year from the herd. The control reproductive number R_c including the test and slaughter intervention as proportion p with a test of sensitivity se was computed as:

$$R_c = \frac{\alpha * \beta}{(\alpha + \mu + se * p)(\mu + se * p)} \qquad (17)$$

The associated costs were estimated in a summaric way, assuming an incremental cost of comparative intradermal or interferon gamma (BOVIGAM®) testing of 3 Euro per animal. The cost of compensation at 80% of the market value varies from 470 Euro for local breeds to 970 Euro for improved breeds (61). For the current estimation of the cost of BTB elimination in Morocco, we used a single value of 500 Euro of compensation per slaughtered animal. The cumulated costs of elimination were discounted by an annual discount rate of 5% and expressed as net present value (NPV). Models run with and without interventions were simulated using data from 2013 onwards.

The OIE recommended cut-off for SICTT interpretation is 4 mm, however, many studies showed that a severe cut-off of 2 mm increased the sensitivity of the test (62, 63), without affecting the specificity compared to the recommended cut-off (64). Consequently, we decided to consider both 2mm and 4 mm cut-offs in the model, and to compare the respective results. The present model considered both options of SICTT cut-off at 2mm and 4mm.

Results

Model properties

The reproductive ratio of the cattle to cattle transmission of bovine tuberculosis without intervention was 1.325. For the total cost, the birth rate of cattle b_c was the most sensitive parameter influencing the dynamics of BTB transmission (Figure 6.2). High birth rate values lead to an increased cattle population yielding higher costs for elimination. For the time to elimination, the sensitivity of the test was the most sensitive parameter. Low test sensitivity (i.e. with cut-off at 4mm) leads to low

detection of infected animals and therefore less culling which leads to a longer time to elimination.

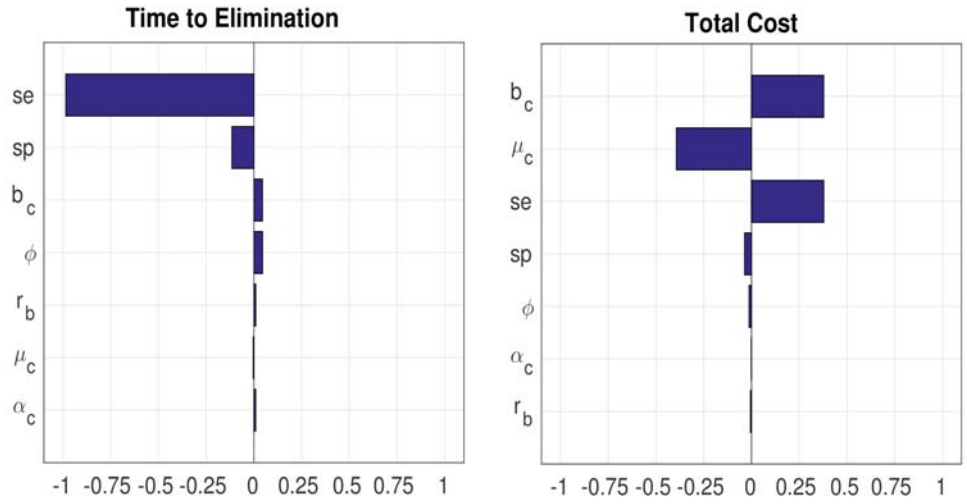

Figure VI.2. PRCC sensitivity analysis of total cost (left) and time to elimination (right) on parameter values

The simulation of a test and slaughter intervention led to a decline in BTB prevalence depending on the proportion p of testing (Figure 6.3). The time to elimination, i.e. the time to reach an individual animal prevalence of less than one in a thousand, ranged from 75 years for p = 20% to 12 years for p =100%. For values of p > 60%, the time to elimination was below 20 years (Figure 6.3). The reproductive number decreased rapidly below one with an increasing proportion test and slaughter p (Figure 6.4). With 60% testing and culling, the prevalence of exposed and active human cases BTB decreased from 3.5 per 1'000’000 to less than 1 in 1'000’000 at the time of freedom from disease after 20 years (Figure 6.5).

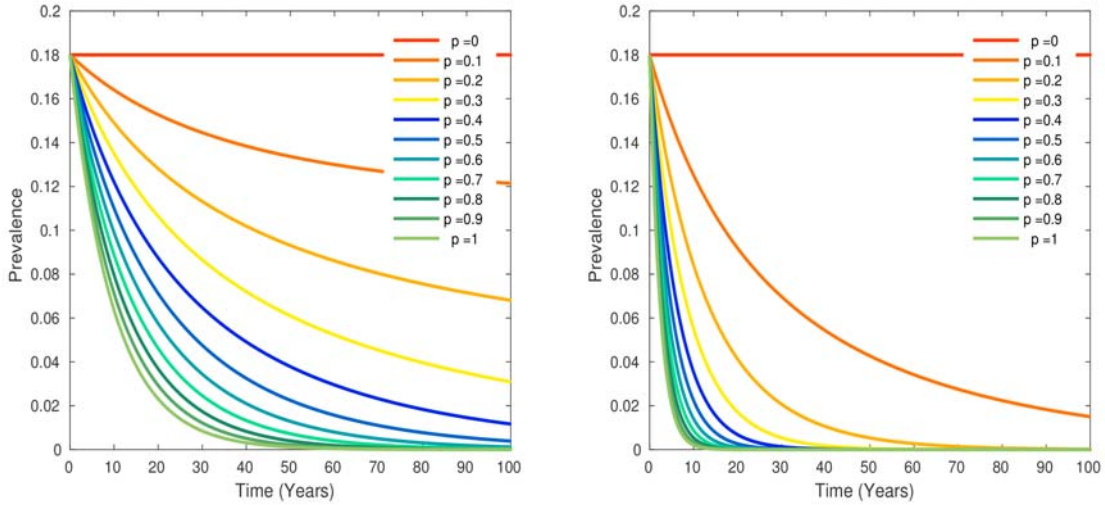

Figure VI.3. Prevalence of tuberculin positive cattle depending on the proportion of test and slaughter between 0 and 1 (in steps of 0.1) with sensitivity and specificity of the 4mm cutoff test (left) and the 2mm cuttoff test (right)

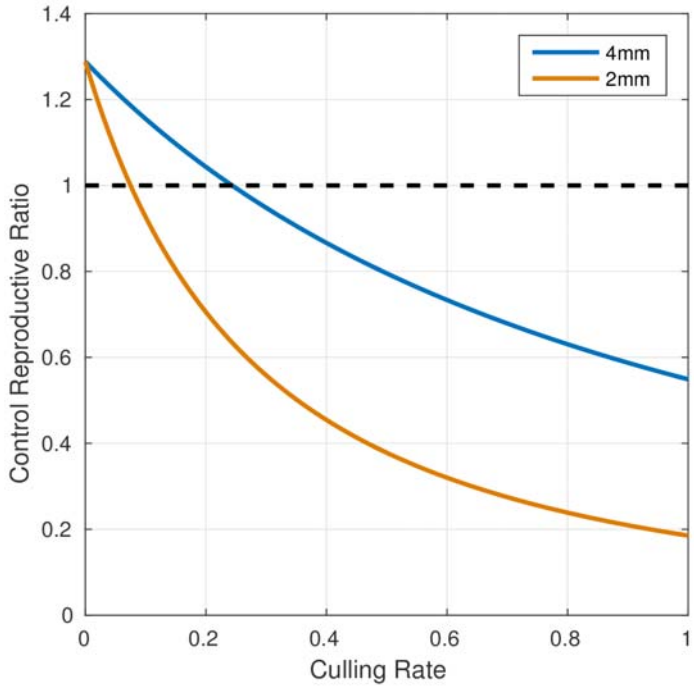

Figure VI.4. Relationship between reproductive number and proportion of test and slaughter for the 4mm cutoff test and the 2 mm cutoff test

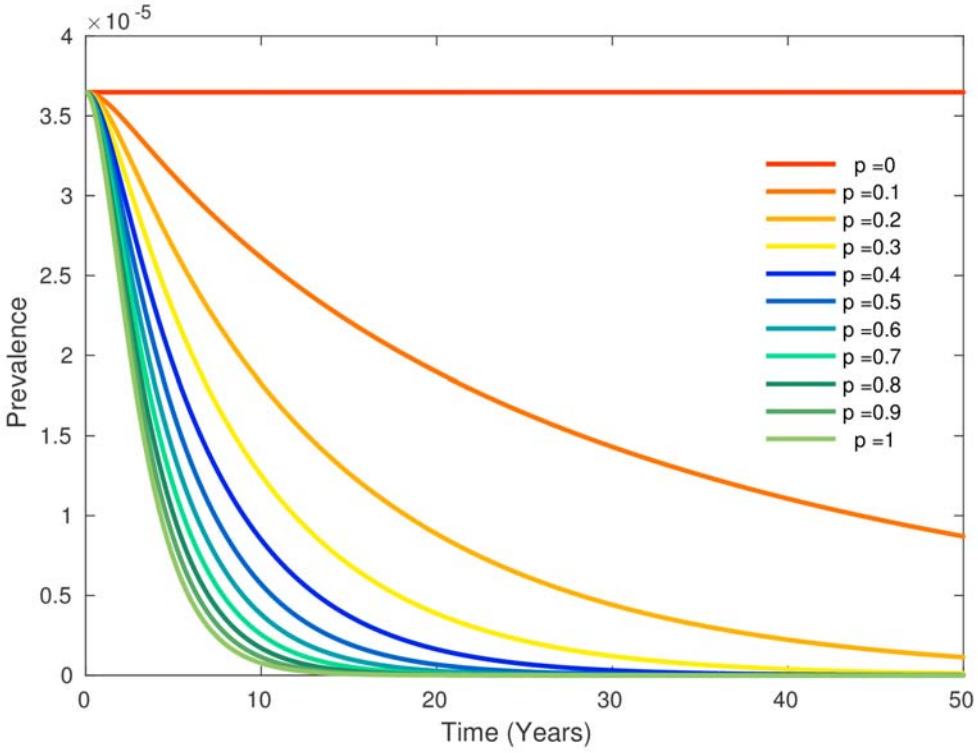

Figure VI.5. Relationship between human prevalence and proportion of test and slaughter

Cost of test and slaughter intervention

The cost of test and slaughter depends on the percentage p of test and slaughter (Table 6.5). Lower p results in lower cumulated costs but longer time until elimination. The cumulated cost is remarkably stable for p values higher than 0.2, ranging between 1.47 to 1.87 billion Euros within a time range of 12 to 32 years to reach freedom from disease. The cumulated cost of BTB test and slaughter intervention and the time to elimination were lower using 2mm cut-off of SICTT compared to the 4mm cut-off (Figure 6.6).

