Bovine Tuberculosis

Disease Monograph Series – 15

Bacteria | *Mycobacterium bovis* | Cattle

IDRC | Bartay

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Acronyms

AU	African Union
AU-IBAR	African Union Inter-African Bureau for Animal Resources
AU-PANVAC	African Union – Pan African Vaccine Centre
BCG	bacilli Calmette–Guerin
bTB	Bovine tuberculosis
CFP	Culture-filtrate Proteins
CMI	Cell-mediated Immune Mechanism
CVO	Chief Veterinary Officer
DALY	Disability-adjusted life year
DG	Director General
DIVA	Differentiation of infected from vaccinated animals
DoI	Duration of immunity
DVS	Director Veterinary Services
ELISA	Enzyme-linked immunosorbent assay
EPTB	Extra-pulmonary TB
FAO	Food and Agriculture Organization of the United Nations
IM	Intramuscular
IN	Intranasal

NGO	Non-governmental organization
OIE	World Animal Health Organization
PTB	Pulmonary TB
PCR	Polymerase chain reaction
PPD	Bovine tuberculin purified protein derivative
SC	Subcutaneous
SHF	Small holder farmer
TB	Tuberculosis
TPP	Target Product Profile
TST	Tuberculin skin test
WHO	World Health Organization of the United Nations

Executive Summary

Disease, etiology, epidemiology and impact

Bovine tuberculosis (bTB) is a chronic bacterial disease of animals and humans caused by *Mycobacterium bovis*. In a large number of countries, it is a major infectious disease of cattle, other domesticated animals, and certain wildlife populations.

Mycobacterium bovis belongs to the *Mycobacterium tuberculosis complex* (MTBC) that causes tuberculosis in humans and animals. The most important members of the MTBC are *M. bovis* and *M. tuberculosis*. In certain regions, *M. caprae* is also relevant. *M. tuberculosis* is avirulent for cattle, although it is able to cause localized lesions. Goats are highly susceptible to *M. bovis* and *M. caprae*. Bovine TB is usually maintained in cattle populations, but a few other species can become reservoir hosts. Most species are considered to be spillover hosts (do not maintain *M. bovis* in the absence of maintenance hosts). *M. bovis* can be transmitted by the inhalation of aerosols, by ingestion, or through breaks in the skin.

Bovine TB generally has a chronic, and often subclinical course. It usually takes many months or even years before clinical signs develop and they depend on the organ or systems involved and the severity of the infection. It is usually characterized by formation of nodular granulomas known as tubercles.

Bovine TB causes significant economic hardship for livestock farmers with estimates of >50 million cattle infected worldwide, costing $3 billion annually. It includes costs associated with reduction in productivity in severely affected animals, testing, culling of affected animals, indemnity payments, movement controls, restrictions on trade, maintenance of control programs, and research to develop improved control strategies

Zoonotic disease

M. bovis infects humans primarily due to the ingestion of unpasteurized dairy products, and it is more associated with extra-pulmonary TB in humans (EPTB). It is rare in humans in developed countries due to the eradication and control programs they have put in place. Unfortunately, it is still common in less developed countries.

TB caused by *M. bovis* in humans is much less common than TB caused by *M. tuberculosis*. Due to difficulties in diagnostics, is difficult to discern the true global load of human TB caused by *M. bovis*. There were estimates mentioning 3.1% of all TB worldwide to be zoonotic TB, but most recent data mentions 2.8% for Africa. The latest data as shown in Table 7 confirms that it is very low, unless for certain specific hotspots. Differentiation of TB caused by *M. bovis* is relevant, as *M. bovis* is naturally resistant to pyrazinamide, one of the four first-line anti-tuberculosis drugs and prognosis is often poor. The WHO estimates the Disability adjusted life years (DALYs) due to *M. bovis* to be 607,775, and 9 DALYs per 100,000 persons (2015 data).

Incidence / Prevalence

Although bovine tuberculosis was once found worldwide, control programs have eliminated or nearly eliminated this disease from domesticated animals in many countries. Bovine TB is still widespread in Africa, parts of Asia and the Americas, and some Middle Eastern countries. Surprisingly, there is not much recent data on prevalence in the focus countries (with some exceptions such as Ethiopia) and there is virtually no information on the prevalence of *M. bovis* in small ruminants. It would seem the disease is more prevalent in peri-urban dairy settings, than in rural pastoralist areas.

Diagnostics

Diagnosis in the live animal is usually based on delayed hypersensitivity reactions (tuberculin skin test). Traditional mycobacterial culture remains the gold standard method for routine confirmation of infection. Blood tests based on gamma-interferon, lymphocyte proliferation assay and indirect ELISA are also available, but are mainly used to confirm a skin test.

Current tests have many challenges, but there have been recent developments. Most promising is a DIVA skin test developed by AgResearch in New Zealand, which will be commercialized soon, but needs to be validated in developing countries as the exposure to environmental mycobacteria will be different. There are also improved blood tests. Quality and availability of tuberculin is also a challenge.

Control

Bovine TB is usually a notifiable disease, but not in all focus countries. There are four principal approaches to the control of tuberculosis: 1) test and slaughter, 2) test and segregation, 3) Immunization and 4) Chemotherapy. Chemotherapy requires a long time, and is forbidden in the majority of the countries. Immunization has not been very effective, and it does not allow the differentiation between vaccinated and infected animals. It is forbidden in many countries. The main measures used have been test and slaughter, and test and segregation. However, these last 2 measures are very difficult to implement in developing countries.

Presence of bTB in wildlife, generates additional complications for control. This is a big problem in UK and USA, but also presents challenges in Africa, in the livestock-wildlife interface.

Current vaccines

A commercial vaccine licensed for use in cattle for bTB does not exist. The vaccine for human TB, the BCG (a live attenuated *M. bovis* strain), has been used in cattle, and remains the best candidate vaccine for use in the field in the short to medium term. Some advantages include that the vaccine is safe, inexpensive and is commercially

produced for human application. The safety of BCG has been demonstrated for multiple host species, including cattle in numerous trials over the past 90 years. The major caveats which have restricted its use, is that BCG protection is not complete. It limits mycobacterial burden and associated pathology in cattle but does not prevent infection. It also sensitises animals to respond in traditional TB tests. Some of these limitations are understandable for countries undergoing eradication, but in settings where the disease is a real problem, there is not an eradication or control program in place (the DIVA capability is not a major issue) and there is not a better option, BCG seems a reasonable alternative. However, there is not enough experience in using the vaccine in those settings, and there are important knowledge gaps in how to best use the vaccine in those scenarios, for example efficacy of a smaller dose (which would reduce the cost), and duration of immunity. Field trials for an integrated control of bTB with BCG vaccine should be recommended where the conditions are appropriate. This will also provide the opportunity to evaluate BCG effect on disease transmission.

The highlights of the recent developments on the use of BCG are shown in Table 10.

Research and potential new vaccine candidates

There is a need for a livestock vaccine that prevents infection and provides sterilizing immunity, that has DIVA capabilities and has a longer duration of immunity. Relatively large amounts are being invested by developed countries like UK and USA, where bTB control is a challenge, and vaccines for livestock and/or wildlife would be of help. However, they are focused on their needs, that might be slightly different from the ones of the developing countries.

Research is being conducted using different approaches: improvement of the current BCG, strategies to improve BCG vaccination by boosting it with different antigens, live attenuated vaccines using mainly *M. bovis* (and in some instances *M. tuberculosis*), DNA, adjuvanted proteins and virus vector vaccines. A summary is presented in Table 13. Many of the candidates have DIVA capability. So far, the best results in cattle have been achieved with strategies using combinations of BCG with subunit vaccines (heterologous prime-boost strategies) as shown in Tables 13 and 14.

Current research projects include several combination vaccines or strategies. For example, work is being done by Dr Waters and his team on developing a novel strategy in which an attenuated *Mannheimia haemolytica*-vectored subunit vaccine is administered intranasally and simultaneous with parenteral BCG. Other groups are working on combination with Brucellosis (mainly from Argentina and Brazil), and a group has published about a combination with bovine viral diarrhea.

There are potential synergies with human TB vaccines, so developments in that area should be watched carefully.

Suggested characteristics of an ideal bTB vaccine, can be seen under the Target Product Profile in Section 9.

Clinical disease overview

Bovine tuberculosis (bTB) is a chronic bacterial disease of animals and humans caused by *Mycobacterium bovis*. In a large number of countries, it is a major infectious disease of cattle, other domesticated animals, and certain wildlife populations. It is a zoonosis

Etiology & Epidemiology

Bovine TB is caused by *Mycobacterium bovis.* The genus *Mycobacterium* consists of about 50 species, most of which are environmental saprophytes that exist and multiply in a wide variety of substrates such as soil, water and plants, domestic and wild mammals and birds. Certain members of the genus, such as those that make up what is known as the *M. tuberculosis* complex, are also known as "tubercle bacilli" due to their causing tuberculosis in humans and animals. These are obligate parasites and are usually transmitted only by infected mammalian hosts. The mycobacteria of the *M. tuberculosis* complex are characterized by 99.9% similarity at the nucleotide level and identical 16S r RNA, but they differ in phenotypes and host tropism.

***Mycobacterium tuberculosis complex* (MTBC)**

Host adaption of the MTBC is not strict. All members have been found to cause disease in humans, and many have been isolated from mammals that are not considered to be the primary host (see Table 1 and Figure 1). This led to the concept of a "maintenance" host, to which each species of the MTBC is adapted, and "spillover" hosts, in which the disease can be found but is not necessarily maintained [1]. The most important members of the MTBC are *M. bovis* and *M. tuberculosis*. In certain regions, *M. caprae* is also relevant.

Table 1: Ten leading disease losses globally by livestock disease units (LSU) loss

	Primary Host	Other hosts	Comments
M. tuberculosis	Humans	Sometimes reported in animals	Obligate human pathogen.
M. africanum	Humans	Rarely reported in animals	Obligate human pathogen. Limited to West Africa
M. canetti	Humans		Opportunistic human pathogen. Associated to the Horn of Africa. "smooth TB bacilli".
M. bovis	Cattle	Other domestic and wild mammals, humans	
M. caprae	Goats and sheep	Cattle, red deer, wild boar and to a limited extent, humans.	Found almost exclusively in continental Europe but information from the Middle East, Asian, or African countries is missing [2]
M. microti	Rodents (voles)	Not considered an important pathogen for domestic or wild animals	
M. pinnipedii	Seals and sea lions		
M. mungi	Banded mongooses	Unknown	Isolated in Botswana from mongooses living near humans in Chobe District, Botswana
M. orygis	Not known	Not clear but includes: Oryxes, waterbucks, gazelles, cows, rhesus monkeys	Proposed in 2012 [3]

Sources: **http://wwwnc.cdc.gov/eid/article/19/6/12-1012_article**
http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3309669/
http://www.nature.com/nrmicro/journal/v7/n7/box/nrmicro2165_BX2.html
http://rstb.royalsocietypublishing.org/content/royptb/367/1590/850.full.pdf
http://www.ncbi.nlm.nih.gov/pmc/articles/PMC1248478/
http://wwwnc.cdc.gov/eid/article/16/8/10-0314_article

Tuberculosis in livestock

Mycobacterium bovis, causes tuberculosis in cattle, but may also affect other species, including humans. *M. bovis* is the most universal pathogen among the mycobacteria, and produces progressive disease in many domestic and wild animals, as well as humans.

Bovine TB is usually maintained in cattle populations, but a few other species can become reservoir hosts. Known maintenance hosts include brush–tailed possums (and possibly ferrets) in New Zealand, badgers in the United Kingdom and Ireland, bison and elk in Canada, and kudu and African buffalo in southern Africa. Most species are considered to be spillover hosts. Populations of spillover hosts do not maintain *M. bovis* indefinitely in the absence of maintenance hosts, but may transmit the infection between their members (or to other species) for a time. Some spillover hosts can become maintenance hosts if their population density is high. Species reported to be spillover hosts include sheep, goats, horses, pigs, dogs, cats, ferrets, camels, llamas, many species of wild ruminants including deer and elk; elephants, rhinoceroses, foxes, coyotes, mink, primates, opossums, otters, seals, sea lions, hares, raccoons, bears, warthogs, large cats (including lions, tigers, leopards, cheetahs and lynx) and several species of rodents. Most mammals may be susceptible

M. tuberculosis was already shown in the 19th century to be avirulent in cattle. Localized lesions might develop, but infection does not result in progressive disease. More recently, Whelan et al [4] confirmed it with strain *M. tuberculosis* H37Rv. They proposed that the immune status of the animal, or genotype of the infecting bacillus, may have significant bearing on the virulence of a strain for cattle. There is currently no evidence of animal-to-animal transmission of *M. tuberculosis* or *M. africanum* [5] in cattle, but they may cause reactions in tuberculin-tested cattle.

Goats are highly susceptible to *M. bovis* and *M. caprae*. Caprine TB has been underestimated for a long time, although it causes economic losses in endemic areas, and goats in contact with cattle might act as reservoirs of bTB [6]. Interestingly, caprine TB is not an OIE noticeable disease.

Transmission

M. bovis can be transmitted by the inhalation of aerosols, by ingestion, or through breaks in the skin. The importance of these routes varies between host species.

Cattle shed M. bovis in respiratory secretions, feces and milk, and sometimes in the urine, vaginal secretions or semen. The risk of infection from infected cattle is dependent on them shedding the organism. Bacteria are shed consistently soon after the initial infection; at a later stage shedding becomes intermittent, and large numbers of organisms may be shed in the late stages of infection. Asymptomatic and anergic carriers occur. In most cases, M. bovis is transmitted between cattle in aerosols during close contact. Some animals become infected when they ingest the organism; this route may be particularly important in calves that nurse from infected cows. Cutaneous, genital, and congenital infections have been seen but are rare. Not all infected cattle may transmit the disease. Humans with open tuberculosis caused by M. bovis can infect cattle by the aerogenous route when

handling them or by contaminating the closed environment of a stable by spitting or urinating. The primary mode of spread of bTB between herds is by the introduction of infected animals. In certain areas, wild animals can be the main reservoir for domestic animals and play a key role.

