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Bovine Tuberculosis Outreach Day

Faculty of Veterinary Sciences

Old Faculty Building Auditorium,
Faculty of Veterinary Science,
Onderstepoort

2 March 2015

Bovine Tuberculosis Outreach Day Agenda

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Session 1	Introduction to BTB	Session Chair: Peter Buss
	What is the Origin of BTB in South Africa?	Anita Michel
	How is BTB transmitted between animals and between properties?	Nick Kriek
	Historical review of BTB control in South Africa and present situation	Alicia Cloete & Donald Sibanda
Session 2	Movement of animals- a vehicle for spread of BTB	Session Chair: Mpho Maja
	Introduction of BTB into wildlife and current status quo	Peter Buss & Anita MIchel
	Translocation of game in South Africa	Alex Lewis
Session 3	Diagnosis and Control	Session Chair: Darrell Abernathy
	Current status of BTB control in wildlife in South Africa	Lin- Mari deKlerk Lorist
	Why is diagnosis of BTB so difficult?	Paul van Helden & Michele Miller
	Guest Speaker- Implications of BTB in Wildlife in Spain	Christian Gortazar-Schmidt

Introduction

The BTB Outreach Day was organized by the TB in Wildlife Study Group to address questions concerning the spread and current geographical distribution, affected species, and challenges in diagnosis and control of bovine tuberculosis in wildlife and livestock in South Africa. Veterinarians, wildlife managers, regulatory agency staff and other interested parties were invited to attend the one day symposium. The TB in Wildlife Study Group was established more than 20 years ago and consists of researchers and field veterinarians who, in collaboration with wildlife managers and policy makers, conduct scientific investigations to further the knowledge base on tuberculosis in wild animals in South and southern Africa.

This publication is the compilation of the presentations made at the BTB Outreach Day. Based on the global interest in this topic, the Wildlife Disease Association provided a small grant for the production and distribution of the materials to advance the goal of sharing scientific knowledge worldwide.

Acknowledgements

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The Origin of Bovine TB in South Africa Indigenous or Foreign?

Prof. Anita Michel, Department of Veterinary Tropical Diseases, University of Pretoria

Bovine tuberculosis (BTB) is a chronic, debilitating, infectious disease, which mostly affects the respiratory tract, but can be found in many organ systems, depending on the route of infection. BTB is caused by the bacterium *Mycobacterium bovis* (*M. bovis*). This disease leads to major economic losses to livestock production. Domestic cattle are the primary reservoir species globally.

In addition to cattle, a number of wildlife species have been reported to be maintenance hosts for BTB. These include African buffalo, greater kudu, warthog, and lechwe in Africa; European badger and Eurasian wild boar in Europe; brushtail possum and feral water buffalo in New Zealand; white-tailed deer and wood bison in North America. *M. bovis* is a multi-species pathogen, and can infect any mammal species, although few are capable of serving as reservoir hosts. Most species infected will be dead-end hosts. In South Africa, BTB has spilled over to a number of wildlife species including leopard, cheetah, baboon to name but a few.

BTB is an alien disease in South Africa, probably being introduced by European cattle imports, mostly during the 19th century. The first reference to BTB in the country was in a report by Hutcheon in 1880 in the Annual Veterinary Report. Awareness of the disease in South Africa led to BTB being one of the first notifiable livestock diseases in South Africa (Diseases of Stock Act, 1911). In an attempt to stem further introductions, cattle imported in the early 20th century from Europe (mostly UK), Australia, and South America were subjected to tuberculin skin tests upon arrival and positive animals destroyed which was, however, not sufficient to stop further introductions of infected cattle. Currently, BTB is widespread on the African continent (Figure 1).

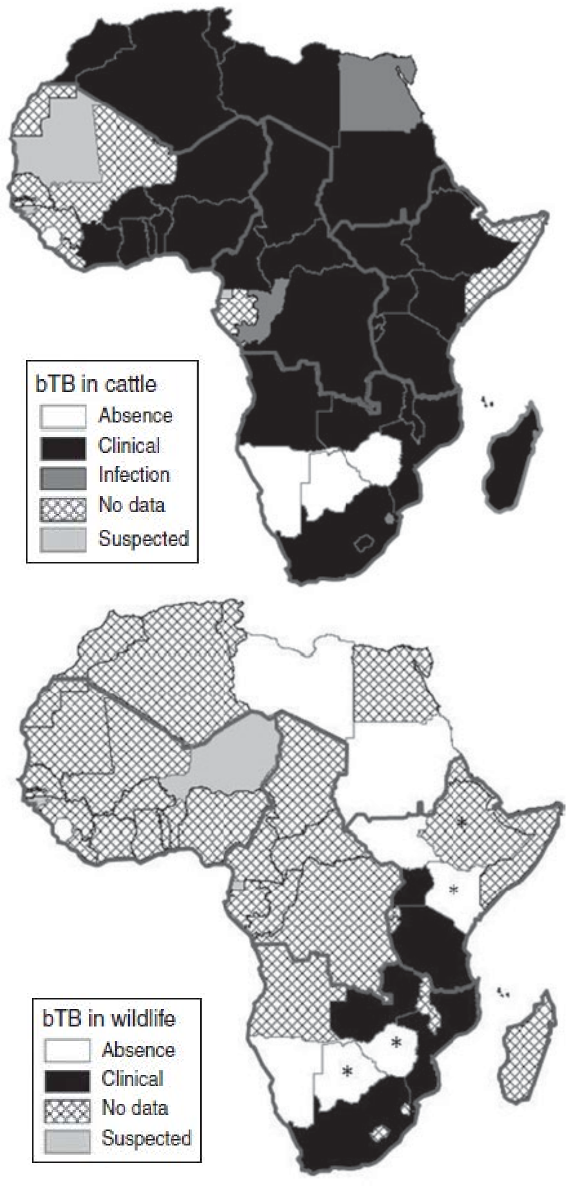

Figure 1. Presence of BTB in cattle (a) and wildlife (b) on the African continent. From: M. De Garine-Wichatitsky, A. Caron, R. Kock, R. Tschopp, M. Munyeme, M. Hofmeyr, A. Michel. 2013. A review of bovine tuberculosis at the wildlife-livestock-human interface in sub-Saharan Africa. Epidemiology & Infection, volume 141, 7, pp. 1342-1356.