Table VI.5. Relationship of proportion of animals included in test and slaughter and the cumulated cost and time freedom from disease (Individual animal prevalence <1/1000)

Proportion of test and slaughter p	Cumulated cost of control in Euro* (4mm Test)	Time to reach freedom from disease in years (4mm Test)	Cumulated cost of control in Euro* (2mm Test)	Time to reach freedom from disease in years (2mm Test)
0		> 100		> 100
0.1		> 100		> 100
0.2		> 100	1.87 * 10^9	75
0.3		> 100	1.68 * 10^9	44
0.4		> 100	1.60 * 10^9	32
0.5		> 100	1.55 * 10^9	25
0.6		> 100	1.53 * 10^9	20
0.7	1.99 * 10^9	82	1.51 * 10^9	17
0.8	1.94 * 10^9	69	1.49 * 10^9	15
0.9	1.90 * 10^9	59	1.48 * 10^9	13
1	1.84 * 10^9	51	1.47 * 10^9	12

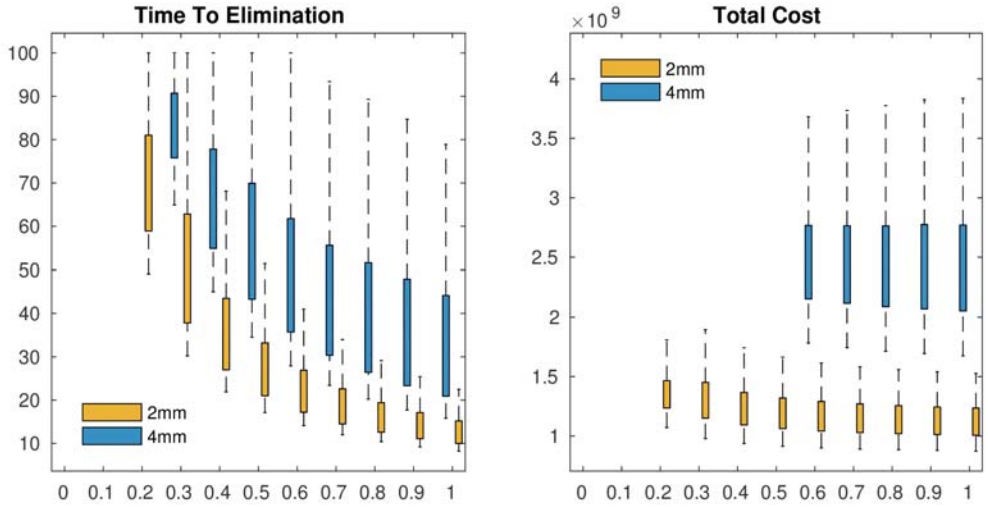

Figure VI.6. Total Cost of the interventions that reach elimination for different proportions of tested animals and 2mm cutoff test (yellow) and 4mm cut off test (blue)

Discussion and Conclusions

This manuscript presents the first cattle to cattle and cattle to human compartmental deterministic mathematical model. Differential equations were used to describe different pathways within and between compartments at human and animal level. Sensitivity analysis of the model has been used to determine the most sensitive parameter. Additionally, different scenarios of test and culling interventions were simulated by considering different proportions of tested population per year (p).

Model properties

To our knowledge this is the first model describing cattle to cattle and cattle to human transmission of BTB, although an African buffalo-human model has been published (65). Time series similar to those for brucellosis in Mongolia (66) are unfortunately not available but the available data allows for a parameterization under the assumption of endemic stability similar to Ethiopia (67). The estimated reproductive number of 1.325 is in the range for both low risk areas (R_0= 0.6-1.4) and high risk areas (R_0= 1.3-1.9) reported for the United Kingdom (68) but is lower than the 1.7-2.2 reported by Bekara et al. for France (69). Using a sensitivity analysis, the birth rate of cattle (b_c) was determined to be the most sensitive parameter of the model.

A key challenge in this model was to distinguish between exposed and infected cattle because the diagnostic test utilized was the intradermal tuberculin test. We used the proportion of cattle with active TB (13.5%) among cattle tested positive by tuberculin skin test, as reported by Ngandolo et al (52), to calculate the number of infected cattle. We did not address the sensitivity of tuberculin testing or within herd transmission because there is no data available on validation of tuberculin testing in Morocco or on within herd BTB transmission in Moroccan husbandry systems.

Further microbiological data is required to better describe BTB prevalence in humans in Morocco. Patients treated for active BTB do not completely clear all organisms from their body, with some bacteria persisting in bone marrow (56). Therefore, in contrast to our model, humans do not become completely susceptible again but technically are subject to re-infection (57). We argue that this has only a minor impact on total human BTB prevalence, but re-infection should be considered to refine the model.

Risk exposure of animals and humans to BTB could change according to sex and age groups. Because our model does not take into account such stratification, specific results for age groups were not provided.

Test and slaughter intervention

BTB prevalence was found to reach less than one per thousand in less than 20 years when the proportion of tested cattle was above 30%. The annual cost for this

potential intervention was nearly 78 million Euros. Intervention in cattle was found to impact the prevalence of human TB due to *M. bovis*, which decreased from 5 per 10'000 to 1 per 10'000 after 17 years.

The economic assessment presented here is preliminary, and a detailed cost and cost-effectiveness analysis will be published separately. However, our analysis informs Moroccan bovine tuberculosis control policy on the time horizon, range of cost and optimal levels of intervention. An effective control programme will depend on the human resources and technical and logistical capacity of the veterinary services to implement testing and slaughtering of animals. If the proportion of cattle subjected to test and slaughter was greater than 60%, freedom from disease would be reached in less than 20 years. Our model simulates the removal of individual animals rather than whole herds. Past experience in Europe has shown that whole herd removal is critical for effective elimination in low prevalence situations (46). In addition, a herd based model of the Moroccan cattle population could potentially lead to a lower intervention cost, as it is more realistic.

A recalculation of the intervention cost taking into account stratification by breed, sex and age should be undertaken, as it could lead to a different cost estimation of BTB control strategy.

Shortage of human resources should be considered for intervention planning, a maximum of 40% cull rate might be feasible; however, it would be costly in view of current Moroccan economic situation. One may think that test and slaughter implementation would lead to a reduction in cattle population and its by-product. But on the other hand, the increased import of cattle from other countries, with enhanced control measures, could maintain the current population density. In the meantime, as dairy products are provided mostly from highly controlled farms where BTB has a very low prevalence, we could argue that milk production would not be significantly affected.

Towards One Health

The WHO includes BTB amongst the seven neglected zoonoses which are perceived to be severe threats to public health (1). Further molecular epidemiology investigations in Morocco are needed in order to clarify local and national human BTB/TB ratios. To reach this goal, closer collaboration, at the national and international level, between the human and animal health sectors through a One Health approach is highly recommended. Operations in these two sectors remain largely independent in Morocco. Communication must be enhanced to establish a One Health approach, which has proven efficacy in health service delivery and potential for economic savings in zoonosis control (70, 71).

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VII. Cost estimate of bovine tuberculosis to the livestock sector of Morocco

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Abstract

In Morocco, cattle production is one of the most important components of the agricultural economy. The development of this sector is faced by many problems, like poor infrastructure, lack of services and climate change, in addition to infectious diseases like bovine tuberculosis. Bovine tuberculosis is a chronic zoonosis caused by *M. bovis*, which has the ability to infect a wide range of animals, as well as humans

The first objective of this study is to develop a demographic model for the cattle population of Morocco based on real data. The second objective is to estimate the cost of BTB to the Moroccan economy, considering the losses in the animal sector.

In order to reach the objectives, a deterministic model was conceptualized in Vensim considering 5 age classes for females and 3 age classes for males. One year was considered as a time step. In addition, using the matrix model, cattle population was simulated for 18 years.

The productivity losses triggered by BTB (5%) were estimated for 18 years, applying Leslie matrix with and without BTB. An endemic stable state of 18% was considered for this estimation. Cattle Moroccan population was compared with and without the disease, and the loss in term of animal numbers was then calculated. The number of cattle lost due to BTB was then transformed into monetary value. The total amount lost due to BTB in 18 years, considering the productivity loss is 913 Million Euro.

The present working paper present the first BTB cost estimation for Morocco. The analysis presented here is preliminary; a complete analysis will be done for the full paper.

The cost estimation of BTB is an important step, which will contribute to the advocacy of BTB control to decision makers. In addition, the inclusion of the societal cost of human health of BTB into the total cost estimation will constitute a strong argument to promote the animal-human collaboration in order to control and eliminate BTB in Morocco using a One Health approach

Keywords: Morocco, Cattle population, Deterministic model, Matrix model, Cost of BTB

Introduction

In Morocco, cattle production is one of the most important components of the agricultural economy; it generates 35 milliards of Moroccan Dirhams (MAD) (1 Euro = 10.87 MAD, (1)) per year which represents more than 44% of the agricultural product. This sector contributes heavily to the development of the Moroccan economy by generating many job opportunities, more than 2.5 million persons work in the animal production sector. The development of this sector is faced by many problems, like poor infrastructure, lack of services and climate change. A large number of Moroccan small farmers follow the ambitious “Plan Maroc vert” (PMV), using an integrated and global approach towards sustainable social and ecological development. This program has multiple objectives, as to increase the animal production aiming at the production of 5 million tons of milk by 2020 (2).

While the livestock production system is growing in Morocco, cattle are facing many infectious diseases which cause economic losses. Some of these diseases, e.g. bovine tuberculosis are zoonotic and cause a human health burden in addition.

Bovine tuberculosis is a chronic zoonosis caused by *M. bovis*, which has the ability to infect a wide range of animals, as well as humans (3). Even though, the preferential host of *M. bovis* is cattle (4), *M. bovis* have been described in several wildlife hosts like the badger (*Meles meles*), and the Possum (*Trichosurus vulpecula*) (5). In Morocco, *M. bovis* have been recently described in wild boar (6), however, there is still no strong evidence about the exact role of wild boar in BTB transmission and persistence in Morocco.

M. bovis can be transmitted between animals through the inhalation of aerosols and the ingestion of contaminated feed (7), while the transmission to humans is possible via the contact with infected cattle in addition to the consumption of unpasteurized milk and milk products (8).