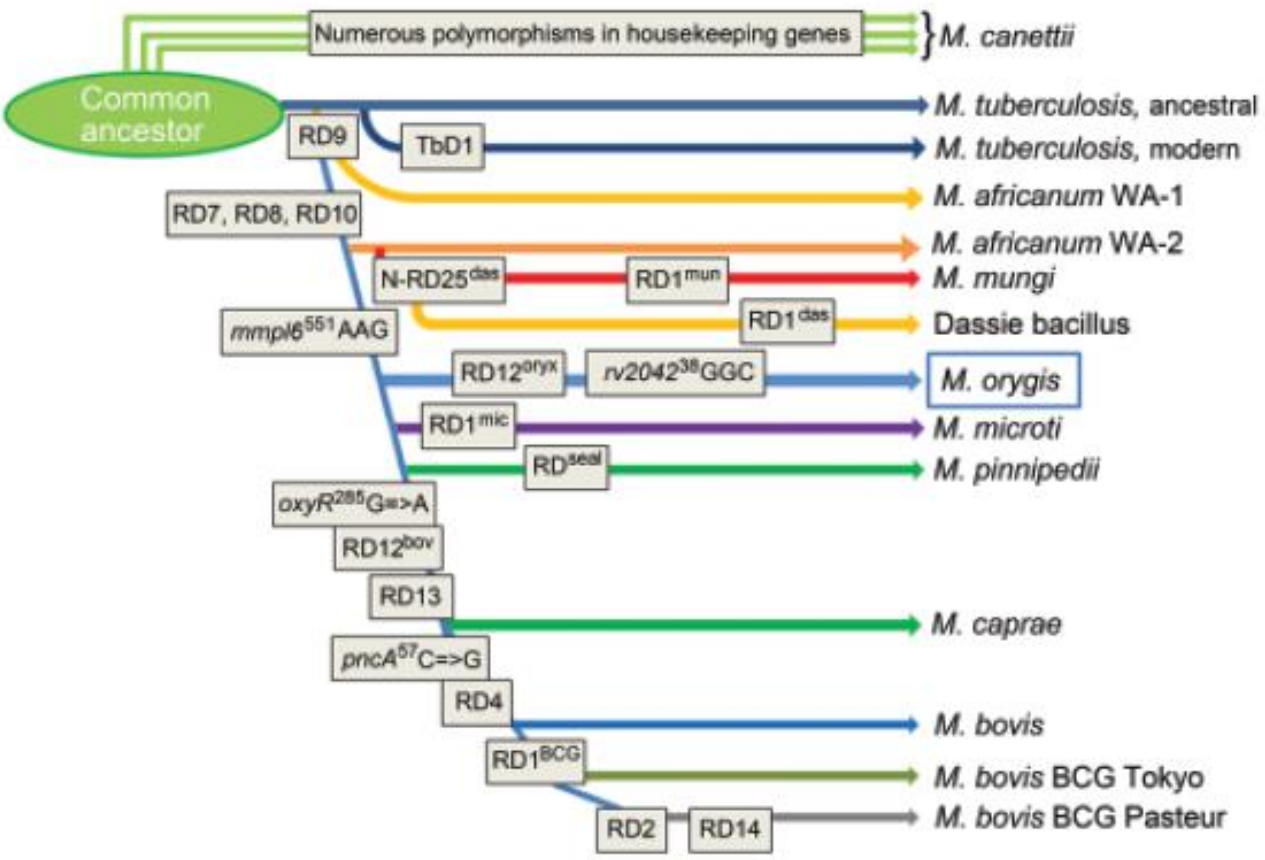


Figure 1: Updated phylogeny of the Mycobacterium tuberculosis complex. Source: van Ingen et al, 2012 [3]

Ingestion appears to be the primary route of transmission in pigs, ferrets, cats and probably deer. Cats can be infected by the respiratory route or via percutaneous transmission in bites and scratches. Nonhuman primates are usually infected by inhalation. Aerosol transmission also seems to be the main route of spread in badgers, but transmission in bite wounds can be significant. Badgers with advanced disease can shed M. bovis in the urine, and organisms have been found in the feces.

M. bovis can survive for several months in the environment, particularly in cold, dark and moist conditions. At 12-24°C, the survival time varies from 18 to 332 days, depending on the exposure to sunlight.

Clinical Signs

Bovine TB generally has a chronic, variable, and often subclinical course. Occasionally it can be acute and rapidly progressive. It usually takes many months or even years before clinical signs develop. In most infected cattle the disease is inapparent, and it is only being detectable by the tuberculin test. If clinical signs are manifested, their nature depends on the organ system or systems involved and the severity of the infection. It is usually characterized by formation of nodular granulomas known as tubercles. Any body tissue can be affected, but lesions are most frequently observed in the lymph nodes (particularly in the head and thorax), lungs, intestines, liver, spleen, pleura, and peritoneum.

In countries where control is active and aggressive, the disease is primarily respiratory in nature and the extent of the lesions is limited. In countries where the disease is not actively controlled, advanced disease and generalization are common.

In the late stages of the disease, common symptoms include progressive emaciation, a low–grade fluctuating fever, weakness and inappetence. Animals with pulmonary involvement usually have a moist cough that is worse in the morning, during cold weather or exercise, and may have dyspnea or tachypnea. In the terminal stages, animals may become extremely emaciated and develop acute respiratory distress. In some animals, the retropharyngeal or other lymph nodes enlarge and may rupture and drain. Greatly enlarged lymph nodes can also obstruct blood vessels, airways, or the digestive tract. If the digestive tract is involved, intermittent diarrhea and constipation may be seen. In some animals, the only symptom may be abscesses of unknown origin in isolated lymph nodes, and symptoms may not develop for several years. In other cases, the disease may be disseminated, with a rapid, fulminating course.

Diagnosis

Bovine TB infection in cattle is usually diagnosed in the live animal on the basis of delayed hypersensitivity reactions. Traditional mycobacterial culture remains the gold standard method for routine confirmation of infection.

- Post-mortem: Bovine TB is characterised by the formation of granulomas (or tubercles). They are usually yellowish and either caseous, or caseo-calcified. They are often encapsulated.
- OIE recognized tests:
    a) Identification of the agent: Isolation of mycobacteria on selective culture media, and their subsequent identification by cultural and biochemical tests or DNA techniques such as PCR.

b) Delayed hypersensitivity test using bovine tuberculin purified protein derivative (PPD), which is the prescribed test for international trade. It involves measuring skin thickness, injecting bovine tuberculin intradermally into the measured area, and measuring any subsequent swelling 72 hours later. It is known as the Tuberculin skin test (TST). The comparative intradermal tuberculin tests with bovine and avian tuberculin is used to differentiate animals infected with *M. bovis* and those sensitized to tuberculin due to exposure to other mycobacteria or related genera.

c) Blood based test: gamma-interferon assay (ELISA) and lymphocyte proliferation assay which measure cellular immunity, and indirect ELISA, which measures humoral immunity. They are usually used as ancillary tests to maximize the detection of infected animals or to confirm or negate the results of the intradermal test.

- Most commonly used in low & middle income countries:

  a) At field level: the hypersensitivity test (with tuberculin provided by the national laboratory).

- Main challenges in diagnostics:

  a) The most commonly used test, the hypersensitivity test has some important challenges:

  - Tuberculin has become more difficult to obtain, and its availability and quality has become an issue. Shortages are not rare: http://www.ashp.org/menu/DrugShortages/CurrentShortages/Bulletin.aspx?id=973
  - Technical issues with tuberculin: low degree of standardization, imperfect test accuracy, variations due to tuberculin doses, PPD preparation, site of application, etc…Tuberculin should be standardized in terms of activity for in vivo and in vitro detection assays.
  - Implementation issues: difficulties in administration and interpretation of results, need for a second-step visit 72 hours later for the reading. The genetic background of the animal can also influence the reaction to tuberculin [7].
  - It is not a DIVA test, allowing differentiation between vaccinated and infected animals. Up to 80% of BCG vaccinated calves have been shown to react in the tuberculin test 6 months post-vaccination [8]. Encouragingly, this decreased to 10-20% by 9 months' post-vaccination and protection against TB was not dependent on maintenance of a tuberculin skin test response.
  - The standard TST is estimated to be able to detect around 40-80% of infected animals.

  b) The sensitivity of serodiagnostic tests needs to be increased.

- Recent developments: (note: an exhaustive review hasn't been done, as it is not the focus of this monograph).

a) Probably one of the most important recent developments are the DIVA tests. They have been developed using antigens from the MTBC which are not expressed or secreted by BCG and can be used instead of bovine PPD in the skin test [9] or in the whole blood IFN-γ release assay [10]. The first two antigens used in DIVA tests were the early secreted antigen target 6 kDa protein (ESAT-6) and culture filtrate protein 10 (CFP10) which are encoded in the RD1 of *M. bovis* and *M. tuberculosis* (not present in BCG). A further protein, Rv3615c (not secreted by BCG), and more recently Rv3020c were added to DIVA tests to enhance sensitivity, while not being recognised by non-infected or BCG-vaccinated cattle. A recent assessment of the whole blood IFN-γ DIVA test incorporating ESAT-6, CFP10, and Rv3615c antigens was undertaken in 75 BCG vaccinated, *M. bovis*-infected cattle and 179 BCG-vaccinated, non-infected animals, revealing estimates of 96.0 % sensitivity and 95.53 % specificity.

   A recent publication described the display of these antigens on polyester inclusions beads in order to decrease the concentration of antigen needed, and the cost. Polyester beads simultaneously displaying all four proteins were produced in a single fermentation process, and polyester beads displaying three or four mycobacterial proteins were shown to have high sensitivity for detection of *M. bovis*-infected cattle and induced minimal responses in animals exposed to environmental mycobacteria or vaccinated with BCG [11]. This research has been led by AgResearch (New Zealand) http://www.agresearch.co.nz/news/international-interest-in-nz-tb-test/. Dr Buddle commented that they are hoping that commercial production for the new skin test reagent would commence in the next 6-12 months, but it may be possible to supply the skin test reagent at an earlier time-point. For more details, see information from Dr Buddle in Section 7. Other methods for a cocktail-based serological diagnosis have been developed, like the mulit-antigen print immunoassay (MAPIA).
   http://www.ncbi.nlm.nih.gov/pubmed/23038072

b) Worldwide, *M. bovis* strain AN5 is used for bovine tuberculin production, but commercially available tuberculins differ greatly in their quality. Attempts are being made to improve their quality, for example, a new lymphocyte proliferation assay for potency determination of bovine tuberculin PPD has been recently published. http://www.ncbi.nlm.nih.gov/pubmed/25935213

c) Alternative tools to read out the skin test like infrared thermography have also been evaluated [12].

d) ELISPOT: Xu et al have established an ELISPOT for bovine interferon-gamma (BoIFN-γ), and applied it in the diagnosis of bovine tuberculosis (bTB).
   http://www.ncbi.nlm.nih.gov/pubmed/26062340

e) The indirect ELISA produced by IDEXX, and the gamma interferon ELISA produced by Prionics AG, have been registered by the OIE. The first one in 2012, and the later in 2015.
   http://www.oie.int/our-scientific-expertise/certification-of-diagnostic-tests/the-register-of-diagnostic-tests/ For more details on the IDEXX test:

https://www.aphis.usda.gov/animal_health/animal_diseases/tuberculosis/downloads/vs_idexx_webinar_presentations.pdf

- Main needs for diagnostics:

  a) A DIVA field test that differentiates vaccinated animals from infected ones.

  b) A field test that could be read at a shorter interval, avoiding a second visit.

  c) Cheap diagnostic assays with superior sensitivity in comparison to the skin tests.

  d) There is evidence that zoonotic tuberculosis is increasing due to *Mycobacterium bovis* in areas where *M. bovis* is endemic. Tuberculosis caused by *M. bovis* requires a different treatment. A test that could differentiate *M. tuberculosis* from *M. bovis* would be of relevance for physicians.

- Differential diagnosis:

  Bovine TB should be differentiated from Contagious bovine pleuropneumonia, *Pasteurella* or *Corynebacterium pyogenes* pneumonia, aspiration pneumonia, traumatic pericarditis, caseous lymphadenitis or melioidosis in small ruminants, and chronic aberrant fluke infestation.

Zoonotic disease

M. bovis can infect humans, primarily by the ingestion of unpasteurized dairy products but also in aerosols and through breaks in the skin. Raw or undercooked meat can also be a source of the organism. *M. bovis* in humans is usually an accidental dead-end host. Person-to-person transmission is rare in immunocompetent individuals, but *M. bovis* has occasionally been transmitted within small clusters of people, particularly alcoholics or HIV-infected individuals. Rarely, humans have infected cattle via aerosols or in urine.

M. bovis used to be a significant cause of human TB, primarily in children who consumed raw milk. It decreased markedly following the introduction of pasteurization and meat-control practices. Bovine TB is now rare in humans in developed countries due to the eradication and control programs. Unfortunately, is still common in less developed countries, which generally lack bovine TB control programs and where exposure to infected animals or consumption of non-pasteurized products is expected to be more frequent.

TB caused by *M. bovis* in humans is much less common than TB caused by *M. tuberculosis*, and the estimated prevalence of cases caused by *M. bovis* in Europe today has fallen considerably from the 30% recorded before the introduction of milk pasteurization procedures and “test and slaughter” control programs in cattle. It is difficult to discern the true global load of human TB caused by *M. bovis*, because TB caused by *M. tuberculosis* and TB caused by *M. bovis* are indistinguishable clinically, radiologically, and histopathologically. Additionally, there is a lack of local diagnostic capacity to detect extra-pulmonary TB, the major form of bTB in humans. The

only way to determine the role of each pathogen is to identify isolates to species level. However, isolation and confirmatory culture of the pathogen is not routinely performed in the regions where human infections by *M. bovis* are more prevalent, thus making identification to species level problematic. *M. bovis* is also expected to be underdetected when it coinfects a person already infected with *M. tuberculosis*. Although thought to be infrequent, coinfection with *M. bovis* was detected in 3 of 189 TB-infected patients (1.6%) in a prevalence study carried out in an urban area of Brazil between 2008 and 2010. Consequently, the figure provided by the World Health Organization (*M. bovis* was responsible for 3.1% of all TB cases in humans) may not reflect the real dimension of the problem [13]. For more details, please see Section 3c, and for the impact see Section 4.

Differentiation of TB caused by *M. bovis* is relevant, as *M. bovis* is naturally resistant to a major anti-TB drug, pyrazinamide, one of the four first-line anti-tuberculosis drugs [14] and prognosis is often poor.

In addition to *M. bovis*, other members of the MTBC can also infect humans and therefore become involved in zoonosis. *M. caprae* is the causal agent of caprine TB. Exposure can be airborne or through ingestion of contaminated dairy products. It is widely distributed in Europe, and it is responsible for 13–31% of the zoonotic TB cases. The incidence of zoonosis by *M. caprae* is lower than that of zoonosis by *M. bovis*.

M. microtti infects small rodents, such as voles, wood mice, and shrews, and its prevalence varies (from 9% to 31%) depending on the season and country. Zoonosis caused by this entity is rare. *M. pinnipedii* is the causal agent of TB in pinnipeds such as seals and sea lions, and as a zoonotic agent has only rarely been described.

There are several organizations dealing with TB research in humans. Some examples:

- The global fund: **http://www.theglobalfund.org/en/tuberculosis/**
- TB alliance: **http://www.tballiance.org/rd/scientific-vision** . Dedicated to the discovery and development of better, faster-acting, and affordable tuberculosis drugs that are available to those who need them.
- WHO: http://www.who.int/immunization/research/development/tuberculosis/en/
- WHO TB facts: **http://www.who.int/mediacentre/factsheets/fs104/en/**
- Stop TB partnership: **http://www.stoptb.org/**

Incidence and Prevalence in Selected Countries

Global

Although bovine tuberculosis was once found worldwide, control programs have eliminated or nearly eliminated this disease from domesticated animals in many countries. Nations currently classified as tuberculosis-free include Australia, Iceland, Denmark, Sweden, Norway, Finland, Austria, Switzerland, Luxembourg, Latvia, Slovakia, Lithuania, Estonia, the Czech Republic, Canada, Singapore, Jamaica, Barbados and Israel.

Eradication programs are in progress in other European countries, Japan, New Zealand, the United States, Mexico, and some countries of Central and South America. Although bovine tuberculosis has been eradicated from the majority of U.S. states, a few infected herds continue to be reported, and a few states may periodically lose their disease-free status. In particular, a focus of infection in wild white-tailed deer has complicated eradication efforts in Michigan. Similar problems exist with infected badgers in the U.K. and Ireland, and infected brush-tailed opossums in New Zealand.

Bovine tuberculosis is still widespread in Africa, parts of Asia and the Americas, and some Middle Eastern countries.

Incidence bovine TB data by country

There are two main sources, OIE and AU-IBAR (which includes only Africa), but data are not always similar.

1- Source: OIE.

Data of outbreaks reported to the World Animal Health Organization (OIE) are shown in Tables 2 and 3. Data are not always reliable, as many countries doesn't seem to report, or to be reporting consistently over time. **http://www.oie.int/wahis_2/public/wahid.php/Diseaseinformation/statusdetail**. Similar information but presented in a different manner can be seen in Annex 1. Number of cases reported to the OIE by disease and by country: - No information, + Present but quantitative data not known, ? Disease suspected

Table 2: ASIA – Bovine TB outbreaks notified to OIE from the Asian countries of interest.