In order to understand the origin and spread of BTB in Africa, studies have been undertaken to characterize and compare the BTB strains occurring in cattle in east, west, and southern Africa at a genetic level. While the African-1 *M. bovis* strain is found predominantly in west African countries, and the African-2 strain in east Africa, the European-1 strain is of European origin (European-1). This strain has been traced back to cattle in the UK, as well as Australia, supporting the theory that BTB is a foreign disease in South Africa. Wildlife species are often resistant to endemic indigenous diseases. However, BTB is an alien disease in South African wildlife. These species are naïve to infection, and there is little chance that wildlife will become resistant in the foreseeable future based on how recent the disease has been introduced. Therefore, understanding the movement of animals, and potentially disease, is crucial to preventing further spread of BTB.

Transmission of *M. bovis* Between Animals and Properties

Prof. Nick Kriek, Department of Pathology, University of Pretoria

"We focus on those snippets of information that we do possess, or can easily obtain, but ignore all of the elements that are missing, leaving us with the impression that we understand everything we need to..." Daniel J. Simon, *The Invisible Gorilla*

What do we really know (or not know) about *M. bovis* and transmission between animals? Our understanding is impacted by the quality of data that are available. This is influenced by assumptions and urban legends, the development of new diagnostic techniques and refinement of our methodology and application, as well as changing realities in livestock and wildlife management, and the role that wildlife plays in South Africa. Our current understanding of *M. bovis* transmission should be re-evaluated in light of these factors. The mode of transmission, in probably all instances, has been derived from, and is based on assumptions. Unless *M. bovis* strains are typed, few assumptions can be made about the epidemiological factors determining the source of the outbreak and spread of disease. Studies in cattle have shown that 1 CFU of aerosolized *M. bovis* was capable of causing pulmonary pathology, typical of BTB, in one-half of infected animals (Dean et al., 2005). There was no difference in severity of lesions when different *M. bovis* doses were used. However, studies suggest that it takes approximately 10 million times more organisms to infect animals orally. These results suggest that ingestion is much less important than aerosol infection, although other portals of entry have not really been thoroughly investigated.

Mycobacteria are able to survive for extended periods in the environment; 300 days or more under controlled temperate conditions. The nature of environmental contamination is dependent on the relevant host species shedding the bacteria. Under African environmental conditions, *M. bovis* could be isolated from tissues for a maximum of 6 weeks and from feces for 4 weeks (Tanner & Michel 1999). One study could not find detectable levels of *M. bovis* in trough water used by infected buffalo, suggesting that organisms are not shed in high quantities from nasal or oral secretions (Michel et al., 2007). Infectivity of *M. bovis* in aerosols depends on the release rate of the pathogen, aerosol droplet size, number of organisms inhaled and infectious dose. There is less than 1% survival by 12 hours (Gannon et al., 2007).

Transmission between animals is affected by host factors such as the location of lesions, nature of lesions (i.e. open lesions allowing shedding), timing of excretion of organisms (usually intermittent), virulence, route of exposure, host susceptibility, behavior and social ranking, and amount of close contact. Transmission between properties can occur through introduction of infected animals, spread from adjacent property, communal grazing and movement of wildlife.

Recurrence of cases on a property may reflect residual infection, or reintroduction from adjacent property, wildlife or purchase of new animals. There are a number of wildlife maintenance host species that are recognized as playing a role in the epidemiology of BTB worldwide. However, as each country battles a different wildlife host, research on each reservoir species will be required as extrapolation of information from one species to the next may not be appropriate or relevant. Some common risk factors for transmission of TB in wildlife include co-mingling of infected cattle and wildlife, supplemental feeding and baiting of wildlife, inadequate surveillance of at-risk wildlife, unrecognized emergence of alternate wildlife species as successful maintenance hosts because of the lack of attention given to wildlife by regulatory authorities. Transmission may also occur with translocation of wildlife, when population densities reach beyond normal carrying capacity, with encroachment on wildlife habitat, and when there are unintended interactions between host ecology, pathogen, and environment. Species may have differing roles as spill-over or maintenance hosts in different settings. Predicting transmission is complicated by the lack of data describing contact between various wildlife and livestock species, as well as the potential for bi-directional transmission.

Inadequate knowledge of the disease and the epidemiological drivers results in failure of management and control programs. Programs should be fit for purpose and designed to eradicate BTB by taking into account all available scientific information and adequately addressing all known risk factors. As a clearer epidemiological picture emerges, additional measures can be incorporated into current programs, addressing all sources of infection. Significant progress should then be possible and BTB control and eradication may be achieved. So what role do *M. bovis*-infected wildlife play as a source of infection for cattle in South Africa? A comprehensive national monitoring system for *M. bovis* in wildlife that is logistically and fiscally sustainable could yield economic benefits for livestock and game health management. Studies show evidence of clonal expansion for some ancestral strains as well as co-infections with two or three *M. bovis* strains on some of the cattle and game farms, which suggests independent introductions of infected animals from multiple and epidemiologically unrelated sources (Hlokwe et al., 2014). One study revealed that five different genotypes (spoligotypes) of *M. bovis* were shared between cattle and wildlife. Beside cattle, at least 21 different wildlife species in South Africa have been reported with BTB. There is strong evidence of inter- and intra-species transmission of *M. bovis* in South Africa, which needs further investigation.

History of Bovine TB Control South Africa 1953-2000

Dr. Alicia Cloete, State Veterinarian,
Department of Agriculture, Forest and Fisheries

In the 1950's in South Africa, accreditation and interim BTB testing schemes for cattle were voluntary. Several factors contributed to this including a lack of state veterinarians and a focus on diseases such as FMD as priority. However, the appointment of a Departmental Committee of Investigation in 1955 signaled the serious intent to control this disease.