The net growth rate of a population is the difference between the per capita birth and mortality rates. In a demographic matrix model a projection matrix P is multiplied with a population vector V composed of age and sex classes as $V_{(t+1)} = P \times V_{(t)}$. The composition of the population is in equilibrium if the relative proportions of the age and sex classes are stable. V is then an Eigenvector of P and the dominant Eigenvalue of P represents the net growth rate of the population (9). A mathematical model is a representation of “reality” (10). Deterministic models of a population using differential equations represent the flow of individuals between different age and sex classes which are considered as compartments. Per capita birth and survival rates constitute the elements of the projection matrix of a matrix model(11).In this way cattle populations can simulated as a set of coupled differential equations or as a matrix model. (10). The simulation of cattle populations provides the backbone to animal health economic assessments, aiming at providing a framework of concepts,

procedures and data to support the decision making process in optimizing animal health management. It actually deals with quantifying the financial effects of animal diseases, develop methods for optimizing decisions when animals, herds or populations are affected, in addition to determining the costs and benefits of disease control measures (12)

Bovine tuberculosis is a zoonosis which affects livestock industry, public health sector and wildlife reservoir. BTB has also indirect effects on trade restrictions in endemic countries. Tourism and other areas of public and private interest may also be affected indirectly by BTB infection.

BTB affects cattle productivity, it causes a decrease in milk and meat production, in addition to a fertility reduction, it could also causes unknown indirect consequences for the whole ecosystem and economic activities by affecting wildlife (13).

Cattle productivity losses due to BTB were for the first time investigated by Meisinger et al. in 1969 (14) and afterwards by Denes et al in 1986 (15) and by Bernues et al in 1997(16). Actually, all the studies investigating quantified effects of BTB on livestock were conducted in developed countries (13). Considering those studies, the milk production loss due to BTB was estimated to range from 10% to 12% of total milk yield. Total meat production loss (losses due to emergency or illness slaughter + losses triggered by the normal slaughtering and routine meat inspection) were estimated to range between 6% and 12%. A replacement loss of 15% in infected animals was registered. In addition BTB have been shown to cause 5% decrease in fertility. An estimation of the losses caused by BTB per animal per year have shown 1/5 calf loss, and 1/5 replacement loss in infected animals.

The quantification of BTB burden by its cost calculation is not well investigated, it has been performed for some countries, such as Turkey (17) and Argentina (18), and more recently Ethiopia (19). Although, the cost of the control of BTB requires further assessments, Zinsstag et al (13) summarized economic studies on BTB control. It was then emphasized that benefit-cost analysis are very scarce. A model cost benefit analysis was performed in Canada, and showed an extreme high Benefit-cost ratio of 33/1. Similar studies were also performed in the USA, and found to be cost effective (20). While in the United Kingdom (UK), the opinions are contradictory, Sheehy et al in 1991 performed a benefit-cost analysis, and showed that the UK BTB control program is cost beneficial, it is to be highlighted that the benefit from preserving international market access was included in the analysis (21). On the other hand Power and Watts found that BTB control was not profitable when the control of badgers was included in the analysis (22). More recently, an analysis of the current costs spend on BTB control showed that BTB control in the UK is not effective and not profitable. The authors argue that even the public health benefit is negligible provided the mandatory pasteurization of milk (23). In Ethiopia, the cost of BTB ranged from 75.2 Million USD to 358 Million USD in the urban area, where the prevalence of BTB is higher, while the cost ranged from 500’000 to 4.9 million USD

for the rural area, where BTB prevalence is lower. The cost analysis of BTB in Ethiopia concluded that the control of BTB is not one of the priorities considering the financial component and the expensive control intervention required (24). However, these analyses did not take into account the cost to human health.

The prevalence of BTB among human TB has been reviewed by Muller et al in 61 countries, for whom the data is available so far. Overall median proportions of zoonotic TB observed were 2.8% in Africa, and 1.4% outside of Africa (25). In addition, WHO declared that *M. bovis* is responsible for 3.1% of all TB cases in humans (26).

Cost analysis and cost-effectiveness studies investigating the cost of different treatments and diagnostic strategies relative to human TB have been much performed, mostly in the developed countries (27–32). In addition, studies investigating direct costs of human TB were performed as well, in the USA, the cost of TB patient considering direct costs which are mainly covered by public sector, in addition to the loss in productivity was estimated to 17'000 USD for non-MDR patients, 134'000 USD for MRD patients and 430'000 for XDR patients (33). In Germany MDR and XDR TB patients cost were 82150 Euros, and 108733 respectively (34). In developing countries, the cost of human TB patients is much lower than the cost in developed countries; actually, in Uganda, the cost of the disease for one human TB patient ranges from 391 USD to 911 USD considering community-based care and hospital-based strategy respectively (35). In Zambia the cost of human TB from the patient perspective was found to be 25 USD (48% of the patient median monthly income) (36). Considering the direct and indirect costs triggered by TB, the cost of TB for the household in rural area in Nigeria was found to be 592 USD (37% of the median annual household income) (37). The cost of TB considering directly observed treatment in Brazil is 336 USD for the patients and 726 USD for the facility, direct and indirect costs were also considered (38).

Recently, cattle to cattle and cattle to human transmission model was developed for Morocco, Using this model, the cost of BTB elimination was estimated between 1.87 to 1.48 billion Euro with a time frame of 13 to 20 years to reach freedom from disease. This model showed also the positive impact of a BTB intervention in cattle on the zoonotic human TB prevalence (39).

Objectives:

The first objective of this study is was develop a demographic model for the cattle population of Morocco based on real data. The second objective was to estimate the cost of BTB to the Moroccan economy, considering the losses in the animal sector.

Materials/Methods

Overview of Moroccan cattle population

Cattle production in Morocco has an important role in the national economy; it participated to more than 50% of the national red meat supplying and almost 90% of Moroccan milk supplying (40). Statistics of cattle population are managed by the statistical section of the ministry of Agriculture; this data is reported to the veterinary services. We considered in our study data from 1995 to 2013. The Moroccan cattle population is composed in majority of cows aged from 3 to 9 years. The growth rate of cattle population from 1995 to 2013 is on average 1.017 with a minimum of 0.905 and a maximum of 1.097.

Moroccan cattle are composed of local breeds, pure imported breeds (represented in more than 80% by Holstein breed) and crossbreeds between local and imported called ameliorated breeds. The cattle population in Morocco in the 1970's was composed in majority of local breed. After the implementation of the milk program In 1975 and the meat program in 1978, Morocco began large scale importation of European breeds (Holstein in majority). In this way the Moroccan cattle herd experienced a transition dominantly local breeds (4 different Moroccan local breeds), to almost an equilibrium between the local breeds and the improved breed (pure and crossed) (40).

According to the data between 1995 and 2013, the birth rate ranged from 31% to 36% with an average of 32%. According to the livestock management service, the mortality of the calves is 8%, while it's 4% for heifers and 2% for cows. The milk production is variable and depends from many factors like the age and the breed of the cow, e.g.: the imported breeds have a higher milk production than the local breeds (41).

Cost analysis

Animal health

Losses triggered by BTB in the animal production were analyzed. Bovine tuberculosis caused losses in the milk production, a decrease in fertility, in addition to losses in the carcass weight and the meat and entrails condemned in the slaughterhouse.

No studies are available for Morocco estimating specific losses in milk and in fertility. Consequently, we will consider in our analysis, estimations from previous studies, which will be validated by Moroccan experts.

As a preliminary analysis, we considered the decrease in productivity caused by BTB. After the implementation of the Moroccan cattle population model using Vensim (42) and a Leslie Matrix, the population was compared with and without BTB, the fertility affected by BTB prevalence was calculated as following:

Fertility (with BTB) = Fertility (without BTB) * (1-(BTB prevalence*reduction in the parameter)) (24)

The differences between the number of cattle of the population without and with BTB were calculated. Using estimated values of the prices of cattle considering sex and age categories, the total amount lost because of BTB for 18 years was estimated.

Human health:

Bovine tuberculosis has also a human health burden, the exact human burden of *M. bovis* in Morocco is not yet known, however, a study performed on MRD tuberculosis patients showed a prevalence of 17.8% of *M. bovis*, we could though consider the proportion of MDR patients among the total TB cases in order to calculate a potential prevalence of *M. bovis* among human TB cases in Morocco.

The cost of the human burden of BTB will then be estimated considering the societal cost of a human TB patient (public and private costs), and the proportion of *M. bovis* among the overall human TB patients.

In order to estimate the societal cost of human TB for the patient and the hospital; first, we conducted a socio economic survey with human TB patients in Rabat hospital (Appendix 1), considering direct and indirect costs of TB. The survey had several parts: 1) socio demographic information, 2) *M. bovis* related risk factors, 3) expenses triggered by the disease, and 4) socio-economic consequences related to the disease. Second we conducted an individual interview with the head of the financial service in order to estimate the cost of TB patients for the hospital, considering that human TB treatment is free in Morocco. It is to be highlighted that the total amount spent by the hospital for each patient includes the direct and indirect costs (staff salaries, external charges).

A total of 112 surveys were conducted, a detailed analysis will follow afterwards. The survey will provide the following information for each TB patient:

- Amount spent by the patient before the hospitalization
- Amount spent by the patient during the hospitalization (direct costs)
- Amount spent by the patient during the hospitalization (indirect costs)
- Amount of the money spent by the hospital
- Hospitalization period
- Invalidity period
- Socio economic status

Results

Model

Data from 1995 to 2013 were used for the model; data of the years 1997, 1999, 2006, 2010 and 2012 were extrapolated from the year before and after. Using data from 2008 to 2013 (Table 1) the proportion of slaughtered cattle was calculated using the cattle meat production, the average carcass weight as given by the national data (43).

Table VII.1. Cattle meat production and the proportion of slaughtered cattle (2208-2013) (44)

Year	Cattle meat production (T)	Number of slaughtered cattle (av=220 Kg)	Total cattle population	Proportion of slaughtered cattle (%)
2008	180000	818’182	2‘814‘000	29
2009	190000	863’636	2‘545‘300	34
2010	192000	872’727	2‘791‘650	31
2011	194000	881’818	3‘038‘000	29
2012	197000	895’455	3‘105‘500	29
2013	198000	900’000	3‘173‘000	28

An average proportion of 30% of the total Moroccan cattle population is slaughtered per year (Table 7.1). This proportion was applied to the total cattle population from 1995 to 2007 in order to have a total slaughtered population.

Deterministic model

The deterministic model was conceptualized in Vensim (42) considering 5 age classes for females (calves, heifers, primipara, cow and old cow) and 3 age classes for males (Male calf, Young bull and bull), for each category we considered the birth rate, the slaughtered rate, and the transition rate from the previous compartment (Figure7.1). For every age and sex class a differential equation was written, ignoring density dependence.. In our model we used a time step of one year. The parameters and the variables used in the model are presented in Table 7.2 and 7.3, with their respective units and values.