Country	2005	2006	2007	2008	2009	2010	2011	2012	2013	2014	2015
Bangladesh	-	-	+	+	+	+	+	+	+	+	-
India	+	+	+	+	+	+	+	+	-	-	-
Indonesia	+	+	-	0	-	-	-	-	-	-	-
Myanmar	+	>3	6	5	3	1	>3	+	1	0	-
Nepal	0	0	0	0	+	3	0	0	0	0	-
Vietnam	?	?	?	?	?	?	?	?	?	0	-

Table 3: AFRICA – Bovine TB outbreaks notified to OIE from the African countries of interest.

Country	2005	2006	2007	2008	2009	2010	2011	2012	2013	2014	2015
Burkina Faso	>1	+	+	+	+	+	+	0	+	+	+
Ethiopia	+	+	+	+	+	+	+	+	0	0	-
Ivory Coast	+	0	0	>3	18	>12	24	+	>13	>5	+
Kenya	-	-	-	-	?	0	>1	+	0	0	0
Madagascar	+	+	+	+	+	7	2	4	1	3	1
Malawi	+	+	+	+	+	+	+	+	-	-	-
Mali	+	+	+	-	+	-	-	-	-	-	-
Mozambique	2	>3	12	19	15	33	21	14	36	>21	-
Rwanda	-	0	+	+	+	+	+	+	?	-	-
Senegal	-	-	-	-	-	-	-	-	?	>1	+
South Africa	28	6	12	9	33	46	37	>19	36	19	-

Tanzania	+?	+?	+?	0	+	+	>2	+	+	+	+
Uganda	+	?	?	?	?	?	+	+	+	+	-
Zambia	-	+	1	7	15	14	+	5	14	11	-

The OIE, also includes zoonoses data. The number of human cases and deaths are reported by the countries. Data from the countries of interest, can be seen in Table 4 below.

http://www.oie.int/wahis_2/public/wahid.php/Countryinformation/Zoonoses

Table 4: Human cases and deaths due to Bovine TB as reported to the OIE

Country	2005	2006	2007	2008	2009	2010	2011	2012	2013	2014
Bangladesh										
India	C: +, D: +		C: +, D: +	C: +, D: +						
Indonesia										
Myanmar										
Nepal						C: +, D: +				
Vietnam				C: +, D: +						
Burkina Faso		C: +, D: +								
Ethiopia	C: +, D: +	C: +, D: +	C: +, D: +	C: +, D: +	C: +, D: +	C: +, D: +		C: +, D:+		
Ivory Coast		C: +, D: +								
Kenya			C: 2,694, D: 869	C: 2,443, D: 0	C: 1,575, D: 60	C: +, D: +			C: +, D:+	C: +, D:+
Madagascar				C: +, D: +	C: +, D: +	C: +, D: +	C: +, D: +	C: +, D: +	C: +, D: +	
Malawi	C: 20,926								C: +, D: +	
Mali				C: +, D: +		C: 4,734, D:0	C: 4,854, D: 248	C: 5,573, D: 462		C: +, D:+
Mozambique		C: +, D: +				C: +, D: +				
Rwanda		C: +, D: +	C: +, D: +			C: +, D: +			C: +, D: +	
Senegal										
South Africa			C: +, D: +				C: +, D: +	C: +, D: +	C: +, D: +	C: +, D:+
Tanzania		C: +, D: +	C: +, D: +	C: +, D: +					C: +, D: +	C: +, D:+
Uganda			C: +, D: +							
Zambia							C: +, D: +			

C: Cases, D: Deaths

2- Source: AU-IBAR.

The African Union Inter-African Bureau for Animal Resources also has a notification system. Data are published in the Pan African Animal Resources Year Books. (**http://www.au-ibar.org/pan-african-animal-resources-yearbook?showall=&limitstart**=). Similarly to the OIE, many countries do not seem to consistently report the outbreaks. Note that the number of outbreaks reported often does not match those reported to the OIE.

Table 5: Number of bovine TB outbreaks per year as reported to AU-IBAR and published in the Pan African Animal Resources YearBook. NS= Not specified

Country	2005	2006	2007	2008	2009	2010	2011	2012	2013	2014	2015
Burkina Faso											
Ethiopia							1				
Ivory Coast				4	39	10	24				
Kenya		1						144		2	
Madagascar			2	1	NS		4				
Malawi		1	18				34	1			
Mali					1						
Mozambique		6	6	2	15	2	7		2	2	
Rwanda											
Senegal										1	
South Africa		6	15	16	5	37	37	9	36	23	
Tanzania			6				1	10			
Uganda				1	1	4	9	10			
Zambia			1	4	5	5	3	16	10	6	

2005: Bovine TB was not included in the 2005 report.
2006: Most African countries do not do routine diagnosis for bTB, and the reported cases are usually from the slaughterhouse.
2008: The outbreaks of bTB mainly affected cattle (77.3%). 20.3% of the outbreaks were unspecified, ovine were involved in 0.6% and wildlife in 1.9% of the outbreaks. Pigs were also involved in Benin.

Regional

Prevalence bTB data by country (from 2000 onwards)

- Sources: PubMed, and internet engine searches (English and French when applicable).
- Efforts have been made to include the year of the study, and not the year of the publication. If they are known to be different, the year of publication is included in the reference.
- For grey literature, links have been included when possible.
- Note that not all papers have been read in full. In many cases, only the abstracts have been read. Critical evaluation of the papers for inclusion has not been conducted.
- Humans: Please see section C for total human TB cases and for zoonotic TB reports from Africa and Asia. If data for zoonotic TB has been found for a specific country, it has also been included under the country in this section.

A report published by ILRI in 2012 (Mapping of poverty and likely zoonoses hotspots - **https://cgspace.cgiar.org/bitstream/handle/10568/21161/ZooMap_July2012_final.pdf**), based on a systematic literature review of the last 10 years for Africa, South Asia and South East Asia (also some studies from the Middle East were included), found that overall 7.4% of livestock were positive. Overall prevalence was: bovines 8%, camels 11%, shoats 2%, pigs 15% and wildlife 5%. (Note: it is referred as prevalence in developing countries, but based on the methodology described, not all the developing countries have been included).

Figure 2 shows the bovine tuberculosis in the countries reported in the study.

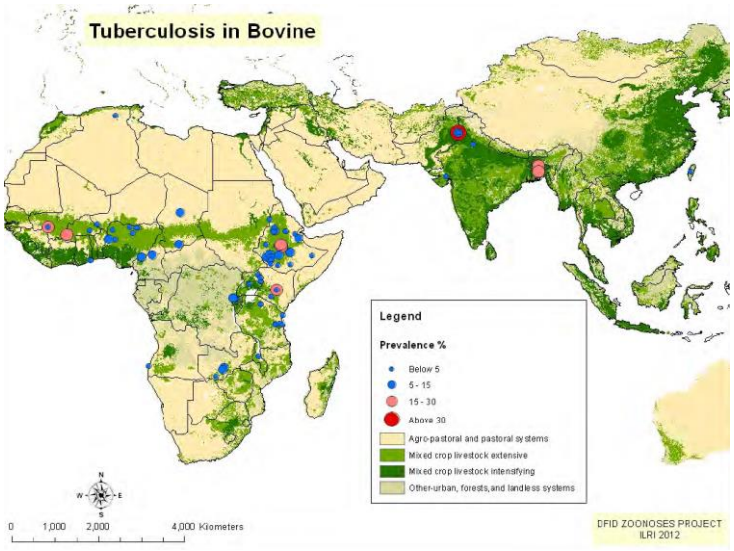


Figure 2: Tuberculosis prevalence in countries included in ILRI 2012 report on "Mapping poverty and likely zoonoses hotspots"

ASIA

Bangladesh

Livestock:

Year	Area	Species of animal	No. of samples tested	% positive	Reference
2013	Bangladesh Livestock research institute*	Sheep and goats	Sheep: 273 Goats: 155	Caudal skin test Sheep: 9.15 Goats: 1.29 Comparative cervical test Sheep: 1.46 Goats: 1.29	Rahman et al, 2013
2012-2013	Sirajganj district	Cattle	270	Antigen Rapid Bovine TB Ab test: 7.78	Mahmud et al, 2014
2012	Rangpur	Dairy farms	150	Caudal skin test: 24.7**	Tahmid Uddin, 2014
2012	Mymensingh Sadar	Cattle	101	Antigen Rapid Bovine TB Ab test: 5.94	Hoq Mondal, 2012
2012	Bangladesh livestock research institute*	Buffaloes	49	Caudal skin test Reactors: 6.12 Suspicious: 10.20 Comparative cervical tuberculin Reactors: 4.08 All suspicious cases proved false positives	Hassain, 2012
2009-2011	Dhobaura upazila of Mymensigh district	Cattle	649	Caudal skin test 2.34	Sarker et al, 2015

*: Even if the authors extrapolate the data to national prevalence, it is questionable that results from one farm can be translated at national level, as there is no enough data. However, it is a good start.

**: Note that the paper mentions 33.73% but calculations do not seem correct. 37 positives out of 150= 24.7%.

Humans:

In 2010, Nakajima et al tested 350 clinical isolates, and no *M. bovis* was identified [14].

India

Livestock:

Year	Area	Species of animal	No. of samples tested	% positive	Reference
2010	Himachal Pradesh	Cattle	440	Individual: 14.31 Herd: 16.67	Thakur et al, 2011
2008	North India	Cattle	768 from suspected positive farms	54 isolates were identified. *M. bovis*: 40 isolates *M. tuberculosis*: 14 isolates	Srivastava K, 2008*

* Study not indicative of prevalence, but confirms infection of cattle with *M. tuberculosis*

In 1994, it was estimated that the prevalence of TB in bovines ranged from 1.6 – 16% in cattle, and 3 – 25% in buffaloes (http://ntiindia.kar.nic.in/ntibulletin/NTI%20BULLETIN%202006-2011/NTI%20BULLETIN%20Vol%2043_3_4_2007/pages/pdf/Zoonotic%20Importance%20of%20tuberculosis.pdf)

Data from Danish Ali and Bhoj R Sing, Division of Epidemiology, IVRI, Izatnagar, India. Published on June 2015: http://www.slideshare.net/singh_br1762/bovine-tuberculosis-epidemiology-control-in-india

Animal tuberculosis in Indian states

PLACE	YEAR	PERCENTAGE	NUMBERS TESTED	SPECIES
BANGALORE	1973	20 %	DAIRY HERD	BOVINES
HARYANA	1970 - 1979	1.9 %	11990	CATTLE
HARYANA	1970 - 1979	3.8 %	1099	BUFFALOES
MEGHALAYA	1982	8.94 %	302	CATTLE
UTTAR PRADESH	1985	13.25 %	1268	BOVINE CARCASSES
PUNJAB	1994	0.33 – 11.7 %	1390	BOVINES
ORISSA	1997	1.6 – 11.5 %	-	POSITIVE REACTORS
-	-	3.5 – 22.8 %	ABBATOIR SURVEY	-

PLACE	YEAR	PERCENTAGE	CONCERNED POPULATION	SPECIES	REFERENCES
BOMBAY	1914	7.6 %	DAIRY FARM 674	CATTLE	Joshi et al 1914
CALCUTTA	1970	4.64 %	TUBERCULOUS COWS [64]	CATTLE	Guha & Sarkar .
-	1969	1.6 - 16 %	-	CATTLE	Lall 1969
-	1969	3 - 25 %	-	BUFFALOES	Lall 1969
NORTHERN INDIA	2006	15.76 %	DAIRY HERDS	BOVINE	Mukherjee
WESTERN INDIA	2006	0.65 - 1.85 %	DAIRY HERDS	BOVINE	Mukherjee
HIMACHAL PRADESH	2010	14.31 %	OVERALL PREVALENCE	BOVINE	THAKUR et al
HIMACHAL PRADESH	2010	16. 67 %	FARM PREVALENCE	BOVINE	THAKUR et al

ACES	INCIDENCE OF CASES	REFERENCES
PUNJAB	0.2 % - 16.3 %	Datta , Iyer et al
BIHAR	1.06 %	Soparkar & Dhillon et al
MADRAS	1.8 %	Lall et al
BOMBAY	12 %	Diwedi & Singh et al
TAMIL NADU	34.58 %	Dhinakaran et al 1991
KARNATAKA	30 - 35 %	Dhinakaran et al 1991
OGANISED DAIRY FARMS	1.93 % [cattle] 6.39 % [buffaloes]	Shah 2002 .

Humans:

In 2010, Jain reported that out of 155 patients enrolled as suspected extra-pulmonary TB, 147 were PCR positive [15]. Of those, 85.71% were PCR positive for *M. tuberculosis,* 9.52% for *M. bovis,* and 4.76% were coinfections of the two.

Indonesia

No information was found.

Myanmar

No information was found.

Nepal

- Livestock:

Year	Area	Species of animal	No. of samples tested	% positive	Reference
2012		Buffaloes and cattle		Buffaloes: 9.08 Cattle: 5.78	Joshi et al, 2012*
2012	Animals reared by TB infected persons	Bovine	50	10	Garie and Subedi, 2012
2011-2012	Western Chitwan	Cattle and buffaloes	100	Cattle: 13.6 Buffaloes: 15.4	Pandey et al, 2012
2004		Cattle and buffaloes		5	Pun et al, 2004**

*: Paper not available, but data mentioned by Dhakal, 2013.

**: Paper not available, but data mentioned by Jha et al, 2007.

- Humans:

Gurung et al, 2010: EPTB accounts for 10-15% of call cases of TB. From a total of 513 patients with EPTB, 54 were culture positive. On characterization of the isolates, 48 (88.9%) were identified as *M. tuberculosis*, 4 as *M. bovis* (7.40%) and 2 (3.7%) as *M. avium/intracelulare.*

Vietnam

- Livestock:

A serological study in 2004 of 1,201 dairy cattle, reported negative results for *M. bovis* (as mentioned by Carrique-Mas and Bryant, 2013).

AFRICA

Burkina Faso

Livestock:

Year	Area	Species of animal	No. of samples tested	% positive	Reference
2014	Ouagadougou & Bobo-Dioulasso	Bovine carcasses	1,499	6.8	Tarnagda et al, 2014
2012	Ouagadougou	Dairy cattle	1,420	6.05	Boussine et al, 2012
2004	Hamdallaye	Dairy cattle	325	27.7	Traore et al, 2004
1999	Bobo-Dioulsasso	Bovines	a) 174 from 6 herds b) 64 milk samples c) 199 slaughtered animals d) Meath inspection records: 4,525	a) 13% positive, 16% indeterminate. Only one herd negative out of 6. b) 26.5% positive culture for *M. bovis* c) 19% lesions suggestive TB., and from those, 47% were positive to *M. bovis* d) 10%	Vekemans et al, 1999

Sanoue et al, 2014, report the genotyping of *M. bovis* isolated from cattle and humans, and concluded that *M. bovis* circulates between Burkina Faso and its neighbouring countries, and that *M. bovis* is transmitted mainly between cattle, but also between cattle and humans.

Humans:

Godreuil et al reported in 2007 a study on 120 Mycobacterium isolates from patients with pulmonary TB, none of them was *M. bovis*.