During the 1960's, research was underway to investigate diagnosis using blood and milk serology. There had been some success with isoniazid (INH) with selected cases treated under state veterinary supervision. Testing of cattle slowly increased during this decade and the larger sampling size led to a calculated decrease in prevalence. Approximately 1-1.5% of the estimated cattle population (10 million) were tested. However the first cases of BTB were found in wildlife; greater kudu in the Eastern Cape in 1965-66, and impala in 1967-68. Tuberculous lesions in pigs were causing economic losses due to condemnation at the abattoir, although these were caused by nontuberculous mycobacteria. Rifampicin and ethambutol were discovered to be effective against mycobacteria but were expensive.

The BTB Eradication Scheme was launched on May 14, 1969, with the following statement; "Until recently the combating of bovine tuberculosis depended on the voluntary participation of cattle owners in either the interim or accreditation schemes. All tests for the latter scheme were carried out by State Veterinarians as a free service, while testing under the interim scheme was performed chiefly by private veterinary practitioners at owners' expense. Disposal of reactors was the responsibility of owners. The State gave no compensation or any other financial aid. During the report year the first steps towards an extension of the campaign aimed at eventual total elimination of the disease were taken." Modifications in the new scheme were contracting of private practitioners to perform TB testing for both the interim and accreditation schemes, trace back to the herd of origin for reactors, abattoir slaughter of reactors, milk examination, infected herds were placed under official control with compulsory testing and elimination of reactors, and the state compensated reactors that were slaughtered.

During the 1970's, there was a focus on clean herds. There was an increase in accredited herds, increase in private veterinarians contracted and stock inspectors trained for TB testing. The budget was also increased and a diagnostic testing scheme was introduced. For the first time since the inception of the BTB eradication scheme, there were more than a thousand farmers in each of two regions of the Division of Veterinary Services that could boast of having BTB-free herds (Cape and Highveld).

Lack of funds and personnel started hampering expansion of the eradication scheme during the 1980's, although the scheme showed steady growth. Mini-campaigns were introduced to screen areas for infection rather than concentrating on already clean herds. There was a change in approach regarding BTB eradication. The emphasis changed regarding cattle participating in the schemes – herd-diagnostic and annual diagnostic schemes increased at the expense of accreditation. In 1986-87, a new system was implemented in which all cattle were tested within a 5 year period. Herds were reallocated from the annual diagnostic scheme to herd diagnostic tests. The aim was to locate infection and concentrate on eradication rather than maintaining the BTB-free status of herds. This responsibility shifted to the owners with changes in health legislation. With the contribution of 182 trained TB officials, there was a decrease in participation by private veterinarians. Research showed that the caudal fold test had limited usefulness in South Africa. The Animal Disease Act (Act 35 of 1984) came into effect on October 1, 1986. During the subsequent years, private practitioners were not equipped to handle the increased number of tests required as a result of the privatization efforts by DAFF. Many farmers were unwilling to test because they now had to pay for the tests themselves. By 1988-89, there was a significant decline in the number of herd tests performed under the accreditation and annual diagnostic test programs. There was resistance by farmers to privatization of maintenance tests on herds that had proven negative test history. The Bovine Tuberculosis Control Scheme (R.1953 of 30 Sept 1988) came into effect during this time.

A new BTB issue emerged during the 1990's in Kruger National Park. BTB was diagnosed in buffalo in 1990 and there was evidence of spread to other species including cheetah, lion, baboon, and kudu in 1995-96. Although initially found in the south, by 1996-97, BTB had spread to the buffalo in the northern areas of the park and lions were found to be infected in the late 1990's.

Changes were also occurring in the animal health sector. National and provincial veterinary structures of veterinary services were implemented during 1996-97 in which the National Directorate Animal Health was responsible for imports/exports, and monitoring standards of veterinary services in the country, while the Provincial Veterinary Services focused on delivery of services. Financial constraints hampered activities in most provinces. A large part of the budget was allocated for communities previously neglected as far as veterinary services were concerned. Disease reporting was unsatisfactory, resulting in underreporting of most diseases and unreliable statistics.

Progress of the BTB eradication scheme was limited by severe staff and vehicle shortages, disease prioritization, funding problems, and the availability of tuberculin. Tuberculin tests performed by private veterinarians declined since dairy farmers were reluctant to pay for regular testing and many switched to beef production.

For the first time, communal areas were included in the BTB eradication schemes in the 1990's. However, communal farmers did not see a direct benefit of participating in the scheme. By facilitating communication through farmers' unions and funding from the National Directorate Animal Health, communal cattle herds were tested starting in 1997-98.

Game farming was emerging as a major economy in South Africa during the 1990's. Game farmers were reluctant to have their animals tested on a voluntary basis because of the consequences should there be BTB found on the farm. However, BTB in wildlife, especially buffalo, was recognized as a possible source for reinfection of cattle. This necessitated constant surveillance along wildlife-cattle interfaces.