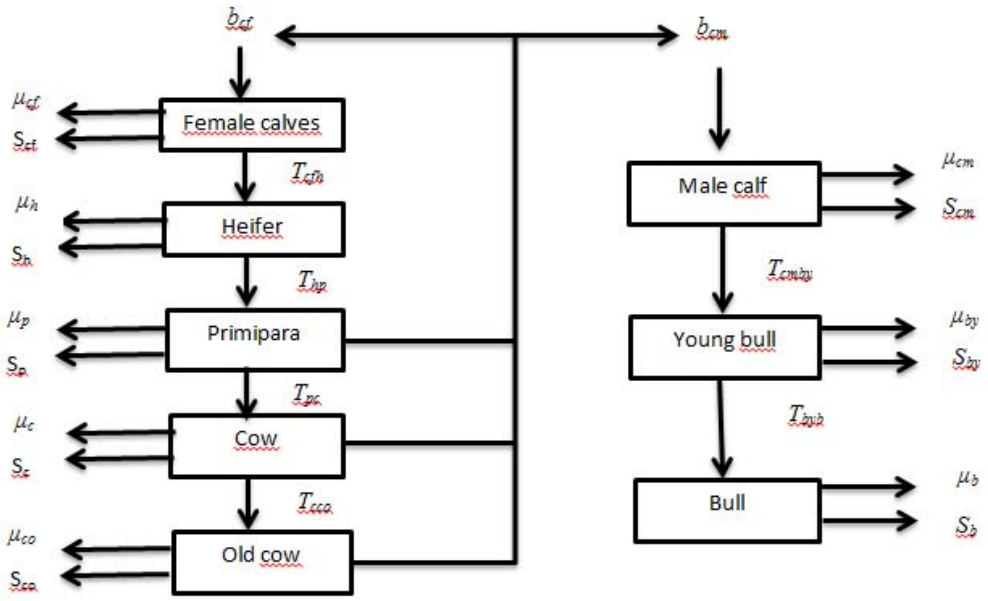

Figure VII.1. Schematic diagram for Moroccan cattle population model

The total cattle population Pc is divided to 8 age and sex compartments, female calves (C_f), Heifer (H), Primipara (P), Cow (C), Old cow (C_o), Male calf (C_m), Young bull (B_y), Bull (B). At a time (t), the total population will be:

$$P_c(t)= C_f(t)+H(t)+P(t)+C(t)+ C_o(t)+ C_m(t) + B_y(t) +B(t) \rightarrow \textbf{(1)}$$

Table VII.2. Interpretation and value of the parameters used in the model as starting values for the optimization

Parameters	Interpretation	Value	Unit	Source
b_{cf}	birth rate female calves	0.375	1/Year	Calculated from data
b_{cm}	birth rate male calves	0.375	1/Year	Calculated from data
μ_{cf}	mortality rate female calves	0.08	1/Year	(41)
μ_h	mortality rate heifer	0.04	1/Year	(41)
μ_p	mortality rate primipara	0.03	1/Year	Assumption
μ_c	mortality rate cow	0.02	1/Year	(41)
μ_{co}	mortality rate old cow	0.05	1/Year	Assumption
μ_{cm}	mortality rate male calves	0.08	1/Year	(41)
μ_{by}	mortality rate young bulls	0.03	1/Year	Assumption
μ_b	mortality rate bulls	0.03	1/Year	Assumption
S_{cf}	slaughter rate female calves	0	1/Year	Assumption
S_h	slaugther rate heifer	0	1/Year	Assumption
S_p	slaugther rate primipara	0.01	1/Year	Assumption
S_c	slaugther rate cow	0.2	1/Year	Assumption
S_{co}	slaugther rate old cow	0.8	1/Year	Assumption
S_{cm}	slaughter rate male calves	0.1	1/ Year	Assumption
S_{by}	slaughter rate young bulls	0.8	1/Year	Assumption
S_b	slaughter rate bulls	0.95	1/Year	Assumption
T_{pc}	primipara to cow rate	1	1/Year	Calculated from data
T_{cco}	cow to old cow rate	1/6	1/Year	Calculated from data
T_{cby}	calf to young bull rate	1	1/Year	Calculated from data
T_{byb}	young bull to bull rate	0.5	1/Year	Calculated from data

Table VII.3. Interpretation and unit of the compartment variables considered in the model

Variables	Interpretation	Unit	Cattle number (1995)	Source
C_f	Female calves	Animal	363'337	National statistics_Morocco
H	Heifer	Animal	172'107	National statistics_Morocco
P	Primipara	Animal	191'230	National statistics_Morocco
C	Cow	Animal	1'070'888	National statistics_Morocco
C_o	Old Cow	Animal	114'738	National statistics_Morocco
C_m	Mal calf	Animal	340'548	National statistics_Morocco
B_y	Young bull	Animal	207'792	National statistics_Morocco
B	Bull	Animal	28'860	National statistics_Morocco
P_c	Total population	Animal	2'489'500	National statistics_Morocco

The differential equations below represent the number of animals per compartment considering the animals in the other compartments and the different parameters:

Female calves

$dC_f/dt=b_{cf}*(P+C+C_o)-(C_f*T_{cfh})-m_{cf}-S_{cf}$ → (2)

Heifers

$dH/dt= (C_f*T_{cfh})-(H*T_{hp})-m_h-S_h$ → (3)

Primipara

$dP/dt = (H*T_{hp})-(P*T_{pc})-m_p -S_p \rightarrow (4)$

Cow

$dC/dt=(P* T_{pc})-m_c-S_c \rightarrow (5)$

Old cow

$dC_o/dt = (C*T_{cco})-m_{co}-S_{co} \rightarrow (6)$

Male calf

$dC_m/dt=b_m*(P+C+C_o)-(C_m*T_{cmby})-m_{cm}-S_{cm} \rightarrow (7)$

Young bull

$dB_y/dt = (C_m*T_{cmby}) -(B_y*T_{byb})-m_{by}-S_{by} \rightarrow (8)$

Bull

$dB/dt=(B_y*T_{byb})-m_b-S_b \rightarrow (9)$

Optimization and implementation of the Matrix model

Several calibrations were performed using Vensim (42), Similarly to Zinsstag et al (45), the Powell nonlinear maximum-likelihood optimization algorithm was used to fit the model to the data (46). Parameters were optimized on the basis of the goodness-of-fit, called ‘‘payoff’’ in Vensim software. The payoff compares the log likelihood of the current model with the log likelihood of a perfect model. The best model is the one with smallest payoff. The parameters from the best calibration were used for the Leslie matrix.

Using the parameters optimized by Vensim, we implemented the model using Leslie matrix as described above.

Table VII.4. Cattle productivity parameters used in the projection matrix

Parameter	Central value
Fertility rate for female	0.376
Fertility rate for male	0.344
Survival female calves	0.517
Survival heifers	0.531
Survival primipara	1
Survival cows	0.28
Inverse years as cow	0.83
Inverse years as old cow	0.5
Survival male calves	0.39
Survival young bulls	0.01
Inverse years as young bulls	0.5
Inverse years as bulls	0.84

Using the matrix model, cattle population was simulated for 18 years, the results obtained were compared to the official national data (Figure 7.2).

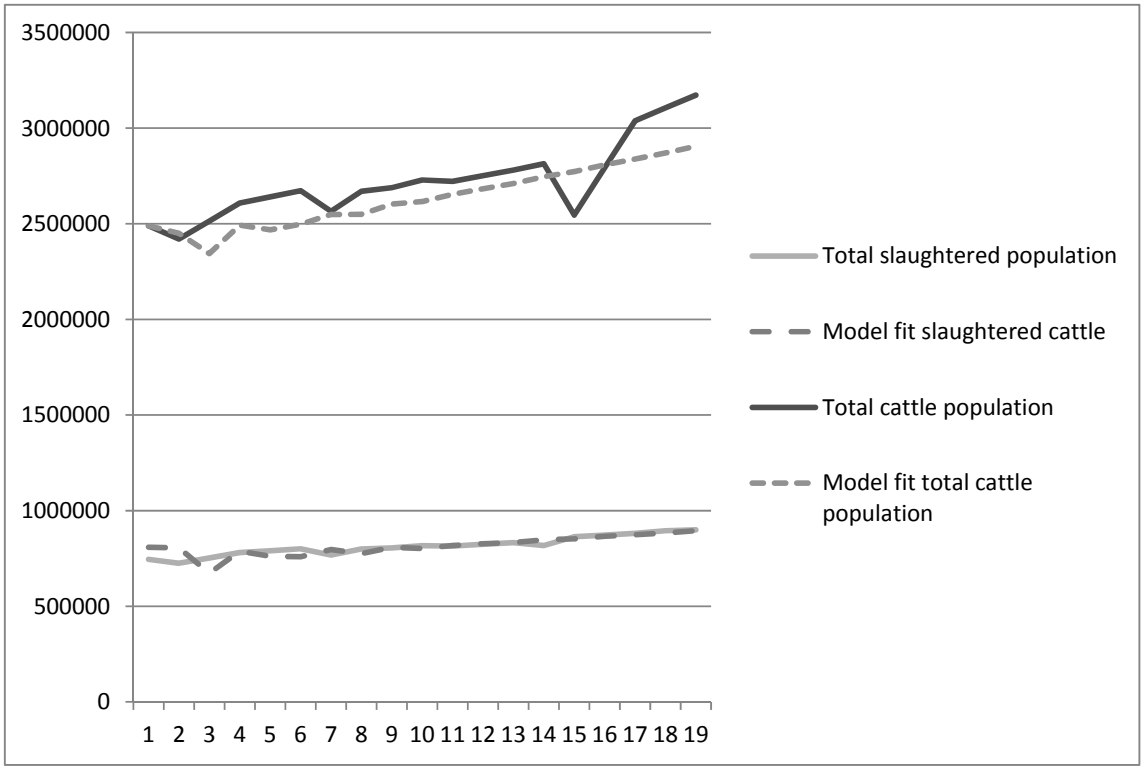

Figure VII.2. . Comparison between Moroccan total and slaughtered cattle population from national official data and data simulated using the deterministic model

The productivity losses triggered by BTB were estimated for 18 years, taking as a starting date 1995, and applying Leslie matrix with and without BTB (considering 5 % loss in productivity). An endemic stable state of 18% was considered for this

estimation. Cattle Moroccan population was compared with and without the disease, and the loss in term of animal numbers was then calculated. The number of cattle lost due to BTB was then transformed into monetary value, considering the average value of animals in every sex and age category. For the final paper, the discounting of monetary value will be included.