Ethiopia

Livestock:

Year	Area	Species of animal	No. of samples tested	% positive	Reference
2013	Addis Ababa municipal abattoir	Cattle	500	Post mortem: 5 Molecular confirmation: 1.2	Mekibeb et al, 2013
2012	Central Ethiopia	Cattle	2,033 cattle from 287 herds	Individual: 1.8 (4 mm cut off), and 4.7 (2 mm cut off) Herd: 9.4 (4 mm cut off) and 20.2 (2 mm cut off)	Ameni et al, 2013
2011	Akaki and Metehara abattoirs	Camels	906	Pathology: 10.04	Mamo et al, 2011
2009-2010	50 Km radius Addis Ababa	Dairy cattle	2,956 animals from 88 herds	Small farms (1-10 animals): Individual: 9.2 Herd: 23.5 Medium farms (11-50 animals): Individual: 20.5 Herd: 63.6 Large farms (>50 animals): Individual: 41.3 Herd: 90.5	Firdessa et al, 2012
2008-2010	Negelle, Filtu, Mojo and Addis Ababa abattoirs	Cattle, camels and goats with suspected TB	207 samples from: Cattle: 50 out of 5,250 Camels: 81 out of 694 Goats: 76 out of 1,744	Culture positive: Cattle: 36 out of 50 Camels: 3 out of 81 Goats: 9 out of 76	Gumi et al, 2012

2009	Meskan, Gurage region, Central Ethiopia	Cattle and small ruminants	Cattle: 1,214 Small ruminants: 406 (63 goats, 343 sheep)	4 and 2 mm cut off used: Cattle: 1.6 (4 mm) and 6.8 (2 mm) SR: 0 (4mm), and 0.41 (2 mm)	Tschopp et al, 2011
2007-2009	Hamer Woreda, South Omo Zone	Cattle (Zebu) and goats	Cattle: 499 Goats: 186	Cattle: 0.8 (4 mm cut-off) and 3.4 (2 mm cut off). Herd prevalence was 33.3 and 83 with the same cut-offs. Goats: 0	Tschopp et al, 2010
2006-2008	Abattoirs in Gonder, Woldiya, Gimbi, Butajira and Jinka	Cattle	32,800	4.7	Berg et al, 2009
2007-2008	Hawassa town and surroundings	Cattle	413	Individual: 11.6 Herd: 48.7	Regassa et al, 2010
2007-2008	Achefer, Bahirdar Zuria and Adet (Western Gojam)	Cattle	1,220 72 milk samples	15.7 (Higher when owned by TB patients) 25% milk samples TB positive.	Fetene et al, 2008
2007	Selalle and Holeta	Cattle	5,424	13.5	Ameni et al, 2007
2007	Adama Town, Central Ethiopia	Cattle	524	Individual: 11 Herd: 15	Ameni and Erkihun, 2007
2006-2007	Oromia, Amhara and Southern Nations	Cattle	2,216 from 73 villages	Meskanena Mareko: 7.9 Woldia: 1.2 Bako Gazer: 4.3 Bale Mountains: 2.0 Overall: 3.1 Herd: 67	Tschopp et al, 2009
2004-2005	Central Ethiopia	Cattle	1,041	Owners with active TB: 24.3	Regassa et al, 2008

				Owners with no TB: 8.6	
2004	Addis Ababa abattoir	Cattle	1,350	1.5	Asseged et al, 2004
2004	Hossana municipal abattoir	Cattle	751	4.5	Teklul et al, 2004
2000-2001	Boji district (Western Ethiopia)	Zebu (traditional husbandry)	780	Individual: 4.1 Herd: 51	Laval and Ameni, 2004
1975-2006	Literature review	Cattle		Range 3.4% (small holder production system) to 50% (in intensive dairy productions). Range of 3.5% to 5.2% (slaughterhouses in various places of the country)	Shitaye et al, 2007

*: Prevalence is difficult to calculate, as only culture data is given. The paper focus more on the typing of the isolates.

Humans:

The majority of the publications do not cover prevalence of zoonotic TB, but the proportion of *M. bovis* isolates from human cases.

- Getahun et al 2015, reported that 92 isolates collected between 2010 and 2011 were all *M. tuberculosis*. Most of the isolates were collected in rural and pastoralist population where raw milk consumption is widely practiced, therefore, the absence of *M. bovis* was unexpected and contradicted earlier studies. The fact that eligible individuals were selected on signs of pulmonary TB, and *M. bovis* tends to produce EPTB, might have influenced the results.
- Korma et al 2015, reported that out of 200 cases of suspected EPTB in Addis Ababa, they obtained 59 cultures and only one was *M. bovis* (1.7%).
- Nuru et al 2015, reported that out of 168 isolates from Bahir Dar City (Nortwest Ethiopia), 2 (1.19%) were *M. bovis*. Both were isolated from TB lymphadenitis cases.
- Seyoum et al 2014, characterized EPTB in Dera Woreda, North Showa, and out of 145 cases, only 1 (0.9%) was positive for *M. bovis.*
- Firdessa et al, 2013 mentions that in Ethiopia, 33% of all new TB cases are EPTB (while the global average is 15%). Out of 964 cultures from patients recruited between 2006-2010, only 4 (0.4%) were *M. bovis*, and all were isolated from pulmonary TB.

- **Gumi et al 2012**, reported that in a study conducted 2008-2010 in Oromia and Somali Regional States in pastoral and agro-pastoral communities, out of 163 isolates, only 3 were *M. bovis,* and all of them were from pulmonary TB.
- **Beyene et al, 2009** looked at 156 cultures from patients with EPTB, and no *M. bovis* was identified.
- **Fetene et al, 2008** reported that *M. tuberculosis* (74.5%), *M. bovis* (14.9%) and atypical mycobacteria (8.5%) were identified from sputum and fine needle aspiration specimens of tuberculosis patient cattle owners in Western Gojam in 2007-2008. 5% of *M. bovis* were identified in cases of pulmonary TB, and 8% in cases of EPTB.
- **Kidane et al, 2002**, performed PCR in 35 EPTB samples from Southern Ethiopia, and found 6 (17.1%) to be *M. bovis.*

Ivory Coast

Livestock:

Year	Area	Species of animal	No. of samples tested	% positive	Reference
2002-2005	Abidjan Port-Bouët slaughterhouse	Various	17,279 condemned carcasses (89.3% cattle carcasses)	38.7 cattle carcasses condemned due to TB	Cisse et al, 2008

Humans:

Dosso et all in 1996, during a national survey conducted in 1995-1996 noted that out of 320 humans tested, 0 were positive for *M. bovis.*

Kenya

Livestock:

Year	Area	Species of animal	No. of samples tested	% positive	Reference
2009	Two abattoirs in Nairobi	Cattle	929	19% lesions 3.7% culture	Kuria and Gathogo, 2013

2007	Dagoretti	Dairy Cattle	143	10	Kang'ethe et al, 2007

Humans:

Koech 2001. For his thesis of epidemiology of TB in humans in Narok district, out of 132 cultures, none was *M. bovis.*

Madagascar

Livestock:

Year	Area	Species of animal	No. of samples tested	% positive	Reference
2015	Antanifotsy (Vakinankaratra)	Dairy cattle	429	Doubtful: 0.9% The rest negative	La Gazette de la Grande Ile
1996		Cattle		6.5	Rasolofo-Razanamparany, 1999
? Old document	Mahajunga, Antananarivo and Morondava	Cattle	Skin test: Mahajunga: 431 Morondava: 90 Slaughter: Mahajunga: 916 Antananarivo: 7,164 Morondava: 874	Skin test: Mahajunga: 63.6 Morondava: 3 Lesions: Mahajunga: 51 Antananarivo: 17.2 Morondava: 6.3	Ssartirano et al

Humans:

- Rasolfo Razanamparany et al 1999, reported that the prevalence of *M. bovis* in 1994-1995 was determined at 1.25% for PTB, and 1.3% for EPTB in urban areas.

- **Menard et al 1995**, reported that out of 138 strains isolated from EPTB patients in Antananarivo, only 1 (0.73%) was *M. bovis.*
- **Rasololofo-Razanamparany et al 1995**, reported that in Antananarivo, out of 126 strains isolated from pulmonary TB cases at the Institut d'Hygiene Sociale, 3 (2.38%) were *M. bovis.* In the prison, out of 36 samples, none was *M. bovis.*

Malawi

Livestock:

Year	Area	Species of animal	No. of samples tested	% positive	Reference
2011	Mzimba and Nkhata Bay (Northern region)	Cattle	95 cows from 74 farms	1.1	Tebug et al, 2014
1986		Cattle	2,032 from dip tanks 1,449 dairy cattle	3.85	Bedard et al, 1993

Mali

Livestock:

Year	Area	Species of animal	No. of samples tested	% positive	Reference
2007	Abattoir Bamako	Cattle	3,330	Lesions: 1.8	Muller et al, 2008
2001-2003	Bamako and Mopti abattoirs	Cattle	Bamako: 6,441 Mopti: 872	Bamako: 1.04 Mopti: 4.93	Dao, 2005
2002-2003	Peri-urban Bamako	Dairy cattle	1,087	Skin test: 18.58	Sidibe et al, 2003

Mozambique

Livestock:

Year	Area	Species of animal	No. of samples tested	% positive	Reference
2015	Limpopo National Park	Buffaloes and cattle	Buffaloes: 62 Cattle: 2,445	Buffaloes: BovidTB Stat-Pak: 8.06 Bovigam IFNγ test: 0 ELISA: 0 Cattle (skin test): Inside park: 0.5 Outside park: 1.3	Tanner et al, 2015
2014	Govuro District (Southeast)	Cattle	1,136 from 289 farmers	39.6	Moiane et al, 2014
2008	Govuro district	Cattle	268	61.94	Macucule, 2009

Humans:

Viegas et al, 2015, reported that out of 49 cultures from EPTB patients in Maputo, no *M. bovis* was found.

Rwanda

Livestock:

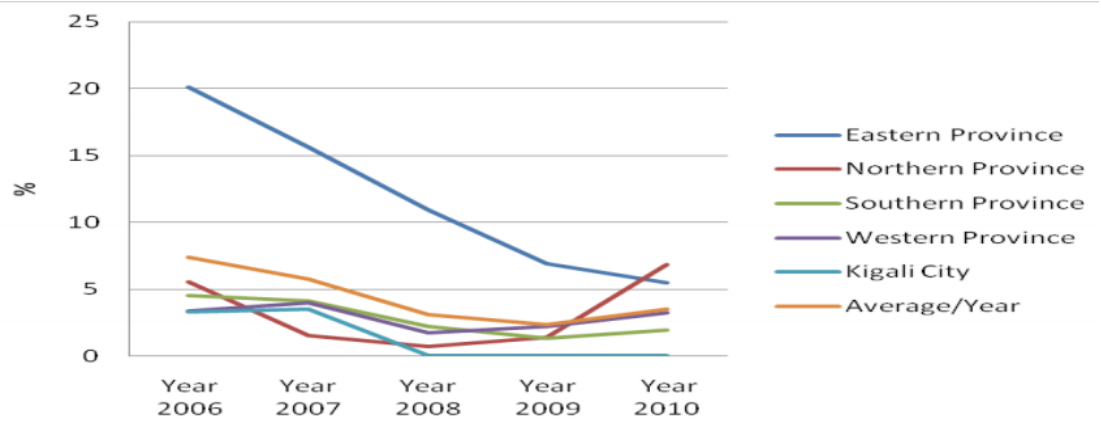
Year	Area	Species of animal	No. samples tested	% positive	Reference
2009	Abattoirs of Nyabugogo-Nyabugogo, Kigali	Cattle	16,753	Lesions: 0.9	Habarugira et al, 2014
2006-2010	National	Slaughter registers	169,488	Eastern Province: 11.8 Northern Province: 3.2	Nshimiyimana et al, 2013

				Southern Province: 2.8 Western Province: 2.9 Kigali Capital city: 1.4 See Table and Figure below for details	

Bovine TB prevalence [5] from 2006 – 2010 by district and provinces of Rwanda. Source: **Nshimiyimana et al, 2013**

Province	District	2006	2007	2008	2009	2010
Eastern Province	Nyagatare	25.53	18.86	11.59	7.25	6.2
	Gatsibo	22.04	17.41	13.93	9.25	5.16
	Kayonza	16.9	13.65	9.47	3.81	4.92
	Kirehe	2.9	8.79	4.8	3.66	2.3
	Rwamagana	0	3.15	3.78	1.94	0
	Ngoma	2.82	2.24	1.71	1.71	1.67
	Bugesera	0.61	0.67	1.26	0.86	2.85
	Sub total	**20.06**	**15.57**	**10.85**	**6.91**	**5.48**
Northern Province	Gicumbi	11.45	1.68	0.78	1.39	0.81
	Rulindo	0.9	1.23	0.6	1.34	0.62
	Gakenke	5.88	0	1.09	1.76	2.42
	Burera	0	7.14	0	0	0
	Sub total	**5.53**	**1.46**	**0.72**	**1.37**	**6.81**
Southern Province	Kamonyi	5.14	2.73	2.16	0.93	2
	Muhanga	0	3.96	0	0	2.13
	Ruhango	10.25	7.08	2.44	2.14	1.42
	Nyanza			0		
	Sub total	**4.5**	**4.13**	**2.18**	**1.32**	**1.87**
Western Province	Ngororero	6.06	6.1	1.7	2.41	3.58
	Rubavu	3.02	2.89	1.7	0	0
	Nyabihu		3.38	1.86	2.06	0
	Rutsiro	0				
	Sub total	**3.35**	**3.96**	**1.72**	**2.24**	**3.19**
Kigali City	Nyarugenge	0	6.25	0	0	
	Kicukiro	0	0	0	0	0
	Gasabo	14.28	0	0		
	Sub total	**3.33**	**3.53**	**0**	**0**	**0**
Average		**18.75**	**14.11**	**8.68**	**5.02**	**3.46**

Progression of the prevalence of bovine TB by province in 5 years period



Senegal

Livestock:

Year	Area	Species of animal	No. of samples tested	% positive	Reference
2005-2008	Dakar abattoirs	Cattle	200,101	0,0185 (37 animals, and 30 were from Mali, one from Mauritania, and only 6 from Senegal)	Diagne, 2009
2000-2003	Kaolack and Fatick	Cattle	479	Doubtful: 1.04	Konte and Unger, 2003 (mentioned by Diagne, 2009)

Humans:

Up to 2004, no cases of *M. bovis* in humans has been reported (Diguimbaye et al, 2004, mentioned by **Diagne 2009**)

South Africa

Livestock:

Year	Area	Species of animal	No. of samples tested	% positive	Reference
2012-2013	Greater Kruger National Park complex	Cattle	1,166	Positive: 0.34 Inconclusive: 2.74	Musoke et al, 2015
2011	Hluhluwe-iMfolozi Park	Buffaloes	19	PCR: 42.1 Stains: 36.8	Laisee et al, 2011
1999-2006	Hluhluwe-iMfolozi Park	Buffaloes	4,733	2.3 – 54.7	Ie Roext et al, 2015
1995	Molopo district (North West Provine)	Cattle	9,675	0.062	Bakunzi et al, 1995
1998 & 1991-1992	Kruger National Park	Buffaloes		1998: North: 1.5 Central: 16 South: 38.2 1991-1992: North: 0 Central: 4.4 South: 27.1	Rodwell et al, 2001

Tanzania

Livestock:

Year	Area	Species of animal	No. of samples tested	% positive	Reference
2011	Serengeti ecosystem	Indigenous cattle	1,103 from 32 herds	Individual: 2.4 by skin test (true prevalence: 0.6)	Katale et al, 2013

				Herd: 50	
2010-2012	Vicinity of Mikumi-Selous ecosystem	Cattle	1,288	3.7	Mwakapuja et al, 2013
2007	Manyara region	Cattle	10,549	0.9 Herd prevalence: 11.8	Cleaveland et al, 2007
2004	Kibaha and Morogoro	Cattle	Kibaha: 181 Morogoro: 259	Kibaha: 1.7 Morogoro: 0.4	Mdegela et al, 2004
2005-2007	Arusha municipality	Cattle	Lung condemnation out of 115,186	0.7	Mellau et al, 2010
2005	Morogoro	Cattle	Cattle: 728	Cattle: 2.5	Durnez et al, 2009
2003-2004	Tanga region	Cattle	642	5.4 Smallholder dairy: 2 Traditionally managed: 0	Swai and Schoonman, 2012
2003-2004	Dodoma rural and Mvomero districts.	Cattle Milk	Cattle: 277 Milk: 38	Cattle: 0 Milk: one isolate *M. tuberculosis* (no *M. bovis*)	Karimuribo et al, 2005
2003	Eastern Tanzania (Morogoro, Coast and Dar-es-Salaam)	Cattle	2,379 animals, 143 herds	Individual: Pastoral: 1 Intensive: 2 Herd level: Intensive: 10 Pastoralist: 17	Shirima et al, 2003
2002-2004	Tanga city abattoir	Cattle	12,444	0.32	Swai and Schoonman, 2012
2001	Southern highlands	Cattle		Ind: 13.2 Herd: 51	Kazwala et al, 2001

1998	Southern highlands	Milk samples	805	*M. bovis* isolated in 0.25% (2 samples)	Kazwala et al, 1998
1997	Lake Victoria zone	Cattle	8,190	0.2	Jiwa et al, 1997

A summary is also given by Katale et al, 2012.