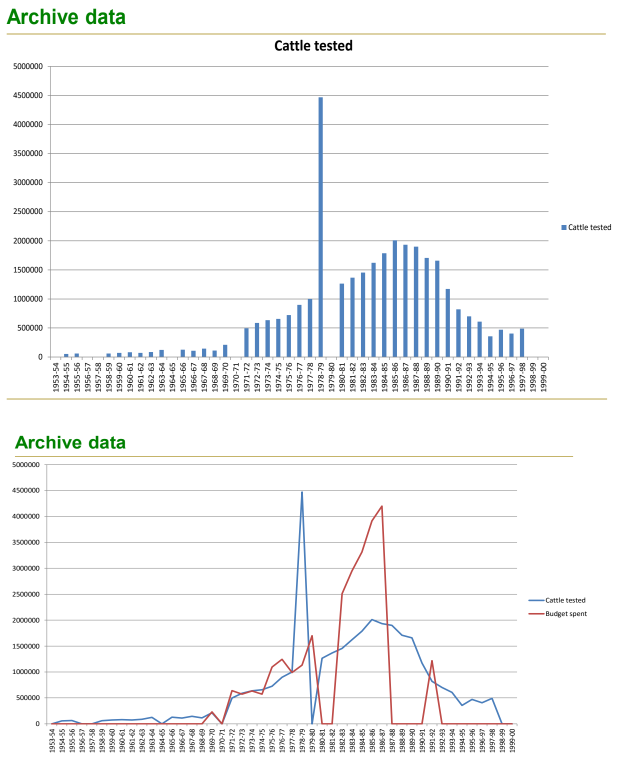

Figure 2. Number of cattle tested in South Africa between 1953 and 2000 (top). Number of cattle tested and budget spent (DAFF) on BTB control program (bottom).

What can we learn from this? We are facing similar problems today in South Africa, such as manpower shortages, budget constraints and disease prioritization. To increase manpower, more private vets need to be contracted. Farmers are reluctant to pay for testing in the absence of an incentive. In order to achieve eradication/control, BTB control will need to funded by the state. Apart from substantial funding, the buy-in from communal farmers, commercial farmers, private veterinarians, and industry is essential to achieve these goals.

Current Bovine TB Control and Proposals

Dr. D.R. Sibanda, Department of Agriculture, Forest, and Fisheries

Bovine TB schemes in South Africa have undergone several changes since the initial introduction of the Bovine TB Eradication Scheme in 1969. Annual diagnostic (maintenance scheme) testing was introduced in 1981-82. Herd diagnostic schemes were implemented in 1986-87, and maintenance tests were privatized at this time. The Bovine Tuberculosis Control Scheme (R.1953 of 30 Sept 1988) was the most recent scheme introduced.

Current BTB management is being reviewed and revised to provide a more effective plan. There are a number of new proposed programs that are in review. The accreditation program has been discontinued due to lack of compliance. The maintenance program (old annual diagnostic) will accommodate herds that need certification. There will be two initial tests at three month intervals performed by the state, and testing every 2 years at the owner's expense. The surveillance program (old diagnostic herd test) will provide information on prevalence, and costs of mini-campaigns will be covered by the state. Testing will be performed at 5 year intervals (except if animals are at risk). Farmers that do not need certification can be accommodated in this program at their own expense. The diagnostic program will include import, export, and individual animal tests, paid for by the owner. The infected herd program is compulsory. Once a herd or animal tested under one of the above mentioned programs becomes positive, this will go into effect. Testing of herds and slaughter of all reactors will be implemented, with a "T" brand placed on any positive animal. Testing will be continued every 3 months until the herd is declared negative after 2 consecutive negative herd tests.

The State will pay for testing until the herd becomes negative, and will compensate the farmer for carcass value of slaughtered animals. Herds will be monitored annually.

The DAFF BTB Working Group is finalizing the updated Tuberculosis manual and working on surveillance information collated from the provinces.

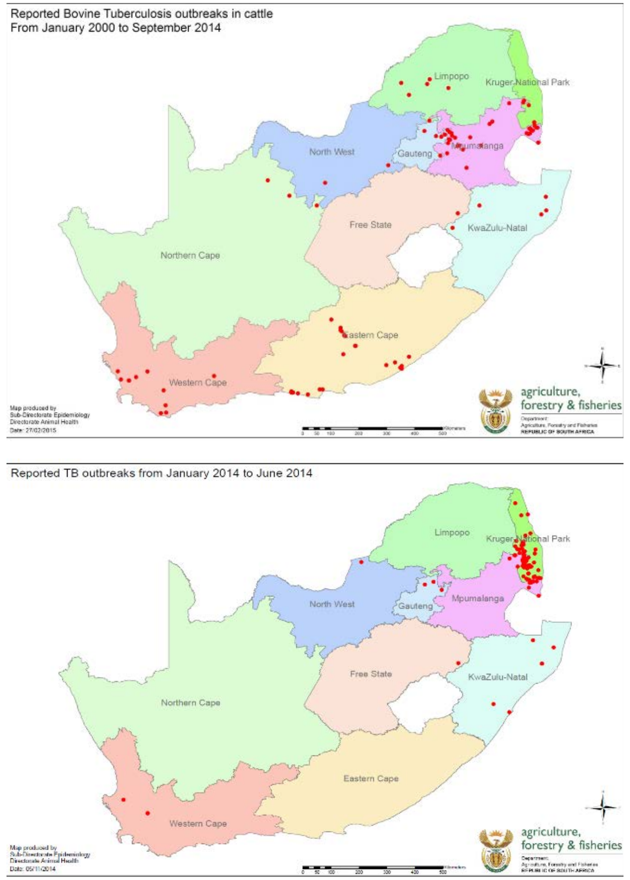

Figure 3. Outbreaks of BTB in cattle (top) and wildlife (bottom) in South Africa.

Between 2000 and 2014, 16,881 cattle were destroyed due to BTB. This caused an economic loss of R126 million, based on average market value for beef cattle. BTB continues to be a problem for South Africa. Some of the challenges and possible solutions for an effective BTB control program are recognizing and managing the risk that wildlife TB presents to the cattle population, establishing joint cooperation between government, private veterinarians and industry to perform surveillance, improve surveillance strategies at abattoirs, improve BTB control and possible eradication in communal areas by examining compensation issues, etc., improving training of animal health staff, and making TB testing compulsory.

Introduction of BTB into Wildlife and Current Status Quo

Dr. Peter Buss, Veterinary Senior Manager, Kruger National Park, South African National Parks

The first recorded cases of BTB in wildlife occurred in the Eastern Cape (Grahamstown). In 1929, BTB was found in a greater kudu and common duiker. In 1940, additional cases were discovered in kudu, springbok, bushbuck, hares and bushpig. More recently, BTB has been reported in multiple wildlife species in Kruger National Park (KNP) and Hluhluwe-iMfolozi Park (HiP). In HiP, mycobacteriosis was diagnosed in a black rhino that died of natural causes in 1970. Chronic granulomas were found in the lungs, lymph nodes and pleura at necropsy and small numbers of acid-fast bacteria were present on histological examination.