Table VII.5. Loss due to BTB considering the price of the animals by sex and age

Animal categories	Price_Min	Price_Max	Average price (MAD)	Loss_Due to BTB (MAD)	Loss_Due to BTB (Euro)
Female calve	5‘000	8‘000	6‘500	101‘273‘580	9‘316‘797
Heifer	15‘000	20‘000	17‘500	134‘065‘153	12‘333‘501
Primipara	15‘000	20‘000	17‘500	67‘559‘147	6‘215‘193
Cow	20‘000	28‘000	24‘000	390‘059‘951	35‘884‘080
Old cow	8‘000	15‘000	11‘500	90‘212‘701	8‘299‘237
Male calf	5‘000	8‘000	6‘500	92‘859‘054	8‘542‘691
Young bull	15‘000	20‘000	17‘500	176‘120‘398	16‘202‘428
Bull	20‘000	28‘000	24‘000	10‘430‘432	959‘561
Total				1‘062‘580‘416	97‘753‘488

Table 7.5 shows the different prices of the different categories of cattle (Marhaben A., Personal communication, 2016), in addition to the respective loss due to BTB. The asset value of the living animals lost due to BTB in year 18 is 98 Million Euro (1 Euro=10.87 MAD).

The estimation of the cumulated losses triggered by BTB in milk production and carcass weight will be estimated in the same way for the final paper. Milk yield data and average carcass weight by sex and age are needed to complete the cost analysis.

Discussion and conclusion:

The present working paper present the first BTB cost estimation for Morocco. The analysis presented here is preliminary; a complete analysis will be done for the full paper. Here we considered only the effect of the loss of fertility triggered by BTB on the asset value of the living cattle population. Deterministic and Matrix models were used in order to create the demographic model of the Moroccan cattle population, the cost of BTB considering the loss in productivity was estimated to be 98 Million Euro in a the year 18. The current optimization of the deterministic model in Vensim will be confirmed using a matrix model MATLAB®.

The cost estimation of BTB is an important step, which will contribute to the advocacy of BTB control to decision makers. In addition, the inclusion of the societal cost of human health of BTB into the total cost estimation will constitute a strong argument to promote the animal-human collaboration in order to control and eliminate BTB in Morocco using a One Health approach.

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VIII. Discussion

The present thesis has 4 components, we first investigated the prevalence of BTB in Sidi Kacem area of Morocco, and afterwards, we conducted a molecular characterization of BTB isolated from two slaughterhouses in Morocco. In parallel, a transmission model for animal-human BTB was realized, followed by a cost estimate of bovine tuberculosis to the livestock sector. The results shown by the present thesis constitutes a step forward in the control of BTB in Morocco; those results will be used for the advocacy of BTB control in Morocco. The results will be disseminated through trans-disciplinary meetings including all the stakeholders in order to enhance the collaboration and the One health approach in Morocco.

In Sidi Kacem in Morocco, 1201 cattle were screened for BTB using SICTT, the prevalence found, when compared to the last national prevalence study performed in 2004, highlighted likely an endemic stable state (20.4% in 2012 vs. 18% in 2004). The slight difference could be due to the study area, which could also explain the higher herd prevalence (56% in 2012 vs. 33% in 2004). Even if the BTB prevalence found in Morocco is higher than that registered in Niger (101) and in Tanzania (95), and lower than BTB prevalence in Mozambique (94) and in Zambia (102), the endemic stable state registered in Morocco is similar to other African countries.

The test used in the prevalence study was the SCITT. In Morocco, the legislation requires the use of SITT first, and SCITT is used as confirmatory test. In the prevalence study, we decided to use the SCITT directly because of logistic matters and to enhance the specificity of the test. Gamma interferon tests have been already used in African settings (190,191), and it could be a good alternative to the SCITT in Morocco to encounter the logistic problems with the SICTT, as the interferon gamma test does not require a second visit (192,193).

Using a random effect model, univariate and multivariate analysis were used in order to perform the statistical analysis of the individual risk factors. Consequently, risk factors that showed significantly higher risk for BTB were identified. Cross-breeds showed a higher risk for BTB compared with the local breed; this could be explained by the higher susceptibility of the cross-breeds to BTB, but needs further studying on the effects of management density of animals. In addition cross-breeds are usually kept in larger herd sizes, in intensive or semi-intensive livestock production systems (LPS), under irrigated rearing systems, which are also factors associated with higher risk of BTB. The previous risk factors have already been linked to higher BTB risk in other settings; in Ethiopia, where intensive livestock production system has shown a higher risk for BTB, with a higher prevalence registered in intensive farms (urban area) in contrast to the rural area which is characterized by extensive LPS (126,127). On the other hand, similarly to our results, the local breed showed a lower risk for BTB, as already described in Mozambique (94).

The livestock production sector in Morocco is quickly growing, due to the ambitious “plan Maroc vert” launched in 2008 in addition to increasing consumption of animal protein in Morocco. Consequently, the livestock production systems are moving towards the intensified and irrigation rearing systems. Those factors, when applied in small to medium herds, with limited means, may have adverse consequences, like a confined environment, with limited access to sun and fresh air, factors that enhance the transmission of mycobacteria between animals and promotes its persistence in the environment. This is in contrast to extensive rearing systems that allow good access to fresh air and sun.

The prevalence of visible BTB lesions in the slaughterhouses of Rabat and El Jadida was 3.7 %, similar to that reported in Algeria (3.6%) (194). The relatively low prevalence of proven *M. bovis* infection found in the abattoirs has to be interpreted with caution. Firstly, the abattoir prevalence reflects more the prevalence in young bulls and old cows rather than the whole population. Secondly, not all granuloma yielded a bacteriological isolate, hence the true prevalence may be much higher than the one observed. Overall, our findings reflect rather the epidemiological situation of a low level endemic transmission similar to other African countries rather than the one of a peri-urban intensive system (55,56).

Surprisingly, no non-tuberculous mycobacteria was found, while in the African context, among slaughterhouse gross visible lesions, 3.3 % in Mali (40), and 9 % in Burkina Fasso (41) were confirmed as non-tuberculous mycobacteria. Additional studies in Chad showed the isolation of NTM slaughterhouse gross visible lesions (33,34). Further molecular analysis with increased sample size and for other slaughterhouses will provide further input in regard to the proportion of non-tuberculous mycobacteria in Morocco

The molecular characterization using deletion typing and spoligotyping of Moroccan BTB isolates showed 43 different spoligotype patterns, of which 33 were already reported in other countries, mostly in Europe. The existence of European spoligotypes in Morocco could be explained by importation of cattle from European countries to Morocco. This element should be considered for a future control strategy, especially in the last stages of the strategy, to prevent a re-increase of the prevalence. In addition, the first introduction of BTB to Morocco is suggested to occur between 1913 and 1950 by the importation in the thirties of European cattle breeds and the introduction of new management systems (195). The fact that Moroccan *M. bovis* has similar spoligotype patterns as European once could be a consequence of this introduction, however, Morocco continue to import cattle from Europe.

On the other hand, spoligotypes of African 1 and African 2 clonal complexes were not reported among our characterized isolates. Considering the localization of African 1 and African 2 in West Central Africa and East Africa respectively, we would conclude that the Sahara could be considered as a potential efficient barrier preventing the introduction of BTB to Morocco from West-Central and East Africa.

Additionally, ten new spoligotypes were reported and submitted to the *M. bovis* database, where they were given new reference numbers.

More research is needed to further characterize the ongoing transmission patterns of bovine tuberculosis in view of the development of a locally adapted elimination strategy of bovine tuberculosis in Morocco (57). In addition, more molecular characterization is needed to investigate the strains circulating in the south and the north of Morocco. Whole genome sequencing could also be useful to gain insight about Moroccan BTB strains, although to date, almost no data is available for whole genome sequences of *M. bovis* in Africa. An overall study considering different countries from Africa would have an added value and give more information about the transmission dynamics of BTB in Africa, and the links between BTB in different African countries.

Co-evolution scenarios have been realized for human TB, to understand the co-evolution of MTBC in regard to the human evolution. It was suggested then that MTBC has been able to adapt to changing in human population and that there is a co-evolution of MTBC with anatomically modern humans for tens of thousands years (Comas et al. 2013). Similarly, a study interested in the co-evolution of MTBC in regard to cattle population will provide an understanding of the origin of *M. bovis*, and the respective co-evolution, this could bring an understanding of the dynamics of *M. bovis.*

Collaboration between African countries for BTB molecular investigation is likely to provide a better understanding of the past evolution of the disease. This understanding could contribute to build more adapted control strategies. An African collaboration could also be efficient for mutual lesson learning, recommendations from different African countries could be bring together and adapted to each context.

The illegal trade of animal from other African countries to Morocco is a crucial point, which will impact the success of a potential control strategy, this element should be rigorously controlled for a most efficient and sustainable BTB control and elimination intervention. Moreover, in Morocco, a big issue facing disease control, especially zoonosis control is the informal trade of diary and meat products. This informal sector is difficult to control, and it is also a key element to consider for a future BTB control strategy.

The identification of the Moroccan cattle is big step that Moroccan veterinary services started a year ago. In the USA, the application of animal traceability is challenging for the abattoir surveillance (114). The identification of the total cattle population will contribute positively into the success and the sustainability of a potential BTB elimination intervention in Morocco, and will provide better abattoir surveillance.

An efficient identification of the Moroccan cattle population could contribute to the control of the illegal trade of animals inside the country, and also with neighboring

countries. In addition, Milk and meat informal trade constitutes a risky behavior, which promotes the possible transmission of *M. bovis* to humans. The control of the informal ways of milk and meat trade in Morocco should be preferentially performed before the implementation of any control or elimination control strategies.

Cattle to cattle and cattle to human compartmental deterministic mathematic model was elaborated for Morocco. The reproductive number was subsequently calculated to be 1.325, in the range of both low risk areas (R_0= 0.6-1.4) and high risk areas (R_0=1.3-1.9) as previously reported in the United Kingdom (196), however, it was lower than the R_0 (1.7-2.2) reported in France (197). Using a senstivity analysis, , the birth rate of cattle bc was the most sensitive parameter for the total cost, influencing the dynamics of BTB transmission. High birth rate values lead to an increased cattle population yielding higher costs for elimination. For the time to elimination, the sensitivity of the test was the most sensitive parameter. Low test sensitivity (i.e. with cut-off at 4mm) leads to low detection of infected animals and therefore less culling which leads to a longer time to elimination.