Humans:

- Katale et al, 2015. Out of 472 TB suspected patients, no *M. bovis* was isolated.
- Cleaveland et al 2007, reported that *M. bovis* was confirmed in seven out of 65 (10.8%) human cervical adenitis cases (EPTB), of which only one came from a household owning infected cattle.
- Kazwala et al 2001, reported that out of 44 isolates from all forms of TB, 7 (16%) were identified as *M. bovis.*
- Mfinanga et al, 2004, in their study in Arusha, out of 65 cultures from EPTB cases in Arusha region during 1999-2001, *M. bovis* was isolated in 10.8% of the cultures.
- Daborn et al, 1997, out of 19 lymph node biopsies (suspected EPTB), 4 (21%) showed *M. bovis* (as referenced by Katale et al, 2012)

Uganda

Livestock:

Year	Area	Species of animal	No. of samples tested	% positive	Reference
2014	Kashaari county (Mbarara District)	Cattle	525 from 63 herds	Reactors: 2.1 Suspects: 15.43 Herd prev: 14.28	Kazoora et al, 2014
2012	Mubende district	Pigs	Approx 1,000	*M. bovis* isolated from 3 out of 150 suspicious lymph nodes	Muwonge et al, 2012
2009	City abattoir	Cattle	16,800	Lesions: 0.5	Asiimwe et al, 2009

2006-2007	Katakwe and Moroto	Cattle	1,470	1.3	Inangolet et el, 2008
2003-2004	Karamoja and Nakasongola.	Cattle	37 herds, 50 animals per herd (total: 1,850)	Herd prevalence: Kotido: 76.6 Moroto: 42.2 Nakapiripirit: 40.9 Nakasongola: 12.3	Oloya et al, 2007
2003-2004	Karamoja and Nakasongola	Cattle	1,864	51.4	Oloya et al, 2006
2002	Mbarara	Dairy cattle	340 herds	Indiv prev: 6 Herd prev: 74.1	Bernard et al, 2005

Humans:

- Assimwe et al, 2008, found that out of 344 isolates of TB patients attending clinics in Rubaga district, only 1 (0.29%)was confirmed *M. bovis.*
- Oloya et al 2007, reported that out of 43 biopsies (EPTB) from patients at Matany and Moroto hospitals in Karamoja, 3 (7%) were *M. bovis.*
- Byarugaba et al 2005, reported that out of 69 cultures from patients at the Mbarara University teaching Hospital, none was *M. bovis.*
- Niemann et al 2002, evaluated 234 isolates obtained 1995-1997 from patients in Kampala, and only 1 (0.43%) was *M. bovis.*

Zambia

Livestock:

Year	Area	Species of animal	No. of samples tested	% positive	Reference
2012-2013	Namwala district	Cattle	96 herds	Herd: 36.4 Individual: 0-14	Tembo, 2013
2012	Namwala abattoir	Cattle	1,680	Lesions: 7.4	Munyeme et al, 2012

2011-2012	Mapepe, Magoye, Monze, Batoka, Kalomo	Cattle Milk samples from PPD +	1,025 cattle and 16 milk samples	Positive: 2.6 Inconclusive: 0.87 3 milk samples (18.7%) positive for *M. bovis*	Pandey et al, 2013
2009	Livestock/ wildlife interface	Cattle	994 from 111 herds	Lochinvar: 5.2 Blue Lagoon: 9.6 Kazungula: 0.8	Munyeme et al, 2009
2008	Southern Province	Cattle traditionally reared	459	4.8	Muma et al, 2013
2004-2008	Kafue basin	Kafue lechwe	119	Lesions: 24.3	Munyeme et al, 2010
2006	Western Province	Carcass condemnation	32,717	0.46	Phiri, 2006
2003-2004	Kafue basin	Cattle	106 herds	49.8	Munyeme et al, 2008
1996	Monze District	Cattle	2,226	Individual: 7.4 Herd: 33	Cook et al, 1996

Humans:

Malama et al 2014, looked at 55 isolates obtained from PTB cases in Namwala district in 2011-2012, and 2 (3.6%) were identified as *M. bovis.*

Human prevalence and incidence data for TB and zoonotic TB

Table 6 shows the incidence and prevalence for TOTAL tuberculosis in humans for the focus countries. The majority of cases will be due to *M. tuberculosis* and not *M. bovis*. It was believed that 3.1% of the total cases of TB, were zoonotic TB. Please see Section 4 (human impact) for more details.

There are 22 countries that are classified as "High burden TB countries". They include 11 of the IDRC focus countries, and more data is available for those countries (first half of Table 6). However, the incidence per 100,000 population is very high for some of the other focus countries, such as Zambia, Malawi and Madagascar.

Table 6: Human TB data 2014

Country	TB Mortality	Prevalence	Incidence	Population	Incidence per 100,00 2014*
Bangladesh	81,000	640,000	360,000	159,078,000	227
Ethiopia	32,000	190,000	200,000	96,959,000	207
India	220,000	2,500,000	2,200,000	1,295,292,000	167
Indonesia	100,000	1,600,000	1,000,000	254,455,000	399
Kenya	9,400	120,000	110,000	44,864,000	246
Mozambique	18,000	150,000	150,000	27,216,000	551
Myanmar	28,000	240,000	200,000	53,437,000	369
South Africa	24,000	380,000	450,000	53,969,000	834
UR Tanzania	30,000	270,000	170,000	51,823,000	327
Uganda	4,500	60,000	61,000	37,783,000	161
Viet Nam	17,000	180,000	130,000	92,423,000	140
Nepal					158
Burkina Faso					54
Ivory coast					165
Madagascar					235
Malawi					227
Mali					58
Rwanda					63
Senegal					138
Zambia					406
All Data from: http://www.tbfacts.org/tb-statistics/					
Except *: http://data.worldbank.org/indicator/SH.TBS.INCD					

Zoonotic TB

From a review of a number of zoonotic tuberculosis studies, published between 1954 and 1970 and carried out in various countries around the world, it was estimated that the proportion of human cases due to *M. bovis* accounted for 3.1% of all forms of tuberculosis: 2.1% of pulmonary TB (PTB) and 9.4% of extra-pulmonary TB (EPTB) [16]. Ingestion of raw dairy products from infected cattle, is more likely associated with the development of EPTB. However, those estimates were thought not to reflect the reality due to the difficulties in correct

diagnosis (see Zoonotic disease in Section 2). Some other reports have speculated that *M. bovis* accounts for 10 to 15 percent of human tuberculosis cases [16], while other estimates range from 0.4 to 8 % [17].

The ILRI Report 212 already mentioned (Mapping of poverty and likely zoonoses hotspots - **https://cgspace.cgiar.org/bitstream/handle/10568/21161/ZooMap_July2012_final.pdf**) concluded that on average, 10.5% of the human TB cases were associated with *M. bovis*.

An attempt to estimate the global occurrence of zoonotic TB caused by *M. bovis* or *M. caprae* infections in humans by performing a systematic review and analysis of relevant scientific literature of the last 2 decades, was conducted by Mueller et al in 2013 [18]. Although information from many parts of the world was not available, data from 61 countries suggested a low global disease incidence. In regions outside Africa included in the study, overall median proportions of zoonotic TB of ≤1.4% in connection with overall TB incidence rates ≤71/100,000 population/year suggested low incidence rates. For countries of Africa included in the study, the observed median proportion of zoonotic TB cases of 2.8% was multiplied with the continental average overall TB incidence rate of 264/100,000 population/year, which resulted in a crude estimate of 7 zoonotic TB cases/100,000 population/year. It is to note that the study found no data for Southeast Asia, including major cattle producing middle- and low-income countries (e.g., India, Bangladesh, Pakistan, Myanmar, Indonesia) as well as others like Kenya, South Africa and Sudan. The authors acknowledged that the results were influenced by the technical constraints, specifically diagnostics and therefore, TB caused by *M. bovis* may be systematically underreported. Table 7 summarizes the data presented by Mueller 2013 [18] and by the ILRI 2012 report.

Table 7: Studies on the proportion of zoonotic TB. Modified from Mueller 2013 [18] and the ILRI 2012 report (link provided in the text above). Updated with all recent and /or additional references mentioned under each country in section 3b. Note it does not include updated references for non-focus countries.
MTBC: *M. tuberculosis* complex, PTB: Pulmonary TB, EPTB: Extra-pulmonary TB

Country	Study	Location	Year	MTBC	*M. bovis*	%
Burkina Faso	Health centers in Ouagadougou and Bobo Dioulasso	PTB	2001	120	0	0.00%
Burundi	Bujumbura, Bubanza Hospital	Both	1987-1994	170	0	0.00%
Cameroon	15 District hospitals West	PTB	1997-1998	455	1	0.22%
Chad	Chari-Baguirmi	Both	2002	10	0	0.00%
Djibouti	Unknown		2002	85	1	1.18%
Egypt	Fever hospital in cities		2002	67	1	1.49%
Ethiopia	Rural and pastoralist areas	PTB	2010-2011	92	0	0.00%
Ethiopia	Addis Ababa	EPTB	2015	59	1	1.69%
Ethiopia	Bahir Dar City (Nortwest)	EPTB	2012-2014	168	2	1.19%
Ethiopia	Dera Woreda (North Showa)	EPTB	2014	145	1	0.69%
Ethiopia	NA	PTB	2006-2010	964	4	0.41%
Ethiopia	Oromia and Somali regions	PTB	2008-2010	163	3	1.84%
Ethiopia	Butajira hospital	EPTB	2005-2006	156	0	0.00%
Ethiopia	Western Gojam	PTB	2007-2008			5.00%
Ethiopia	Western Gojam	EPTB	2007-2008			8.00%
Ethiopia	Southern Ethiopia	EPTB	2000-2001	35	6	17.14%
Ethiopia	Butajira health centre	EPTB	2000-2001	35	11	31.43%
Ethiopia	NA	PTB	NA	48	14	29.17%
Ethiopia	Fitche Hospital TB clinic	Both	2004-2005	42	7	16.67%
Ethiopia	Felegehiwot	Both	2007-2008	47	8	17.02%
Ghana	Korle-Bu teaching hospital	PTB	2003	64	2	3.13%
Guinea B	Unknown		1999	229	4	1.75%
Ivory Coast	TB and rural health centres	PTB	1994-1996	320	0	0.00%
Kenya	Narok		2001	132	0	0.00%
Madagascar	Institut d'Hygiene Sociale, Antananarivo	PTB	1994	126	3	2.38%
Madagascar	Antananarivo prison	PTB	1994	36	0	0.00%
Madagascar	Antananarivo - urban areas	EPTB	1994-1995	156	2	1.28%
Madagascar	Antananarivo - urban areas	PTB	1994-1995			1.25%
Madagascar	Antananarivo, Antsirabe, Fianarntsoa and Mahajanga	PTB	1994-1995	316	4	1.27%
Madagascar	Antananarivo	EPTB	1994-1995	138	1	0.72%
Malawi	Blantyre, Queen Elizabeth Hospital	PTB	NA	30	1	3.33%
Mozambique	Maputo	EPTB	2013-2014	49	0	0.00%
Nigeria	2 hospitals Ibadan		2006	60	3	5.00%
Nigeria	Lagos		1986	91	4	4.40%
Nigeria	Jos	PTB	NA	65	10	15.38%
Nigeria	Lagos		1989	357	3	0.84%
Sierra Leone	Western Area and Kenema Districts	PTB	2003-2004	97	0	0.00%
Tanzania	Arusha	PTB	2014	472	0	0.00%
Tanzania	Arusha	EPTB	1999-2001	65	7	10.77%
Tanzania	NA	EPTB	1997	19	4	21.05%
Tanzania	Arusha		2007	34	7	20.59%
Tanzania	Pastoralist Nort & South		2001	38	7	18.42%
Tanzania	Arusha		2004	34	7	20.59%
Tanzania	Arusha	EPTB	1994	11	4	36.36%
Tanzania	NA	EPTB	NA	53	20	37.74%
Tanzania	Arusha and Southern highlands	Both	1993-1996	44	7	15.91%
Tanzania	Three districts Arusha region	EPTB	1999-2001	65	7	10.77%
Uganda	Kampala		2008	344	1	0.29%
Uganda	Kampala	PTB	1995-1997	234	1	0.43%
Uganda	Karamoja		2007	43	3	6.98%
Uganda	Karamoja	EPTB	NA	24	3	12.50%
Uganda	Mbarara	Both	2004-2005	69	0	0.00%
Uganda	Kampala, Rubaga division	PTB	2006	386	1	0.26%
Zambia	Namwala district	PTB	2011-2012	55	2	3.64%
Bangladesh	Clinical		2010	350	0	0.00%
Pakistan	Hospital Lahore		2012	42	5	11.90%
India	EPTB hospital adjusted for prev EPTB in population	EBTB	2011	155	22	2.90%
India	EPTB hospital adjusted for prev EPTB in population	EPTB	2005	115	53	12.60%
India	TB meningitis	EPTB	2006	37	24	64.86%

Economic and Social Impacts at Global and Regional Levels, and in Selected Countries

Livestock impact

Bovine TB is a health problem worldwide, causing significant economic hardship for livestock farmers with estimates of >50 million cattle infected worldwide, costing $3 billion annually [19]. But due to its zoonotic nature, bTB can have serious consequences for public health.

Financial hardships to livestock owners and farmers, and associated industry include costs associated with reduction in productivity in severely affected animals, testing, culling of affected animals, indemnity payments, movement controls, restrictions on trade, maintenance of control programs, and research to develop improved control strategies.

Impact in Africa

In a questionnaire survey conducted by the OIE in Africa during Dec 2014 – Jan 2015 and sent to the Veterinary Authorities of the 54 African OIE member countries, respondents were asked to estimate parameters that drive economic impacts for 35 priority diseases (including bovine TB). In all, 27 out of 54 countries provided quantitative information.

(**http://www.oie.int/fileadmin/Home/eng/Publications_%26_Documentation/docs/pdf/TT/2015_AFR1_Grace_A.pdf**). The level of responses indicated a high level of lack of information on disease impacts. Expert opinion was used to estimate the average value of adult livestock at USD 379 for cattle. Vaccination and treatments were estimated to cost USD 2 and USD 3 for large animals. Using these approximate estimates, the 35 priority diseases were roughly estimated to cost nearly USD 9 billion a year or 6% of the total value of the livestock sector in Africa. These estimates do not include losses due to lost productivity or to impacts on human health. The results can be seen in Figure 3, and would represent approximately USD 200 million per year for bTB. These results need to be taken with caution, considered the methodology described (using estimated parameters given by respondents) and the limitations. Caution needs to be taken. For example, looking at other diseases, Tambi et al reported in 2006 an annual cost of CBPP of 44.8 million euros (approx. USD 50 million) which is very far from the over 400 million calculated in this study.