This was followed by cases in buffalo (1986), lion (1992), kudu, bushpig, and baboon (1998). It was believed that the source of infection for the wildlife was the communal grazing between buffalo and cattle prior to the park being fully fenced in the 1960's.

BTB has also been found in buffalo in the Spioenkop Nature Reserve. These animals were believed to have been infected prior to or during 1997. Although there was no link to buffalo from other infected areas, the source of infection was thought to be greater kudu which contracted disease from cattle in adjacent areas (Michel et al. 2009).

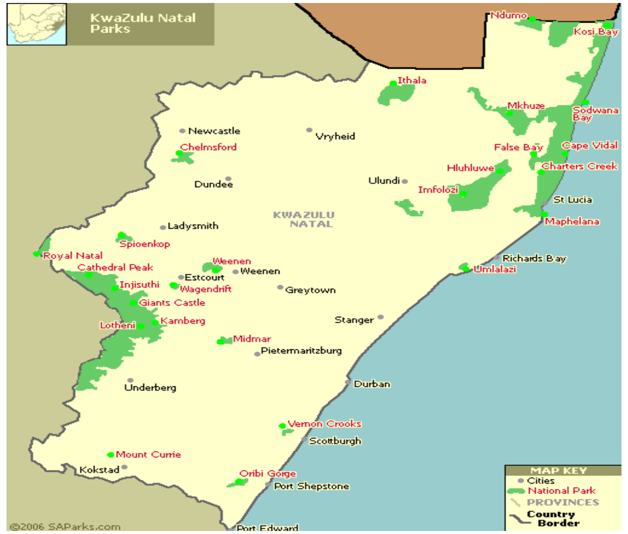

Figure 4. Location of Spioenkop Reserve and Hluhluwe iMfolozi Park.

Figure 5. Spread of BTB in Kruger National Park

Bovine tuberculosis is considered endemic in Kruger National Park. However, the first suspected case of BTB was found in 1977 in an impala. The diagnosis was not confirmed by culture and the finding was considered an isolated incident. During the subsequent years, a large number of carcasses from several species were inspected without an additional case. The index buffalo case was detected in July 1990 in the south-western region of the park by Dr. Roy Bengis. Based on analyses of State Veterinary records, BTB was present in the late 1950's or early 1960's in a dairy herd south of the Crocodile River. Molecular typing of the *M. bovis* found a single strain, suggesting a single introduction. It was believed that *M. bovis* entered the park through interaction of wildlife with infected cattle on the southern boundary. In contrast, there have been at least three distinct strains of *M. bovis* found in HiP, indicating multiple sources of infection.

Monitoring of disease in buffalo was initiated in KNP following the discovery of these cases. The first survey indicated BTB was well established in herds south of the Sabie River. Regular monitoring surveys recorded increases in incidence and prevalence of infected herds as the disease spread northwards. By 2006, BTB was detected in buffalo in the far north of KNP; this had spread approximately 350 km from the initial point of entry. In 2008, a buffalo was found to be infected with the "Kruger strain" of *M. bovis* in Gonarezhou National Park, Zimbabwe.

Since the introduction of *M. bovis* into KNP, the disease has been detected in 15 species, of which buffalo and kudu are known maintenance hosts. There is some speculation that lions may also serve as reservoirs. This is of concern since lions are the top predator in KNP and change in populations may affect both conservation and tourism. Other species that have been found to be infected are warthog, leopard, cheetah, spotted hyena, genet, Chacma baboon, honey badger, impala, banded mongoose, giraffe, blue wildebeest, and wild dog.

Spillover, Spillback and Translocation of Bovine TB in South Africa

Prof. Anita Michel, Department Veterinary Tropical Diseases, University of Pretoria

Spillover of BTB to wildlife from cattle has been well documented based on the experience in KNP. However, this risk was recognized much earlier than the first cases - "The risk of tuberculosis in wildlife increases with their proximity to humans and their domesticated cattle" (Griffith 1928); although the risk is greater for captive compared to free-ranging wildlife. To date, BTB has been spread to 21 species in South Africa.

Some of the possible consequences include spread to a larger number of wildlife species, spread to new geographical locations, unknown consequences in terms of endangered/rare species, potential establishment of new wildlife reservoir species, and spillback from wildlife to cattle. In a paper published in 2013 (De Garine-Wichatitsky et al.), the authors stated that "no case of BTB spillback from wildlife to livestock has been confirmed". However, results from a cross-sectional BTB prevalence study showed that 4/1167 (0.34%) of cattle tested in the Mnisi area, bordering KNP, were positive on tuberculin skin test. Field and molecular epidemiological investigations revealed that the cattle were infected with the same strain (SB0121) as Kruger wildlife, confirming the first spill-back of *M. bovis* from wildlife to cattle in South Africa.

An estimated 70,000 wild animals are captured and translocated every year in South Africa, with an estimated value of R 900 million. There have been four recent events in which the spread of BTB by translocation has been documented. One event occurred in Madikwe Game Reserve in North-West Province. In 2012, 3 buffalo that had tested positive on skin test and gamma interferon assay were slaughtered and found to have TB lesions. *M. bovis* was isolated and genotyped as strain SB0140, which is the strain found in cattle and wildlife in KwaZulu Natal (KZN). The VNTR genotype exactly matched that of a BTB case near Mkuze (Hlokwe et al. 2016). A nyala that had been bought at an auction and transported to Gauteng was also discovered to have BTB after one animal died soon after arrival in 2013. The isolate matched the strain of *M. bovis* from KZN (SB0140).