In addition, a preliminary economic assessment of the cost of a potential BTB intervention in Morocco was performed. It was found that the cost of the intervention depends on the percentage of test and slaughter (the annual coverage). The cumulated cost was remarkably stable for coverage values higher than 40%, ranging from 1.47 and 1.87 billion Euros within a time frame 12 to 44 years to reach freedom from disease. Shortage of human resources should be considered for intervention planning, a maximum of 40% cull rate might be feasible; however, it would be costly in view of current Moroccan economic situation.

More parameters should be considered in the model in order to further refine it, e.g., re-infection of human TB recovered patients was not considered in the model. In addition, further molecular characterization of the BTB/TB ratio in human patients in Morocco is needed in order to be able to quantify the BTB human burden.

The transmission model allowed us to investigate the link between BTB intervention in animals and the prevalence of human TB due to *M. bovis*. With 40 % coverage of test and slaughter, the prevalence of exposed and active human cases due to *M. bovis* decreased from 5 per 10’000 to less than 1 in 10’000 at the time of freedom from disease after 17 years.

The OIE recommended cut-off for SICTT interpretation is 4 mm, however, many studies showed that a severe cut-off of 2 mm increased the sensitivity of the test (198,199), without affecting the specificity compared to the recommended 4 mm cut-off (200). The two cut offs were compared using the model. The cumulated cost of BTB test and slaughter intervention and the time to elimination were lower using 2mm cut-off of SICTT compared to the 4mm cut-off.

Considering the economic situation of Morocco as a middle income country, BTB intervention remains unaffordable. In developing countries, as well as in Morocco,

BTB is a neglected problem, the limited financial and human resources available are mainly engaged in the control of other highly contagious diseases and parasitic diseases. The only BTB control measure which is applied in Morocco is the slaughterhouse inspection of cattle and condemnation of animals (or organs) with BTB suspicious lesions, a measure which remains inadequate for efficient BTB control. In order to deal with this problem, collaboration between the private milk industry and the public veterinary services may be useful to launch a BTB control and elimination program. The milk industry in Morocco, in addition to the meat industry, will benefit from the elimination of BTB.

Deterministic and matrix models were used to perform a preliminary cost analysis of BTB considering the losses in productivity. The amount of 98 Million Euro was found to be the asset value lost due to BTB in the year 18 years, with an endemic stable prevalence of 18%.

The optimization of the demographic model using MATLAB will provide a useful and efficient tool for livestock management in Morocco. The conceptualized and optimized model could be used for the estimation of the cost of infectious disease for the Moroccan livestock, in addition to the estimation of the cost of potential disease control strategies.

One health approaches have shown their efficiency in many settings. In Chad, a OH health approach was used in a vaccination campaign for both pastoralists and their animals which led to a reduction of the cost by 15% (175,176). In addition, an intervention can become highly cost effective when costs are shared between different sectors respective to their benefits (201). In Morocco, human and animal health experts have been introduced to the OH approach; however, the collaboration between the two sectors is currently at a very low level. Moreover, almost no communication exists between the ministries of Health and of Agriculture. In order to enhance the communication and collaboration between the two ministries, many meetings should be held, and a trans-disciplinary (TD) approach could be useful.

Considering that the implementation of BTB control strategy should be motivated by both public health issues and the economic losses triggered. Trans-disciplinary approach is an efficient way to set up a beneficial dialogue between different disciplines, and bring them to work together and collaborate. The benefit from an efficient collaboration between the two sectors should be highlighted using examples from other countries, or models from Morocco, showing the effect of animal interventions in the prevalence of human zoonosis. Zinsstag et al demonstrated, using brucellosis, rabies and influenza examples, that zoonosis control intervention could become cost efficient when the societal component is considered (179). The results of the present thesis could be used as advocacy elements during the TD meetings with human health, animal health experts and decision makers in Morocco.

A potential way to enhance the collaboration between the two sectors would be to set up a common project between human and animal health experts, where all the

partners are actively and commonly leading the project actions. The establishment of sustainable collaborations between the veterinary services and the human health sector could be an efficient way to bring the two sectors together and promote one health approach in Morocco.

In addition, considering the animal health sector in Morocco, contact between livestock keepers, local authorities and veterinary services is characterized by mistrust by the owners. While a solid and sustainable control program cannot be achieved without the contribution of all the stakeholders, and although the local population is considered as a substantial stakeholder, we have to start from the bottom and roll-out an adapted awareness program to make the local population aware about the necessity of a national control program. Awareness campaigns have been shown to be cost-effective and beneficial in order to enhance disease awareness among the population. Besides, a better awareness of the disease is key element for an effective application n and sustainability of control and elimination strategies (120,121).

The implementation of an awareness stainable strategy in Morocco for several zoonoses in Tandem could contribute to a better acceptance of the control strategies by the population afterwards. In a long term, health education could contribute to the Modification of some risky cultural behaviors like the consumption of unpasteurized milk and traditional milk product.

In Morocco, the application of BTB elimination strategy is a long term goal, as it is not logistically and financially feasible currently. Bovine tuberculosis control measures are almost inexistent, expect from the abattoir surveillance. Consequently, further spread of the disease is possible in the future, with potential increase in the prevalence. In the meanwhile, some measures could be implemented like the control of illegal trade of cattle product in Morocco, and a better management of cattle movement.

IX. Perspectives and identified research needs

In order to reach a deeper understanding of molecular epidemiology of BTB in Morocco, further molecular investigations, are needed in other Moroccan slaughterhouses. In addition, information regarding BTB strains in the north and south of Morocco is not available so far.

Furthermore, new molecular techniques such as whole genome sequencing are needed to clarify more the potential links between Moroccan BTB strains and those of Europe and other African countries.

Collaboration with the other African countries, especially the neighboring countries for a deep investigation of BTB dynamics in Africa and the co-evolution of BTB in regard to cattle will provide a better understanding of BTB in Africa.

The results provided by the present thesis could be used to launch TD meetings with all the stakeholders involved in BTB problem in Morocco. Meetings in small level could be first performed, before to set up a national TD seminar, in collaboration with all the stakeholders.

In order to deal with the problem of unaffordability of BTB elimination intervention in Morocco, possible public-private collaborations for BTB control in Morocco have to be identified and explored.

X. Appendix

Appendix 1: Questionnaire for TB patients

Coût direct et indirect de la tuberculose humaine

Publique cible : Patients tuberculeux hospitalisés

1. Identification:……..Code …□□□

1.1. Nom du patient……

1.2. N° de tel :…… ……..

1.3. Lieu de l'enquête :……

1.4. Date d'enquête : ...../……../……..

1.5. Nom de l'enquêteur :

……..

2. Informations sociodémographiques

2.1. Sexe : □ Féminin □ Masculin

2.2. Age (ans) : □□

2.3. Occupation (s)……..

2.4. Secteur d'activité: □ Public □ Privé

2.5. Etat civil: □ Célibataire □ Marié □ Divorcé □ Veuf

2.6. Niveau d'instruction : □ (1. Aucun, 2. Ecole coranique, 3 Primaire, 4 Secondaire, 5 Supérieur)

2.7. Adresse: (Ville/Village / Commune / Province)……..……

……

2.8. Etes-vous indépendant financièrement : □ Oui□ Non

2.8.1 Si Non, de qui dépendez-vous :…….

2.8.2. Si oui,

2.8.2.1. Revenu mensuel moyen ? (en Dhs)……

2.8.2.2. Combien de personnes vivent dans votre foyer ? □□, dont à votre charge……□□

2.8.2.3. Parmi les personnes vivant dans votre maison combien sont :

- Enfants scolarisés □□
- Enfants non scolarisés □□
- Personnes âgées de plus de 60 ans □□.
- Adultes sans emploi □□
- Invalides (incapables de travailler) □□
- Actifs □□

Domaines d'activité	Revenu moyen (en Dhs)

2.9. Description du foyer:

Rural		Urbain	
Habitat traditionnel (terre battue)		Appartement	
Habitat moderne (briques et béton)		Maison individuelle	
Baraque en tôle		Baraque en tôle (bidonville)	
Collectif (plusieurs familles)		Collectif	
Individuel		Individuel	
Superficie approximative		Superficie approximative	
Nb. pièces habitables		Nb. pièces habitables	
Commentaires :…… ……		Commentaires :……. …….....	

3. Connaissance et perception de la maladie par le patient

3.8. Quel est l'origine de la tuberculose selon vous ?.....
.....
...

3.9. Quels sont les voies de transmission de la tuberculose selon vous ?

☐ Contact ☐ Utilisation des mêmes ustensiles de cuisine

☐ Autres (à préciser) ……..

4. **Informations sur les facteurs d'exposition à *M. bovis***

4.8. Distance entre la maison et les locaux d'élevage :

4.8.1. Même bâtiment…….…...…..mètres

4.8.2. Bâtiments différent…….mètres

4.9. Consommation du lait cru / produits laitiers à base de lait cru ☐ Oui ☐ Non

4.10. Contact avec un élevage de bovins ☐ Oui ☐ Non

4.10.1. Cadre professionnel ☐ Oui ☐ Non

4.10.2. Cadre ménager ☐ Oui ☐ Non

4.10.3. Occasionnellement ☐ Oui ☐ Non

4.11. Consommation de viande du circuit informel ☐ Oui ☐ Non

4.12. Consommation du lait provenant du colporteur/laiterie ☐ Oui ☐ Non

5. Facteurs de susceptibilité :

5.1. Consommation de :

5.1.1. La cigarette ☐ Oui ☐ Non

5.1.2. La drogue ☐ Oui ☐ Non

5.2. Autres maladies :

5.2.1. Diabète ☐ Oui ☐ Non

5.2.2. Insuffisance rénale ☐ Oui ☐ Non

5.2.3. Problèmes cardiaques ☐ Oui ☐ Non

6. Information sur la maladie

6.1. Quand est-ce que vous avez été diagnostiqué de la tuberculose : …./…../….

6.2. Combien de docteurs/guérisseurs avez-vous consulté avant de commencer le traitement antituberculeux :

Intervalle	Durée	Médecin /Guérisseur (M/G)	Frais Transport (Dhs)	Frais Consultations (Dhs)	Frais de médication (Dhs)
Apparition de symptômes et 1ère consultation					
1ère et 2ème consultation					
2ère et 3ème consultation					

Déduire intervalle Apparition de symptômes – suspicion de tuberculose: ……

Déduire intervalle Suspicion de tuberculose – confirmation: ……

6.3. Date d'hospitalisation :…../…../….. ; Date probable de sortie : …../…../…..

6.4. Quelle est, selon vous le type de tuberculose que vous avez? (appellation vernaculaire)

……

6.5. Le type de tuberculose selon le médecin traitant :

……

6.6. Les coûts engagés depuis le diagnostic jusqu'à présent :

Phase	Coûts associés (Dhs)	
	Avant diagnostic	Après diagnostic
Diagnostic		
Traitement à l'hôpital		
Traitement au foyer		
Convalescence		
Après la maladie		
Accompagnateur		