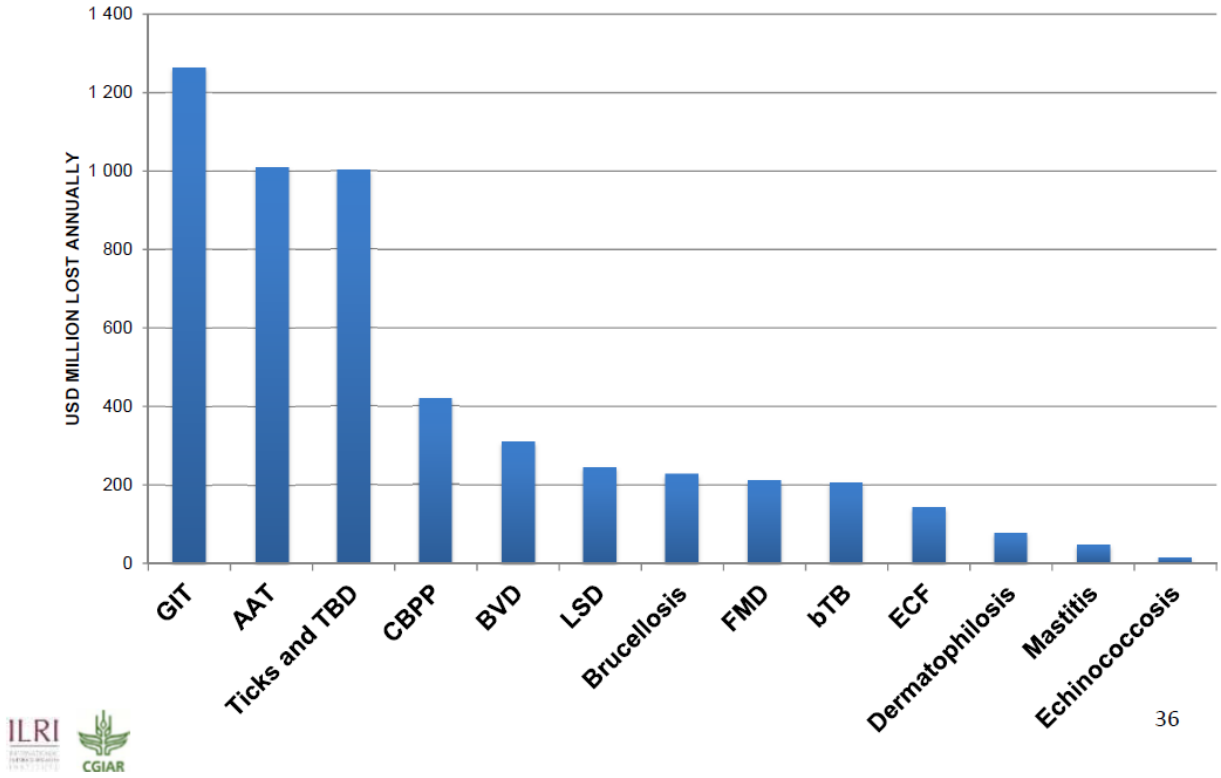


Figure 3: Losses from cattle diseases, as estimated by OIE Africa. Source: Grace, Songe and Knight-Jones, 2015. Impact of neglected diseases on animal productivity and publich health in Africa. OIE Africa Regional Commission.

Analysis by the World Bank:

The World Livestock Disease Atlas – a quantitative analysis of global animal health data [20], published by the World Bank (with cooperation of OIE and FAO) in 2011 is an attempt to understand which livestock diseases cause the heaviest losses, which countries suffers the worst disease-related losses and which livestock species are most affected.

http://wwwwds.worldbank.org/external/default/WDSContentServer/WDSP/IB/2012/02/17/000356161_20120217030841/Rendered/PDF/668590WP00PUBL00Livestock0Atlas0web.pdf

The World Livestock Disease Atlas bases its analysis on the Livestock Units (LSU). Each species has a LSU value, and the losses of LSU have been given a value. See Figure 4. For more information on the methodology description, please refer to the World Bank Atlas itself (pages 6 & 7). Bovine TB is one of the top 10 diseases causing losses for cattle, buffalos and small ruminants, as shown in Figure 5. However, looking at the data in detail, there are few data from sub-Saharan Africa and Asia.

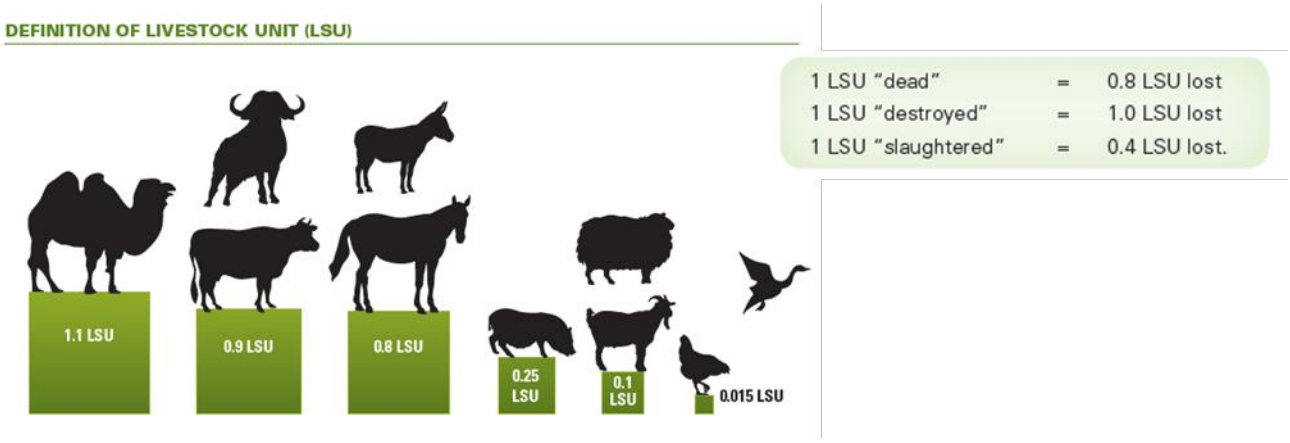


Figure 4: Livestock Units. Source: World Livestock Disease Atlas – The World Bank, 2011 [20].

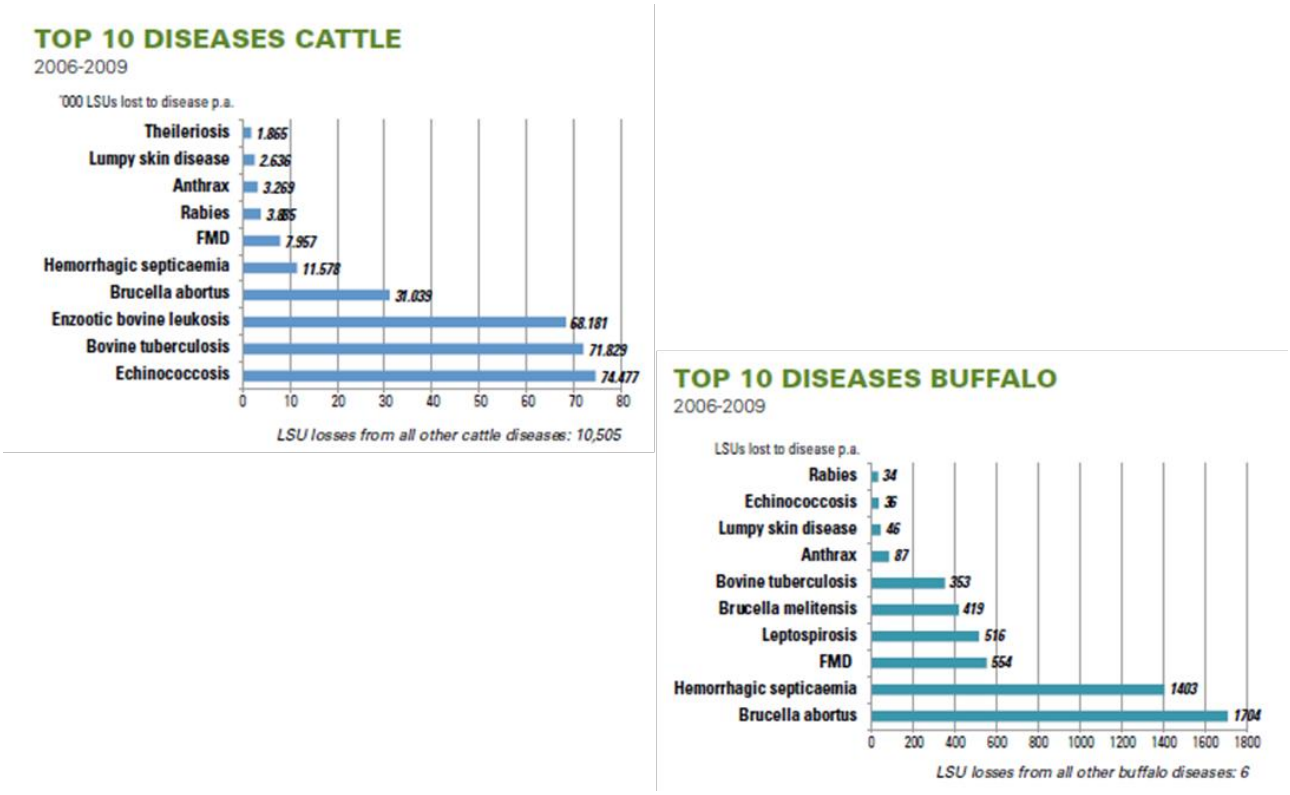


Figure 5: Top 10 diseases in terms of LSU losses for cattle, buffalo, and sheep & goats. Source: World Livestock Disease Atlas – The World Bank, 2011 [20][3].

Wildlife impact

Infection of wild animals by bTB is raising concern worldwide. Its importance relates to conservation issues (impact on wildlife including endangered species), impact on livestock production, impact on public health and economic impact on private game ranchers [21].

Wildlife can play an important role as maintenance hosts, and interfering in the disease control, as demonstrated by the European badger in UK and the Republic of Ireland, and the possum in New Zealand.

In Africa, bTB has been confirmed in livestock in the majority of countries. Wildlife infection is confirmed in seven countries from southern and eastern Africa, apparently spreading in the southern Africa region. *M. bovis* has been isolated from 17 wild mammal species, although maintenance host status has only been shown for buffalo and lechwe, and suggested for greater kudu and possibly common warthog. Buffaloes don't seem to be clinically affected. It is suspected that most other wild species act as dead-end-hosts.

Zoonotic risks are a concern, but no direct spillover from wildlife to humans has been documented, and no case of bTB spillback from wildlife to livestock has been confirmed.

Human impact

Prior to mandatory pasteurization in many countries, *M. bovis* accounted for ~25% of TB cases in children. In Great Britain alone, it is estimated that human consumption of infected cows' milk led to 2,500 deaths and >50,000 new cases of TB per year in the early 1900s. At present, *M. bovis* infection of humans is rare except in developing countries that do not have control programs or mandatory pasteurization directives.

The WHO Global Tuberculosis 2015 Report, says that 9.6 million people fell ill with TB in 2014, including 1.2 million people living with HIV. In 2014, 1.5 million people died from TB, including 0.4 million among people who were HIV-positive (**http://who.int/tb/publications/global_report/factsheet_global_2015.pdf?ua=1**).

Regarding the prevalence of zoonotic TB, as mentioned in Section 3c, there is a scarcity of data. A review of a number of zoonotic tuberculosis studies, published between 1954 and 1970 and carried out in various countries around the world, estimated that the proportion of human cases due to *M. bovis* accounted for 3.1% of all forms of tuberculosis Some other reports have speculated that *M. bovis* accounts for 10 to 15 percent of human tuberculosis cases [16], while other estimates range from 0.4 to 8 % [17]. More recently, a report published by ILRI in 2012, concluded that an average of 10.5% of human TB cases were associated with *M. bovis* in developing countries (not all developing countries were included), and Mueller et al in 2013, concluded that the proportion of zoonotic TB in Africa was 2.8%.

For more details on zoonotic prevalence per country, please see section 3.

Disability adjusted life years (DALY's)

The WHO Estimates of the global burden of foodborne diseases Report, published in December 2015, (http://www.who.int/foodsafety/publications/foodborne_disease/fergreport/en/), estimates the **Disability adjusted life years (DALYs) due to *M. bovis* is 607,775, and 9 DALYs per 100,000 persons**. See Figure 6.

Abbreviations: NoV = Norovirus; Camp = *Campylobacter* spp.; EPEC = Enteropathogenic *Escherichia coli*; ETEC = Enterotoxigenic *E. coli*; STEC = Shiga toxin-producing *E. coli*; NTS = non-typhoidal *Salmonella enterica*; Shig = *Shigella* spp.; Vchol; *Vibrio cholerae*; Ehist = *Entamoeba histolytica*; Cryp = *Cryptosporidium* spp.; Giar = *Giardia* spp.; HAV = Hepatitis A virus; Bruc = *Brucella* spp.; Lmono = *Listeria monocytogenes*; Mbov = *Mycobacterium bovis*; SPara = *Salmonella* Paratyphi A; STyph = *Salmonella* Typhi; Toxo = *Toxoplasma gondii*; Egran = *Echinococcus granulosus*; Emult = *E. multilocularis*; Tsol = *Taenia solium*; Asc = *Ascaris* spp.; Trich = *Trichinella* spp.; Clon = *Clonorchis sinensis*; Fasc = Fasciola spp.; Flukes = Intestinal flukes; Opis = *Opisthorchis* spp.; Parag = *Paragonimus* spp.; Diox = Dioxin; Afla = Aflatoxin.

Figure 6: Scatterplot of the global burden of foodborne diseases per 100,000 population and per incidence case Source: WHO Estimates of the global burden of foodborne diseases, 2015. (Note: axes use log scales). The red arrow points at *M. bovis*. Green arrows point at other diseases of interest for IDRC (*T. solium*, Brucellosis and *E. granulosus*)

Cost effectiveness of control & research strategies

Research and control efforts, however, have proven cost effective. For instance, it is estimated that the US bovine TB eradication program cost ~$3.5 billion from 1917 to 1962 which resulted in a net savings of ~$159 million annually, primarily due to decreased carcass condemnation, improved animal productivity, and reduced animal replacement costs [19]. Some other authors, question the resources invested in the control of zoonotic TB, at least in countries like the UK [22].

Country information

Only information from Zambia was found. Mwacalimba and colleagues [23] developed an economic simulation model evaluating the costs and benefits of bTB control in a wildlife-livestock interface area of Southern Zambia over a 10 year period. They were based on the use of test and slaughter in livestock and promotion of milk pasteurization amongst livestock keeping communities to reduce the zoonotic transmission of bTB through milk. Expected benefits included increased productivity and health in village resident and transhumant cattle, and averted human bTB treatment costs after the fourth year of the project. In monetary terms, at different bTB prevalence estimates in cattle, the simulation outcome showed that the costs of control never exceeded the few benefits considered over the simulated period. However, the benefits are likely to outweigh the costs if wider implications of bTB in humans (infirmity-related productivity losses), livestock and wildlife (reduced productivity and herd value in cattle and diminished tourism potential from bTB-related wildlife mortalities) are taken into account.

Disease Prevention and Control Methods

There are four principal approaches to the control of tuberculosis: 1) test and slaughter, 2) test and segregation, 3) Immunization and 4) Chemotherapy. Chemotherapy is forbidden in the majority of the countries, immunization is not very effective, so the main measures used have been test and slaughter, and test and segregation. However, these last 2 measures are very difficult to implement in developing countries.

Treatment (Control)

Until the discovery of the antituberculosis drug isonicotinic acid hydrazide (INH), there was no practical therapeutic agent available for the treatment of bTB. Reports from Brazil and South Africa suggest that it is feasible to treat cattle with isoniazid. It is claimed a 78% bacteriological cure, and a prophylactic value. It is easy to administer and has relatively few adverse effects. It is used at 10mg/Kg body weight daily for eight weeks.

As all reactors that are treated are not cured bacteriologically, INH treatment cannot be regarded as a means of eradicating the disease. The disadvantages are so great, however (up to 25% refractory cases, emergence of drug resistant strains, elimination of INH in the milk and the danger of relapse when the drug is withdrawn) that the treatment is not allowed in many countries.

Treatment with INH is regarded as a temporary measure, and would only be justified where an attempt is made to salvage animals with unique genotypes.