A black rhinoceros died on a private game farm in North-West Province and the *M. bovis* isolated was the Kruger strain (SB0121). A previously undetected strain (SB2200) of *M. bovis* was isolated from a blue wildebeest that was translocated from another area in Mpumalanga to a private reserve adjacent to KNP (Hlokwe et al. 2014). These events demonstrate the potential risk of moving BTB with translocation of wildlife and the value of molecular epidemiology in tracing the potential source. In conclusion BTB fits the analogy of a ping-pong ball which is successfully passed on between cattle and wildlife, supported by the environment from which it can bounce back to either side.

Translocation of Game in South Africa

Dr. Alex Lewis, private wildlife veterinarian

The wildlife industry is growing in South Africa due to the high return on investment compared to cattle, ability to own wildlife species, and strong hunting industry. In 1992, there were 3500 farms registered for game. By 2014, there were 5000 farms with game only, 4000 mixed farms and 6000 farms not registered. The wildlife industry is now bigger than the sugar and dairy sectors in South Africa. During the past 15 years, the game industry grew at an average rate of 20.3% per year (measured by turnover). Sixty percent of all wildlife in South African is privately owned (i.e., outside national and provincial parks).

In terms of game capture, there are no reliable statistics. It is estimated that 70,000 to 200,000 animals are moved annually. Although not all wildlife are immobilized for translocation, there are ample opportunities to obtain samples for testing. In 2014, there were 5 game auctions in 4 different provinces which sold 1562 animals. There are an estimated 5000-8000 animals immobilized monthly, based on dart sales in 2014. In addition, hunters harvest over 1 million head of game each year. These animals provide accessible opportunities for disease surveillance.

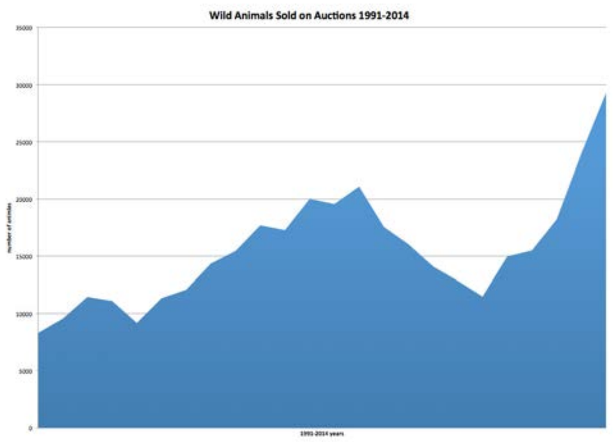

Figure 6. Numbers of wild animals sold at auctions between 1991 and 2014

Tracking of wild animal movements can be implemented through tracking of permits. The integrated electronic permit administration system (IEPAS) has potential to document and provide reports on wildlife translocated throughout South Africa. The Wildlife Ranchers Association of South Africa is beginning data collection to track movement especially of buffalo. This may provide useful information and access to researchers and regulatory officials in the future.

The Current Status of Bovine TB Control in Wildlife in South Africa

Dr. Lin-Mari de Klerk-Lorist, State Veterinarian, Kruger National Park

The Animal Diseases Act No. 35 of 1984 provides for the control of animal diseases and parasites, for measures to promote animal health, and for matters connected therewith. However, wildlife were never included in the BTB and brucellosis eradication schemes. This was due to the misconception of the disease in wildlife at the time that the Act was implemented; "with the exception of free-living badgers in the United Kingdom, wild animals have never been implicated as a reservoir host of tuberculosis and appear to be unimportant, incidental victims of the disease" (Scientific and Technical Review of the OIE, 1988). An additional constraint is that there are no validated tests in most wildlife species.

A recent outbreak of BTB in buffalo illustrates the importance of testing wildlife and not always implicating buffalo as the villain when this disease occurs (Hlokwe et al. 2016). The Madikwe Game Reserve is located in the North-West Province of South Africa, bordering on Botswana to the north. The Madikwe Buffalo Brand is valuable since the herd was disease-free and had valuable genetic stock. Founder animals (53) were sourced from European and American zoos, although the origin was suspected to be from East Africa. Periodically, Madikwe Game Reserve offered live buffalo sales, with the income generated contributing to their conservation efforts. All buffaloes from previous auctions were tested and confirmed free of all diseases. In June 2012, during preparation for another auction, 51 buffaloes were tested and for the first time, a buffalo tested positive for BTB. The young bull buffalo (18 months old) was slaughtered for necropsy evaluation. Histopathology revealed occasional multinucleate giant cells containing intracytoplasmic acid-fast staining bacilli, and culture confirmed *M. bovis*. Review of records showed that 156 buffalo had been removed from the game reserve between 2001 and 2010.

Investigations were undertaken to determine the source of infection. Game introductions into Madikwe between 2006 and 2009 included numerous blue wildebeest (1792), impala (4227), and greater kudu (461). Variable nucleotide random repeat (VNTR) typing of *M. bovis* from the Madikwe buffalo was identical to an isolate from a baboon and very similar to an isolate from a kudu in KZN. Since BTB lesions in kudu often form fistulas and thus contaminate the surrounding environment, it was suspected that *M. bovis* was introduced with translocation of kudu into the reserve. The result was that the game reserve was put under quarantine and lost all income associated with game sales.

Options for management and salvage are underway. Due to the size of the herd, eradication is an enormous undertaking. In a smaller herd, eradication could be achieved after 8 years with 99% certainty if the whole population of 200-250 buffaloes were tested every year and reactors removed.