6.7. Avez-vous eu une récidive / récurrence : □ Oui □ Non

6.8. Si oui,

6.8.1. Combien cela vous a coûté (en Dhs):……

6.8.2. Est-ce qu'il y a eu un changement au niveau de votre travail et/ou votre revenu à cause de la maladie : □ Oui □ Non

6.8.3. Si oui, préciser……

6.8.4. Variation du revenu du à ce changement (en %):……..

6.9. Autres personnes de la famille ou de l'entourage immédiat ayant fait la tuberculose après vous ? □Oui □Non

6.10. Si oui, combien de personnes en milieu familial □□ et en milieu professionnel □□

7. Conséquences socio-économiques

7.1. Combien de personnes par jours vous rendaient elles visitent à l'hôpital : □□

7.2. La distance moyenne parcourue par ces personnes pour venir à l'hôpital : ……..……km/personne

7.3. Période d'invalidité en rapport avec la maladie (en mois) :…….

7.4. Avez-vous chargé quelqu'un pour assurer vos activités professionnelles pendant votre hospitalisation : □ Oui □ Non

7.5. Si oui :

7.5.1. Quelles activités :……..……

7.5.2. Combien ceci vous a coûté par mois ?……..…

7.6. Est-ce que votre revenu a baissé depuis que vous êtes malade : □ Oui □ Non

7.7. Est-ce que vous vous êtes senti victime d'une stigmatisation à cause de votre maladie ?

- De la part des amis □ Oui □ Non
- De la part de la famille □ Oui □ Non
- De la part des collègues □ Oui □ Non

7.8. La maladie a-t-elle causé des problèmes conjugaux □ Oui □ Non

Si oui:

- Problèmes mineurs □ Oui □ Non
- Problèmes majeures □ Oui □ Non
- Séparation temporaire □ Oui □ Non
- Séparation définitive (divorce) . □ Oui □ Non

Conséquences liées à ces problèmes :…….
……
……
……...

Lieux de l'enquête: Hôpital My Youssef, Rabat ; Hôpital XXXX, Kenitra ; CHU Ibn Rochd, Casablanca.

Equipe de recherche :

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- Pr. Fouad Sghrouchni (Institut National d'Hygiene, Rabat, Maroc)
- Pr. Jamal Eddine Bourekkadi (Hôpital My Youssef, Rabat, Maroc)
- Pr. Kamal Marhoum El filali (CHU Ibn Rochd, Casablanca, Maroc)
- Pr Jakob Zinsstag, Dr. Jan Hattendorf (Institut Tropicale et de Santé Publique, Université de Bâle, Suisse)
- Pr. Alexandra Shaw (AP Consultants, UK)

Appendix 2 : Supporting information 1

Supporting Information 1: Calculation of the cattle to cattle transmission rate

The equations for the cattle population are given by

$$\frac{\mathrm{d}S(t)}{\mathrm{d}t} = bS(t) + r_b b(E(t) + I(t)) - \beta\frac{S(t)I(t)}{N(t)} - \mu S(t) - (1 - s_p)pS(t), \tag{1a}$$

$$\frac{\mathrm{d}E(t)}{\mathrm{d}t} = \beta\frac{S(t)I(t)}{N(t)} - \alpha E(t) - \mu E(t) - s_c p E(t), \tag{1b}$$

$$\frac{\mathrm{d}I(t)}{\mathrm{d}t} = \alpha E(t) - \mu I(t) - s_c p I(t), \tag{1c}$$

where $N(t) = S(t) + E(t) + I(t)$ and

$$\frac{\mathrm{d}N(t)}{\mathrm{d}t} = bS(t) + r_b b(E(t) + I(t)) - \mu N(t) - (1 - s_p)pS(t) - s_c p(E(t) + I(t)).$$

If $b > \mu$ and $p = 0$ the total cattle population, N, increases exponentially. In order to calculate the pre intervention endemic equilibrium we therefore define the proportion of susceptible, exposed and infected cattle as

$$s(t) := \frac{S(t)}{N(t)}, \quad e(t) := \frac{E(t)}{N(t)}, \quad i(t) := \frac{I(t)}{N(t)}.$$

Using the chain rule and equation (1b) we get

$$\frac{\mathrm{d}e(t)}{\mathrm{d}t} = (b - r_b)e(t)^2 - \beta i(t)^2 + (b - r_b - \beta)e(t)i(t) - (b + \alpha)e(t) + \beta i(t).$$

and from equation (1c)

$$\frac{\mathrm{d}i(t)}{\mathrm{d}t} = (b - r_b)i(t)^2 - (b - r_b)e(t)i(t) + \alpha e(t) - bi(t).$$

There exists an equilibrium proportion of exposed cattle, e_*, and infective cattle, i_*, such that

$$0 = (b - r_b)e_*^2 - \beta i_*^2 + (b - r_b - \beta)e_* i_* - (b + \alpha)e_* + \beta i_*.$$

and

$$0 = (b - r_b)i_*^2 - (b - r_b)e_* i_* + \alpha e_* - bi_*.$$

We now choose the transmission rate β such that

$$e_* + i_* = \phi,$$

where ϕ is the endemic prevalence before the intervention. This yields

$$i_* = \frac{\alpha\phi}{\alpha + b - (b - r_b b)\phi},$$

$$e_* = \phi - i_*$$

and

$$\beta = \frac{(b - r_b b)e_*^2 + (b - r_b b)e_* i_* - (b + \alpha)e_*}{i_*^2 + e_* i_* - i_*}.$$

Appendix 3: Supporting information 2

Supporting Information 2: Calculation of the cattle to human transmission rate

The equations for the human population are given by

$$\frac{\mathrm{d}S(t)}{\mathrm{d}t} = bS(t) - \beta\frac{I_C(t)S(t)}{N(t)} - \mu S(t), \tag{1a}$$

$$\frac{\mathrm{d}E(t)}{\mathrm{d}t} = \beta\frac{I_C(t)S(t)}{N(t)} - \alpha E(t) - \mu E(t), \tag{1b}$$

$$\frac{\mathrm{d}I(t)}{\mathrm{d}t} = \alpha E(t) - \delta I(t) - \mu I(t), \tag{1c}$$

where $I_C(t)$ is the number of infected cattle at time t, $N(t) = S(t) + E(t) + I(t)$ and

$$\frac{\mathrm{d}N(t)}{\mathrm{d}t} = (b - \mu)N(t).$$

If $b > \mu$ the total human population, N, increases exponentially. In order to calculate the pre intervention endemic equilibrium we therefore define the proportion of susceptible, exposed and infected humans as

$$s(t) := \frac{S(t)}{N(t)}, \quad e(t) := \frac{E(t)}{N(t)}, \quad i(t) := \frac{I(t)}{N(t)}.$$

Using the chain rule and equation (1b) we get

$$\frac{\mathrm{d}e(t)}{\mathrm{d}t} = \beta i_C^* - \beta i_C^* e(t) - \beta i_C^* i(t) - \alpha e(t) - be(t).$$

where i_C^* is the equilibrium proportion of infected cattle. Equation (1c) yields

$$\frac{\mathrm{d}i(t)}{\mathrm{d}t} = \alpha e - \delta i - bi.$$

There exists an equilibrium proportion of exposed humans, e_*, and infective humans, i_*, such that

$$0 = \beta i_C^* - \beta i_C^* e_* - \beta i_C^* i_* - \alpha e_* - be_*.$$

and

$$0 = \alpha e_* - \delta i_* - bi_*.$$

We now choose the transmission rate β such that

$$e_* + i_* = \phi,$$

where ϕ is the endemic prevalence before the intervention. This yields

$$i_* = \frac{\alpha\phi}{\alpha + \delta + b},$$

$$e_* = \phi - i_*$$

and

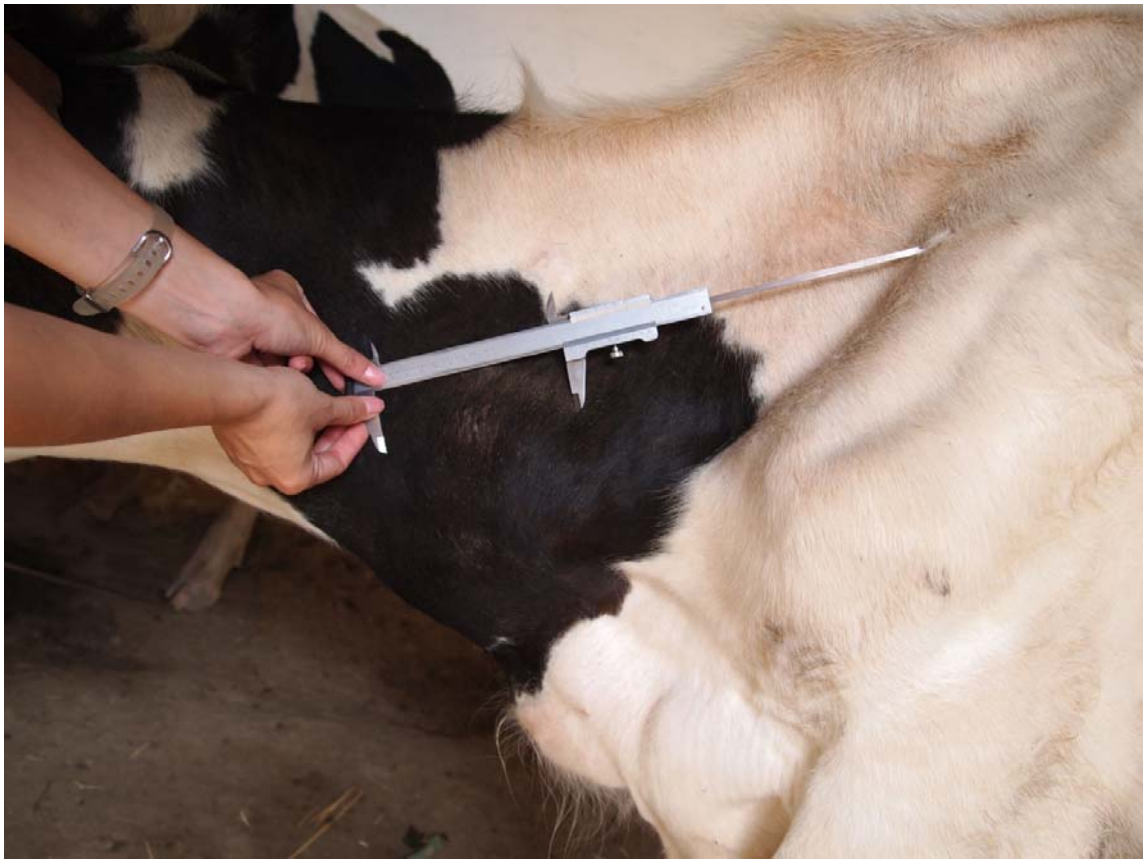
$$\beta = \frac{(\alpha + b)e_*}{i_C^* s_*}.$$