Prophylaxis (Prevention)

Sanitation and disinfection may reduce the spread of the agent within the herd. *M. bovis* is relatively resistant to disinfectants and requires long contact times for inactivation. Pasteurization and abattoir inspection are recognized as successful methods to decrease the spread of the disease to humans.

BCG vaccines, incorporate a culture-attenuated variant of *M. bovis* (Bacillus Calmette–Guérin), for the basis of a human tuberculosis vaccine. The same vaccine has been used in cattle in some high-risk areas, but it does not completely prevent infection. For more details, see Section 6. Unfortunately, vaccinated cattle react on the tuberculin skin test and therefore the vaccine should not be used in countries where control or trade measures are based on such testing. However, this should not preclude its use, especially in developing countries where other measures are not feasible, unless final stages of eradication programs have been reached. Countries that attempted to use vaccination as the basis of a control program have ultimately abandoned the procedure in favor of the test-and-slaughter method.

There are no more effective vaccines for use in livestock or wildlife. New vaccines are being developed and tested. For more details, see Section 8.

Options and strategies for control programs at herd, national, sub-national or regional level

The Food and Agriculture Organization of the United Nations (FAO) has recognized zoonotic bovine tuberculosis as a priority infectious disease which should be controlled at the animal–human–ecosystem interface, through national and regional efforts.

Bovine TB can be controlled by test-and-slaughter or test-and-segregation methods. Affected herds are re-tested periodically to eliminate cattle that may shed the organism; the tuberculin test is generally used.

Infected herds are usually quarantined until no further reactors are detected, and there is no evidence of tubercules in reactors at slaughter, and animals that have been in contact with reactors are traced. Only test-and-slaughter techniques are guaranteed to eradicate tuberculosis from domesticated animals. However, some countries use test-and-segregation programs during the early stages of eradication, and switch to test-and-slaughter methods in the final stage. Once eradication is nearly complete, slaughter surveillance, with tracing of infected animals, may be a more efficient use of resources.

The success of control programs based on the test and slaughter strategy depends on institutional and technical requirements including (as mentioned by Ahmed El Idrissi “Bovine TB” in The Art & Science of Tuberculosis Vaccine Development, 2nd Edition, 2014 **http://tbvaccines.usm.my/finlay**):

- an efficient cattle identification system that allows effective tracing back to the herds of origin of tuberculous animals detected through slaughterhouse surveillance;
- a high standard of meat inspection practices enabling effective surveillance for tuberculous lesions in animals passing through slaughterhouses;
- an animal health information system for recording relevant information including epidemiological investigations, and data analysis to monitor progress and guide decision making;
- a legal framework for enforcing control measures and compensating farmers for the slaughter of tuberculin positive reactors;
- full control of movements of cattle including cross-border transhumance;

- political support with cooperation of stakeholder groups involved and public awareness to ensure the success of the bovine TB control and eradication program;
- public awareness campaigns and sensitization of farmers and the general public on bovine TB hazards and hygiene practices, and awareness of the objective, benefits, challenges and other implications of surveillance and control;
- incentives for farmers to adhere to the eradication program, such as guaranteed milk prices and setting subsidies for disease free herds;
- laboratory diagnostic capability for TB diagnosis based on the isolation and species identification of the bacterium from tuberculous lesions on organs, and,
- financial resources, for adequate and speedy compensation of farmers for losses due to the removal of infected animals.

These requirements are complex, expensive, and difficult to implement for most African countries. The general lack of public resources in developing countries, seriously hampers such control strategies. Also, many control measures will conflict with the customs and habits of the affected communities, so it is essential that the appropriateness of each possible intervention is assessed. The culling of sick animals might be seen as a loss of prestige, for example, while the keeping of livestock outdoors during the night might expose it to too great a risk of theft, and boiling will change the production and flavor of naturally soured milk. When possible, the negative effects of control strategies should be mitigated. The introduction of new probiotic organisms for milk souring may, for example, enable the fermentation of milk to continue as an acceptable practice, while limiting mycobacterial growth and infection [24].

Control of tuberculosis in wildlife

This can be a major component of a control program and a big challenge, in places where wildlife is acting as maintenance host, like badgers in the UK, possums in New Zealand, or wild boar in Spain. Culling to reduce the population density can decrease transmission, but it might not be a very popular measure and may have unanticipated effects, such as increasing the dispersal of the remaining members. Barriers can be used around certain areas to prevent wildlife access. Several control measures are being evaluated, for example vaccination of badgers in the UK, or oral baits with BCG vaccine in wild boar in Spain.

Other measures:

The high prevalence of bTB in African buffalo (*Syncerus caffer*) in regions of southern African has a negative economic impact on the trade of animals and animal products, represents an ecological threat to biodiversity, and poses a health risk to local communities through the wildlife-cattle-human interface. Test and cull methods may not be logistically feasible in many free-range wildlife systems, and with the presence of co-existing bTB hosts and the limited effectiveness of the BCG vaccine in buffalo, there is a need for alternative methods of bTB management. Selective breeding for increased resistance to BTB in buffalo may be a viable method of bTB

management in the future, particularly if genetic information can be incorporated into these schemes. This has been recently explored by Ie Roex et al, 2015: **http://www.ncbi.nlm.nih.gov/pubmed/25985909**.

Disease situation and government policies by country

Tables 8 and 9 below have been completed with the information received from the questionnaires sent to the DG and DVS. For a list of respondents, please see Annex 2.

Table 8 covers the disease situation (if it is notifiable or not), the presence of official surveillance and/or control programs, and the treatment situation. Table 9 refers to vaccination.

The definitions that were given to the respondents are:

[1]Surveillance: is the systematic ongoing collection, collation and analysis of data and the timely dissemination of information to those who need to know so that action can be taken.

[2]Control: a program which is approved, and managed or supervised by the Veterinary Authority of a country for the purpose of controlling a vector, pathogen or disease by specific measures applied throughout that country, or within a zone or compartment of that country.

Table 8: Official status, official programs and treatment for bovine TB in the countries of interest. Information provided by the questionnaire sent to the DG/DVS as part of this monograph. No responses were received from India, Indonesia, Burkina Faso, Ethiopia, Madagascar, Mozambique, Senegal and South Africa.

Country	Notifiable (yes/no)	Official surveillance[1] program (yes/no) (if yes, active or passive)	Official control[2] program (yes/no)	Treatment (Chemotherapy)	
				Treatment authorised (yes/no)	Frequently practiced (yes/no)
ASIA					
Bangladesh	Yes	Yes (targeted)*	No	N/A	N/A
Myanmar (Burma)	Yes	Yes, passive	No	No	No
Nepal	Yes	Yes, passive	No	No	No

Vietnam**	No	Yes, passive	No	Yes	Yes
AFRICA					
Côte d'Ivoire (Ivory Coast)	Yes	Yes, passive but active if outbreaks	Yes	-	-
Kenya	Yes	Yes, passive (abattoir)	No	No	No
Malawi	Yes	Yes, passive and active	Yes	N/A	N/A
Mali	N/A	Yes, passive		No	No
Rwanda	Yes	Yes, passive and active	No	Yes	No
Tanzania	Yes	Yes, passive and active	No	No	No
Uganda	No	No	No	No	No
Zambia	Yes	Yes, passive	No	N/A	N/A

* Not clear if the respondent meant active, or referred to a certain area.
**It is interesting to note, that Vietnam respondent said that treatment was authorized, and used frequently, as well that it was not a notifiable disease.

Table 9: Vaccination for bovine TB in the countries of interest. Information provided by the questionnaire sent to the DG/DVS as part of this monograph. No responses were received from India, Indonesia, Burkina Faso, Ethiopia, Madagascar, Mozambique, Senegal and South Africa.

Country	Vaccination			
	Compulsory vaccination (yes/no)	Who pays for the vaccine (Government, farmers, combination, others-specify)	Who delivers the vaccine (official, private vaccinators or both)	Species vaccinated (cattle, sheep, goats, pigs, poultry)
ASIA				

Bangladesh	No	N/A	N/A	N/A
Myanmar (Burma)	No	-	-	-
Nepal	No	N/A	N/A	N/A
Vietnam*	No	Famers	Private vaccinators	Cattle
AFRICA				
Côte d'Ivoire (Ivory Coast)	No	-	-	-
Kenya	No	N/A	N/A	N/A
Madagascar				
Malawi	No	N/A	N/A	N/A
Mali	No	N/A	N/A	N/A
Rwanda**	No, but tuberculin test	Government	Official	Cattle
Tanzania	No	N/A	N/A	N/A
Uganda	No	N/A	N/A	N/A
Zambia	No	N/A	N/A	N/A

*Again, it is interesting to note the response from Vietnam. It Is surprising and raises concerns as to whether there may have been a misinterpretation or language barrier.
** The reply refers to the tuberculin skin test, but it was left to reflect the country response.

Vaccines Available

BCG vaccine in livestock

Currently, a commercial vaccine licensed for use in cattle for bTB does not exist. The vaccine for human TB, the BCG (a live attenuated *M. bovis* strain), has been used in cattle, and remains the best candidate vaccine for use in the field in the short to medium term [25]. Some advantages include that the vaccine is safe, relatively inexpensive, and is commercially produced for human application. The safety of BCG has been demonstrated for multiple host species, including cattle (strains Danish, Pasteur, Russia, Glaxo, Goteborg) in numerous trials over the past 90 years. The major caveats which have restricted its use, is that BCG protection is not complete. It limits mycobacterial burden and associated pathology in cattle but does not prevent infection. It also sensitises animals to respond in traditional TB tests. Potentially, these issues can now be overcome by using vaccination as part of a control program integrated with other control measures and using DIVA diagnostics [26].

History

Waters et al, published in 2012 a good historical review [19] on the development of bTB vaccines (summarised here). Calmette and Guerin, developed an attenuated *M. bovis* vaccine (bacillus of Calmette and Guerin, BCG) by serial propagation of the bacillus on ox bile glycerine potato medium as a vaccine for *M. tuberculosis* infection of humans. Ironically, BCG was first evaluated and proven effective in cattle circa 1911, ten years prior to delivery of the vaccine to a human infant in 1921.

During the 1920s, von Behring and others in Great Britain, France, Netherlands, Italy, Argentina, United States, and Japan tried immunization of cattle with different approaches and candidates, but besides BCG, none of these efforts proved both effective and practical. In the 1940s *M. microti* was also evaluated with reasonable results, but its use was not recommended due to variations in virulence of *M. microti* in cattle and interference with tuberculin skin tests.

Numerous experimental and field trials were performed in the early to mid 1900s examining the use of BCG in cattle. While variations in study design as well as strains, doses, and routes of both BCG and virulent *M. bovis* challenge complicate comparisons between studies, some conclusions of these early studies are still applicable today. They include:

- a single dose of BCG provides non-sterile immunity to subsequent experimental challenge with virulent *M. bovis* 2–4 months after vaccination
- field vaccination results in variable efficacy (0–80%)
- live bacilli are required for protection

Efficacy results for field trials with BCG performed between 1920 and 1982 ranged from complete to minimal benefit to no benefit. In 1953, an extensive field trial in England demonstrated inconclusive results with the use of BCG to protect cattle; therefore, use of the vaccine was not advised. Field trials with BCG performed during the mid 20th century failed to demonstrate a suitable level of protection. Potential reasons for failure were: generally high doses of BCG (10^8–10^{10} cfu parenterally) were used for vaccination, now known to be less effective than lower doses of BCG (10^3 to 10^6 Cfu parenterally); studies were often performed in regions with very high prevalence of *M. bovis*; and, calves may have been exposed to *M. bovis* very early in life prior to vaccination through consumption of milk from cows with tuberculous mastitis.

More recent studies with BCG in cattle have confirmed prior favorable studies demonstrating reductions in disease severity (i.e., decreased *M. bovis* colonization and associated pathological changes) and considerable level of protection consistently in excess of 50%. Additional conclusions of recent research include:

- BCG is at least effective, and most likely, more effective when administered to neonatal calves compared to vaccination of older calves,
- short interval BCG booster vaccination of young calves does not enhance protection,
- the vaccine may be delivered orally with effective results provided adequate doses are given (see more information on doses below).

Current status and challenges

A very good summary has been recently published by Parlane and Buddle [26] and is the basis for this section. Over the past two decades, a large amount of knowledge has been acquired on the use of BCG through the harmonisation of cattle challenge models, BCG strains, and doses, and there have been an important number of recent developments (Table 10). Against experimental challenge with *M. bovis*, vaccination with BCG via subcutaneous or oral routes has repeatedly resulted in reductions in pathological and microbiological findings, although sterilising immunity is not produced. However, the experimental challenge is more severe than that observed following natural exposure as it is aimed to produce pathology in the majority of the non-vaccinated animals, while mimicking the pathology observed in naturally-infected cattle.

BCG vaccine has been shown to be effective when administered at relatively low doses (10^3 to 10^6 CFU) subcutaneously or at higher doses (10^8 CFU) via the oral route, and by different daughter BCG strains (Pasteur and Danish). The OIE Terrestrial Manual says that a typical dose would be from 10^4 to 10^6 colony-forming units given SC, and the vaccine should be based on the standard reference strain, BCG Pasteur or Danish. Vaccination of neonatal calves (<1 month of age) induced higher levels of protection than those vaccinated at 6 months of

age [27][28]. This may be explained by the high numbers of circulating NK and γδ T cells in young calves which may lead to a robust innate response following vaccination and administration of BCG prior to presensitisation with environmental mycobacteria.

One contentious attribute of BCG in cattle is the duration of immunity. A recent study using a stringent challenge model, demonstrated that BCG immunity may not be of long duration, with protection induced in calves vaccinated at 1 month of age and challenged 12 months later, while no significant protection was observed in another cohort challenged after 24 months [29]. Calves vaccinated as neonates and then revaccinated 6 weeks later had significantly reduced protection compared to those vaccinated once as neonates [27]. A recent study has demonstrated that BCG revaccination of cattle at 2 years after initial vaccination when immunity had waned boosted protection against challenge with *M. bovis*. In contrast, revaccination with either of two TB protein vaccines failed to enhance protection [30]. This study provided encouragement that protection could be produced in cattle following revaccination with BCG at 2 years after initial vaccination, and further studies are now required to optimise the timing of revaccination to provide long-term protection and to evaluate revaccination in the field situation.

Field trials in Mexico, Ethiopia, and New Zealand have provided evidence for BCG to protect against natural exposure to *M. bovis*. In Mexico results showed a 59.4% efficacy [31] and in Ethiopia 56-68% protective efficacy [32]. The trial in Ethiopia also indicated that protection could last at least 22 months. A BCG field trial has just been completed on an isolated farm in New Zealand where *M. bovis* infection was endemic in wildlife (possums, ferrets, wild pigs, and wild deer). Over a 3-year period, five cohorts of cattle (ranging from 6 to 30 months of age) were tuberculin skin tested and reactor animals excluded from the study, but retained in the herd. Just over half of the almost 1,300 test-negative cattle were vaccinated orally with BCG mixed in a lipid matrix (Liporale, University of Otago, New Zealand), and the remainder left unvaccinated. These cattle were subsequently slaughtered at a commercial slaughterhouse when they reached their target weight for beef animals at 3 – 4 years of age. An oral (rather than injected) preparation of BCG was used as it was considered likely to produce fewer BCG-induced positive skin test responses in tests conducted a year or more after vaccination. At the slaughterhouse, the animals were examined for tuberculous lesions and samples collected for bacterial culture. Of >1,200 animals inspected, preliminary analysis indicated that a significantly smaller percentage (~4 %) of vaccinated animals were infected with *M. bovis* compared with ~10 % for the non-vaccinated animals. The efficacy of the vaccine appeared to be high in the first year after vaccination [26].