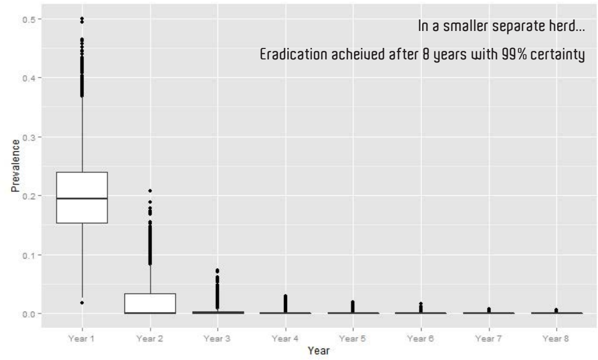

Figure 7. Predicted BTB prevalence over the course of 8 years when a herd of 200-250 buffaloes are tested every year and reactors removed

Lessons learned from Madikwe - Never acquire high risk wildlife species from areas of unknown disease status or already known BTB infected areas and even more so, only obtain wildlife from reputable translocation operators.

Wildlife in South Africa has enormous economic importance. Recreational and trophy hunting industry (along with taxidermy) generate 4.5 billion rand annually. Auctions, translocations, ecotourism and meat production add another 4 billion rand each year. Therefore, the introduction of a controlled disease such as BTB can have devastating consequences.

Although there is concern for the diseases present in wildlife, apart from buffalo, there are no official control or monitoring strategies for these species. Historically, BTB in wildlife appeared to be contained in two endemic areas, the Kruger National Park and Hluhluwe-iMfolozi Park. However, more recently outbreaks of BTB have occurred in wildlife in and around South Africa (figure 8).

Figure 8. Location of BTB outbreaks in wildlife in South Africa.

In summary, actions that need to be taken include integrating control strategies for domestic species and wildlife. These programs should provide specific benefits to farmers participating in the control schemes, such as bigger incentives from industry to farmers that comply. The Directorate of Veterinary Services should support and approve novel research initiatives. Universities should drive better and more focused research projects. The private buffalo industry needs to source funding for research questions related to BTB.

Why is Diagnosis of Bovine TB so Difficult?!

Prof. Paul van Helden, Director and Head, DST/NRF Centre of Excellence for Biomedical Tuberculosis Research, MRC Centre for TB Research, Stellenbosch University

Prof. Michele Miller, NRF South African Research Chair in Animal TB, Stellenbosch University

Diagnosis of TB in animals usually requires a degree of suspicion based on clinical signs. However, TB results in nonspecific signs in most species. Animals are often asymptomatic until disease is advanced. In some cases, weight loss, lethargy, anorexia, respiratory signs (coughing, dyspnea), low grade fever, or enlarged lymph nodes may be present. If TB is suspected, additional investigation is required to confirm the diagnosis. This may include a thorough herd history, diagnostic testing (e.g., tuberculin skin test, interferon gamma assay, mycobacterial cultures), and abattoir inspection and sampling of any test-positive individuals.

Most ante-mortem tests are immunological tests, based on detecting the host's immune response to that particular organism. With *M. bovis* infection, immunological responses may take weeks/months/years to develop. Changes in immune responses occur with progression of infection and development of disease, which will affect test results. However, there are limitations to using immunological tests. These are indirect tests; therefore, the confirmatory "gold standard" results may be lacking. Test performance can be affected by any factor that impacts the immune system (e.g. malnutrition, other diseases, very young or old age) or exposure to cross-reactive organisms. Test performance is usually based on studies in known infected and uninfected animals, so results may not be applicable to individuals that are in different stages of infection. Test results are classified based on predetermined cutoff values. However, measurements are usually a continuum and may vary due to method, laboratory, or personnel reading test.

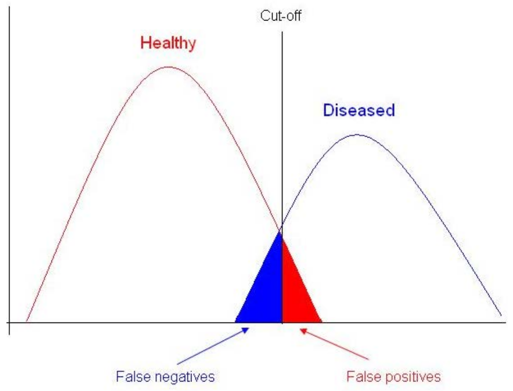

Figure 9. Continuum of test results in healthy and diseased populations, and effect of setting a cut-off value on test interpretation.

There are a number of factors influencing interpretation of test results. The ability of a test to determine infection status depends on the population and test characteristics, how common infection is in the population (prevalence), probability that an animal that is infected will test positive (sensitivity), and probability that an animal that is NOT infected will test NEGATIVE (specificity). There is NO perfect test!

A real world example may help clarify how to interpret test results in different scenarios. In scenario A, assume that a buffalo herd of 1000 animals has a 1% prevalence of BTB (low prevalence herd). If the tuberculin skin test (TST) has a sensitivity of 80%, specificity of 97%, then the accuracy is 96.8% (percentage of true results). Given this prevalence, this means that if the TST is negative, there is a 99% probability that the animal is uninfected (negative predictive value). If the TST is positive, only 21% of these animals are truly infected (positive predictive value). Looking at the table below, a large number of the TST positive animals are actually false positives (30/38).

Table 1. TST results in a low prevalence buffalo herd based on known test sensitivity and specificity

TST Result	Infected	Uninfected	Totals
Positive	8	30	38
Negative	2	960	962
Total	10	990	1000

In scenario B, assume a 10% BTB prevalence in a buffalo herd of 1000 (high prevalence herd). Although the test performance, sensitivity (80%) and specificity (97%), have not changed, the accuracy (95.3%) and predictive values do. In this case, if a buffalo is TST negative, there is a 97.8% probability that it is uninfected (negative predictive value). But in a high prevalence herd, the positive predictive value improves (fewer false positives); therefore, there is a 74.8% probability that a TST positive buffalo is infected. Therefore, it is important to have a herd or population history in order to interpret test results appropriately.

Table 2. TST results in a high prevalence buffalo herd based on known test sensitivity and specificity

TST Result	Infected	Uninfected	Totals
Positive	80	27	107
Negative	20	872	8
Total	100	900	1000

Another challenge in diagnosing TB has to do with the organism itself. TB is caused by infection with a member of the *Mycobacterium tuberculosis* complex (MTBC). However the genus *Mycobacterium* contains over 128 species, the majority (60%) of which have been identified in the last 15 years. These organisms can be divided into fast and slow growers. The pathogenic mycobacteria tend to be slow growers, with the fast growers found mainly in the environment as saprophytes. However, there are environmental mycobacteria that have been shown to cause disease.