Appendix 4: Field and laboratory pictures

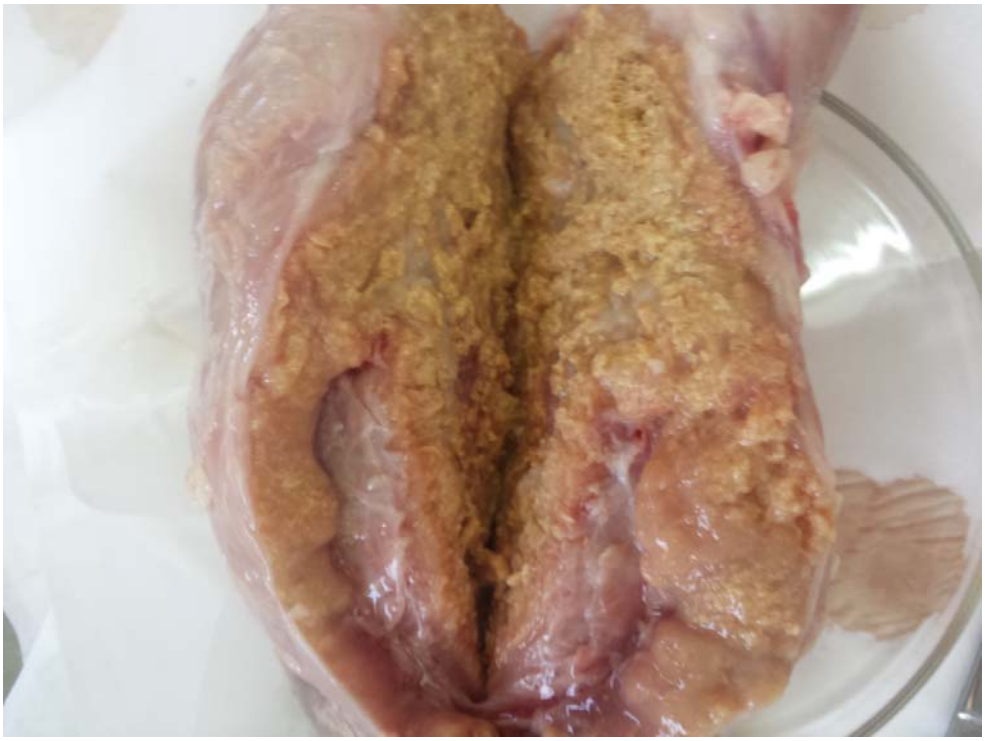
Survey with the household keeper in Sidi Kacem-Morocco

Blood sampling a goat in Sidi Kacem-Morocco

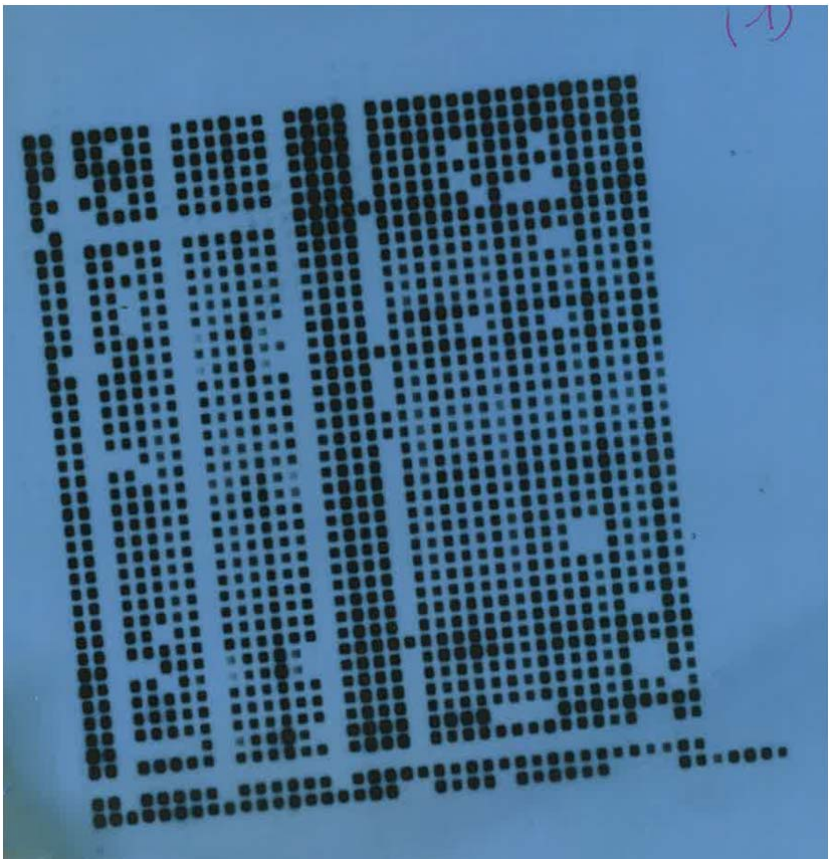
Tuberculin skin test investigation in Sidi kacem-Morocco



SCITT reaction in a female cattle in Sidi Kacem



Bovine tuberculosis granuloma from the abattoir of Rabat



Spoligotyping membrane-Tuberculosis laboratory-Swiss TPH

Resume

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PERSONAL DETAILS

Date of Birth:	29th April 1988
Nationality:	Moroccan
Languages:	English, French, Arabic (Proficient in reading and writing)

CAREER PROFILE

I am an early-career researcher and practitioner in the field of veterinary medicine, with 5 years' cumulative post-graduate experience in the epidemiology of zoonotic diseases. I have carried out research in Morocco and Switzerland and possess strong skills in managing international knowledge networks, research partnerships, program development and policy analysis in the field of veterinary medicine and One Health. Currently, I am working as a postdoctoral researcher in the department of Infectious diseases in the University of Georgia in the USA

AREAS OF EXPERTISE

- Molecular Characterization of zoonotic diseases
- Veterinary epidemiology
- Research project management
- One Health approach and disease control

ACADEMIC AND PROFESSIONAL QUALIFICATIONS

PhD (2013-2016)

Joint PhD. Swiss TPH-University of Basel, Switzerland and the IAV Hassan II in Rabat, Morocco (Magna cum laude).

Prevalence, Molecular Characterization, Transmission Dynamics and Cost Analysis of Bovine Tuberculosis in Morocco.

DVM (2006-2012)

Diploma and Doctorate in Veterinary Medicine, Agronomic and Veterinary Hassan II institute, Rabat, Morocco (High Honors).

Investigations on Bovine Tuberculosis and ruminant brucellosis in the province of Sidi Kacem in Morocco.

Baccalaureate (2003-2005)

Baccalaureate in Experimental Science

Continuous Professional Development and other Qualifications

Environmental Risk Management, Health Governance and Contingency Planning. International Graduate School, Basel – August 2016.

Bioethics and dual use

Preventive and Protective Measure against Chemical and Biological Hazards In Tunisia - April 2016

HONOURS AND AWARDS

- Fulbright research scholar grant: University of Georgia, USA, (July-September 2017)
- International Foundation for Science Grant (N° B 5643): Molecular characterization of Bovine Tuberculosis among slaughtered cattle in Morocco (January 2015-June 2016)

SELECTED PUBLICATIONS AND SEMINAR PRESENTATIONS

- Yahyaoui Azami H, Ducrotoy MJ, Bouslikhane M, Hattendorf J, Thrusfield M, Conde-Alvarez R, et al. The prevalence of ruminant brucellosis and Bovine Tuberculosis in Sidi Kacem area in Morocco. In Press. 2016.
- Yahyaoui Azami H, Aboukhassib H, Bouslikhane M, Berrada J, Rami S, Reinhard M, et al. Molecular Chracterization of Bovine Tuberculosis strains in two slaughterhouses in Morocco. BMC Vet Res. 2016; In press.
- Yahyaoui Azami H, Zinsstag J, Economics of Bovine Tuberculosis: a One Health issue, Book chapter, CABI, 2017, In press
- Abakar F, Yahyaoui Azami H, Justus Bless P, Crump L, Lohmann P, Laager M, et al. Transmission Dynamics and Elimination Potential of Zoonotic Tuberculosis in Morocco. PLOS Negl Trop Dis. 2016.
- Ducrotoy MJ, Yahyaoui Azami H, El Berbri I, Bouslikhane M, Fassi Fihri O, Boué F, et al. Integrated health messaging for multiple neglected zoonoses: Approaches, challenges and opportunities in Morocco. Acta Trop. 2015 Dec; 152:17–25.
- The prevalence of ruminant brucellosis and Bovine Tuberculosis in Sidi Kacem area in Morocco, presented in: 6ème Colloque International Francophone de Microbiologie Animale à Toulouse-France, and XII^{ème} International Congress of Mediterranean Federation for Health and Production of Ruminants- Sassari-Italy**.**
- Molecular characterization of Bovine Tuberculosis strains in two slaughterhouses in Morocco, Presented in 1st Annual Biorisk Management Symposium in Tunis-Tunisia.

SKILLS AND COMPETENCES

- Data analysis: analysis of epidemiological data about zoonoses in Morocco (e.g: Bovine tuberculosis, Brucellosis)
- Laboratory skills: Brucellosis diagnosis: Rose bengale test, culture, PCR; Tuberculosis: Culture, identification via Ziehl Neelsen coloration, PCR, Spoligotyping
- One health expertise: through the participation of several workshops, trainings and courses taught by international experts in OH, and using OH principles during my PhD research
- Good communication skills gained through my experience in the preparation of field work and its implementation
- Good group work skills gained through my field work in an interdisciplinary team.
- Good organizational skills thanks to my participation in the organization of many activities in Morocco and Switzerland.
- Proficiency in laboratory management and security: management of laboratory security measures in the IAV Hassan II in Morocco, tuberculosis laboratory at the Swiss TPH and the laboratory of Infectious Disease at the University of Georgia
- Good skills in research projects management and implementation: participation in the EU project ICONZ (Integrated Control of Neglected Zoonosis)

Computer Skills

- Microsoft Office
- Health Mapper
- Stata
- Epi Info
- Vensim
- Win Episcope
- Ersatz;
- MAXQDA

Interests Traveling, reading and cooking.

REFERENCES

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