Table 10: Recent highlights in the development of TB vaccines for cattle (Source: Parlane and Buddle, 2015)

Year	Development	References
1995	Low doses of parenteral BCG protected against bovine TB	[33]
1999-2001	Use of specific *M. bovis* antigens in whole blood IFN-γ test could differentiate BCG-vaccinated from *M. bovis*-infected cattle	[34][35]

2003-2005	BCG vaccine induced a high efficacy in calves <1 month old.	[27][28]
2003-2005	Combinations of TB DNA or protein vaccine with BCG induced protection greater than with BCG alone.	[36][37]
2009	BCG prime with viral vector vaccine boost enhanced protection against bovine TB.	[38]
2009	Vaccine-induced central memory immune response was a useful correlate of protection	[38][39]
2010	DIVA skin test was developed for differentiation of BGC-vaccinated from *M. bovis*-infected cattle.	[9]
2011	Demonstrated that maintenance of antigen-specific skin test response was not required for protection	[8]
2012	BCG-induced immunity waned between 12-24 months post-vaccination.	[29]
2012	BCG strain overexpressing Ag85B induced better protection than that induced by wild-type BCG strain	[40]
2012	Revaccination with BCG when immunity had waned boosted protection against bovine TB	[30]

An additional challenge associated with BCG vaccination is that it compromises the specificity of tuberculin PPD-based diagnostic tests such as skin tests. Up to 80% of BCG-vaccinated cattle reacted positively to tuberculin skin test 6 months post-vaccination. However, the skin test reactivity rate decreased rapidly to ~10–20% by 9 months post-vaccination. Therefore, the BCG use in cattle and other domestic animals will require the development of a diagnostic test that can be used alongside vaccination to differentiate vaccinated and infected cattle (DIVA test). Please see recent developments under Diagnostics in Section 2.

The BCG cattle vaccine is banned in the EU because it interferes with the tuberculin skin test and there is currently no validated test to differentiate infected from vaccinated animals. For the same reasons it is banned in the USA and many other countries.

(**https://www.gov.uk/government/publications/2010-to-2015-government-policy-bovine-tuberculosis-bovine-tb/2010-to-2015-government-policy-bovine-tuberculosis-bovine-tb**).

Characteristics of the current BCG vaccine are included in the TPP in Table 11.

Research is being conducted to improve the BCG vaccine, and it has been demonstrated that subunit vaccines may boost immunity elicited by BCG in cattle (See Section 8).

Wildlife vaccines

The efficacy of BCG delivered orally has been demonstrated for brushtail possums (in field trials) as well as Eurasian badgers, wild boar, and white-tailed deer (each in experimental challenge studies). Vaccine delivery to wildlife reservoirs is oral, although a parenteral route is being deployed for badgers in England.

The major advantages of use of BCG in wildlife include: (1) the long history of use and safety in humans; thus, there is minimal concern for accidental exposure to humans, (2) proven efficacy when delivered orally in multiple species, (3) low cost of production, (4) commercially available sources of the vaccine and, (5) demonstrated efficacy with a single dose. Other vaccines evaluated for use in wildlife species include heat-killed *M. bovis* for use in wild boar, heat-killed *M. vaccae* in combination with live BCG for use in brushtail possums, and novel attenuated *M. bovis* mutants for use in brushtail possums [19].

Vaccination of wildlife present several unique challenges, especially with delivery. In general, the most practical method of delivery is via oral baits. Difficult to control variables for oral delivery include: dose, coverage (that is, the number of animals receiving the vaccine), age of vaccination, prior exposure to *Mycobacterium* spp. including *M. bovis*, vaccine uptake by non-target species, vaccine viability in the environment, and a controlled mechanism for revaccination in subsequent years. With oral baits, bait attractant and consistency must be optimized for each target host.

Main vaccine needs:

There is a need for a vaccine that:

1. Provides sterile immunity
2. Has a longer duration of immunity
3. Has DIVA capabilities

Human vaccines

The BCG vaccines have been in use since the 1920s and while these protect very young children from the more invasive forms of TB, adolescents and adults are variably protected and remain susceptible to pulmonary diseases caused by *M. tuberculosis*. The efficacy of current BCG vaccines against pulmonary TB disease is highly variable and is approximately 50% on average worldwide, while the efficacy against meningeal and miliary TB in

infants is relatively higher at about 75% in average (WHO Informal Consultation on Standardization and Evaluation of BCG Vaccines Geneva, Switzerland 22-23 September 2009.

http://www.who.int/biologicals/publications/meetings/areas/vaccines/bcg/BCG_meeting_report_2009v7_FOR_WEB_10JUNE.pdf)

Commercial vaccines

There are no commercial manufacturers of bTB vaccines, or of BCG vaccines specifically for cattle. Search of the databases from the Center for Food Security & Public Health (Iowa State University), and Vetvac did not yield any results. The BCG manufactured for humans, is the vaccine that has been used in cattle.

The BCG vaccine is in fact a range of different strains, as it became apparent that the subculture Calmette and Guérin distributed to laboratories around the world in the 1920s were evolving in different ways. It is now produced from samples emanating from 7 sites around the world, each with subtly different molecular and genetic characteristics. Different strains are named after these sites: BCG (Paris), BCG (Copenhagen) etc... This may result in different product characteristics.

The BCG strain used most frequently since the mid-1980s was BCG Pasteur. However, mainly due to licensing issues, recently, the BCG strain of choice has become a freeze-dried preparation of BCG Danish produced by the Staten Serum Institute (Denmark). Equal protective efficacies of these two strains has been confirmed.

Currently the WHO has a list of 5 pre-qualified BCG manufacturers (accessed 18 January 2016):

http://www.who.int/immunization_standards/vaccine_quality/PQ_vaccine_list_en/en/ :

1. BB-NCIPD Ltd. Country of manufacture: Bulgaria. Distributor: Intervax (Canada).
2. Green Signal Bio Pharma Pvt. Ltd. Country of Manufacture: India **http://www.gsbpl.com/index.php/products/bcg-vaccine**
3. Japan BCG Laboratory. Country of Manufacture: Japan **http://www.bcg.gr.jp/english/menu1.html**
4. Serum Institute of India Ltd, India. Country of Manufacture: India **http://www.seruminstitute.com/content/products/product_bcg1.htm**
5. Statens Serum Institut. Country of Manufacture: Denmark. **http://www.ssi.dk/English/Vaccines/BCG%20Vaccine%20Danish%20Strain%201331.aspx**

None of their websites mentions the vaccine being licenced in cattle.

The BCG strain used in the badger and cattle in the UK, is BCG Danish strain 1331 produced by the Statens Serum Institut in Copenhagen, Denmark, which is the strain licensed for human vaccination in the UK (**http://veterinaryrecord.bmj.com/content/175/4/90.full.pdf+html**). The injectable badger vaccine is called BadgerBCG and has been available on prescription in the UK since March 2010. It is the same vaccine that the one used in humans, but at higher doses. Research is ongoing for an oral badger vaccine.

Characteristics of Ideal Vaccine Candidates for Smallholders

The Target Product Profiles (TPPs) reflect the availability and utility of current agents and incorporate features that will be necessary to improve on the current products and to address unmet needs, taking into account the particular requirements of the poorest livestock keepers.

The TPPs are more robust when they include the opinions and consider the needs of the different stakeholders. While efforts have been made to encompass them, the TPP showed in Table 11 below, should be considered a proposal, a live document subject to improvements.

Information on current vaccines has been based on the BCG vaccine produced by SSI Denmark:

http://www.ssi.dk/English/Vaccines/BCG%20Vaccine%20Danish%20Strain%201331/Discription%20of%20BCG%20Vaccine%20SSI.aspx

Table 11: Target Product Profile (TPP) bovine TB – Proposal:

	Attribute	Minimum (current available vaccine)	Ideal
1	Antigen	Live attenuated *M. bovis*	Immunogen with protective antigens for *M. bovis* and *M. caprae (*and *M. tuberculosis?).*
2	Indication for use	Theoretically (no licensed product): Reduction of disease severity and transmission in cattle (*M. bovis*) and goats (*M. caprae)*	For active immunization of cattle, water buffalo, sheep, goats, pigs, camels and wildlife to prevent infection and transmission
3	Recommended species	Humans (currently there is no product licensed in animals)	Cattle, buffalo, sheep, goats and pigs. Also all susceptible animals, including susceptible wildlife.

4	Recommended dose	Cattle: 10^3 to 10^6 CFU SC, or at higher doses (10^8 CFU) via the oral route (High doses are known to be less effective)	Same dose for all species (1 or 2 ml)
5	Pharmaceutical form	Freeze dried (powder and solvent for suspension)	Ready to use solution/suspension
6	Route of administration	SC Has been used oral in wildlife and experimentally in calves	SC or oral (oral route very important for wildlife)
7	Regimen - primary vaccination	One injection	Single lifetime dose
8	Regimen - booster	When immunity wanes. Probably between 1 and 2 years.	Lifelong immunity after primary vaccination
9	Epidemiological relevance	Protection against disease caused by *M. bovis* and *M. caprae*	Protection against infection *M. bovis* and *M. caprae*
10	Recommended age at first vaccination	From one month of age (more effective in younger than older calves)	From 1-2 months of age, when other vaccines are applied.
11	Onset of immunity	As soon as 25 days after vaccination	<7 days
12	Duration of immunity	1 year. No protection after 2 years.	Lifelong immunity
13	Expected efficacy	Reduction of disease severity and >50% protection. Does not prevent infection.	To prevent infection and transmission in 100% of the animals. No disease after virulent challenge.
14	Expected safety		No post-vaccinal reactions at any age. Safe for pregnant animals at any stage. Safe for all sexes at any age.
15	Withdrawal period	Nil	Nil for milk and meat
16	Special requirements for animals		Vaccinate all animals

17	Special requirements for persons	BCG is the human vaccine used at higher doses. People accidentally vaccinated will react to the vaccine.	None
18	Package size	1-5-10 vials. Each vial has 10 adult human doses (20 child doses)	Multiple pack size from 5 doses
19	Price to end user		
20	Storage condition and shelf-life as packaged for sale	2°C-8°C - 18 months	Stable at 30°C for 24 months
21	In-use stability	4 hours	24 hours or greater
22	Other: Interference with antibiotics	Live attenuated *M. bovis*	Immunogen with protective antigens for *M. bovis*

Limitations

Scientific quality: The publications and data from the different research groups, should be carefully evaluated. The use of good science and good experimental design with use of proper controls, adequate numbers, suitable challenge model, reproduction of results by them and by independent groups, and appropriate analysis has not been verified for this monograph. If any of these projects were to be pursued, a detailed peer review taking into account the above considerations is strongly recommended.

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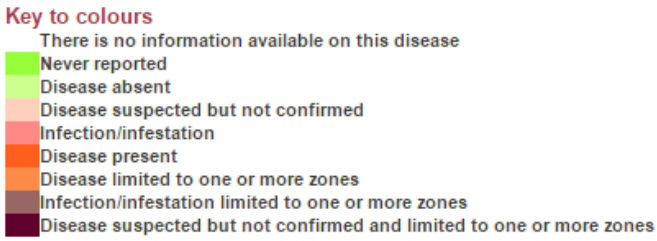
ANNEX 1: Additional data on disease presence and incidence

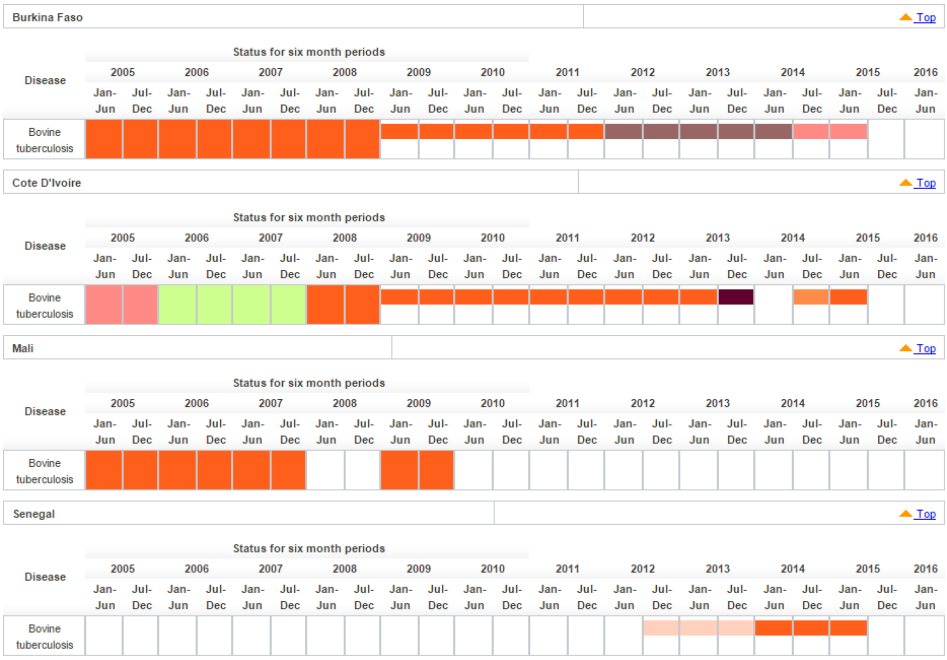
Reports to OIE on bovine TB:



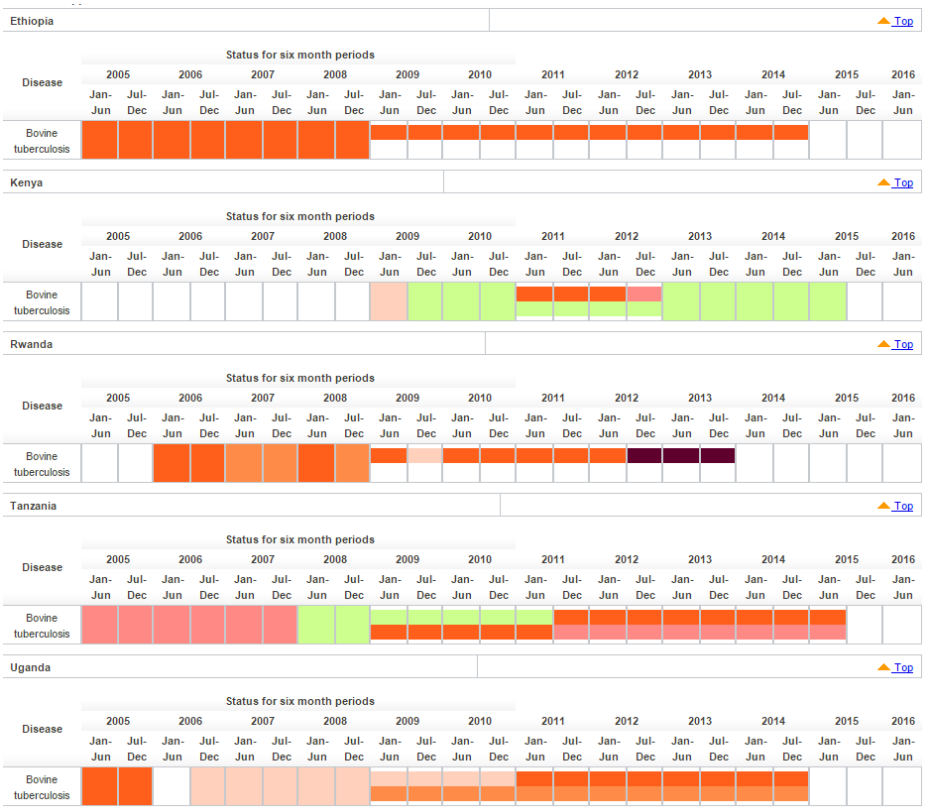
When different animal health statuses between domestic and wild animal population are provided, the box is split in two: the upper part for domestic animals, and the lower part for wild animals.

Bovine TB in Asia: Bangladesh, India, Indonesia, Myanmar, Nepal and Vietnam

Bovine TB in Western Africa: Burkina Faso, Ivory Coast, Mali and Senegal



Bovine TB in Eastern Africa: Ethiopia, Kenya, Rwanda, Tanzania and Uganda



Bovine TB in Southern Africa: Madagascar, Malawi, Mozambique, South Africa and Zambia