Although isolation of the causative organism by culture is necessary for a diagnosis, it is not sufficient. Specific identification requires speciation of the isolate using molecular techniques. This is especially important since there are animal-specific MTBC, such as *M. suricattae*, *M. mungi*, and *M. orygis* that belong to the complex but may have different pathogenesis than *M. bovis*.

In conclusion, when dealing with TB diagnosis, all is not what it at first seems. Make no assumptions, particularly if you are dealing with a new scenario (e.g., TB in a new species). We do not yet know much about disease (as opposed to infection) caused by the non-tuberculous mycobacteria (NTM). The presence of NTMs can complicate diagnosis, particularly serology.

How can TB diagnosis be improved, especially in wildlife? There is a need for ongoing research to improve the sensitivity and specificity of existing techniques, develop novel methods for determining infection and disease, and create diagnostic algorithms and develop strategic testing that will improve (cost-effective) detection of TB. Test results should always be interpreted with consideration of herd/individual history and other clinical findings. Ideally, use the same laboratory for repeat samples to minimize variability which may occur in different labs. Perform multiple tests to increase confidence in results (or use multiple labs). Detection sensitivity is improved when any positive result is used from multiple tests (but decreases specificity). Requiring a positive result from two or more tests usually increases specificity but decreases sensitivity. Confirm positive indirect test results using methods that detect mycobacteria or bacterial components (culture, PCR, antigen detection).

It is important to understand the impact of test results on TB management decisions. Individual animal variation will lead to some false positive and false negative results for any given population. False negative results (animals that are infected but test negative) may lead to persistent infection in the herd/population and incomplete control of TB. False positive results (animals that test positive but are uninfected) can cause significant economic, genetic, social, and other losses if the herd/population is quarantined or culled. Interpretation of test results and ultimately, diagnosis of TB, requires consideration of multiple factors along with a clear objective for application of the test results.

Implications of BTB in Wildlife in Spain

Prof. Christian Gortazar-Schmidt, Head, SaBio (Sanidad y Biotecnología) research group, National Wildlife Research Institute IREC (CSIC-Universidad de Castilla La Mancha), Spain

Similar to the scenario in South Africa, bovine tuberculosis is relevant to major animal-related industries in Spain such as farming and hunting. Losses occur due to inspection and destruction of animal carcasses at slaughter, removal of animals during test and slaughter campaigns, and movement restrictions. Since wild boars are an important species for hunting in Spain, the presence of disease can have a significant impact. For instance, BTB causes 40% of adult wild boar mortality in this country. In Europe, wildlife species such as the Eurasian badger, deer, European bison, and Eurasian wild boar may serve as reservoir hosts for BTB. The distribution of *M. tuberculosis* complex-infected wild boar has been shown to be consistent with distribution of outbreaks of BTB in cattle in France (Richomme et al., 2013). Prior to the start of the cattle TB test and slaughter program in the 1980's, the prevalence of BTB in cattle was constant, with evidence of spillover to wildlife in the 1950's (figure 10). Although cattle TB prevalence in Spain has been decreasing since the late 1980's, there has been an increase in wild ungulate density and associated BTB prevalence, with evidence of spillback and re-emergence in cattle beginning in 2000. Some of the factors contributing to these changes include increased wild ungulate protection and re-introduction and more intensive wildlife management practices such as fencing and feeding.

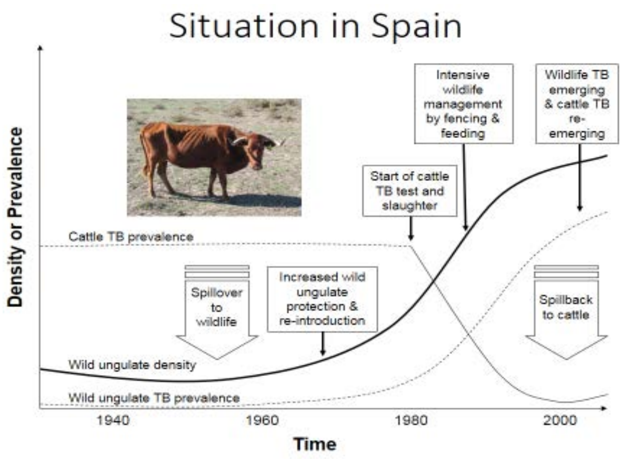

Figure 10. Temporal changes in cattle TB prevalence, wild ungulate density, and wild ungulate TB prevalence in Spain.

M. bovis is a multi-host pathogen. Although cattle serve as the primary reservoir, goats and pigs can also serve as maintenance hosts. In addition, if *M. bovis* is introduced into wildlife, there can be a wildlife cycle that maintains infection between wild boar, red deer and badger in Europe. As the number of different species involved in the maintenance of the pathogen increases, so does the complexity of the cycle, which creates more stability for infection in the system, resulting in greater difficulty in controlling disease.

The bovine reservoir in Spain is more complex than other systems, since there is increasing BTB prevalence when testing dairy, beef, and bullfighting animals. Although no test is 100% sensitive, testing of cattle should be ongoing; the presence of wildlife reservoirs should not be an excuse for relaxing cattle testing! In summary, animal TB is a global problem which, in addition to zoonotic aspects, causes significant economic losses to different sectors.

BTB research in Mediterranean ecosystems is multi-faceted. Field sampling of wildlife provides information on the presence of TB lesions, other infections, and association with body condition. Population monitoring contributes data on abundance and distribution of species. Molecular epidemiological techniques are used to evaluate patterns of infection. Photo-trapping has been used to investigate the presence of different species in areas where disease transmission can occur (for instance, watering and feeding locations).

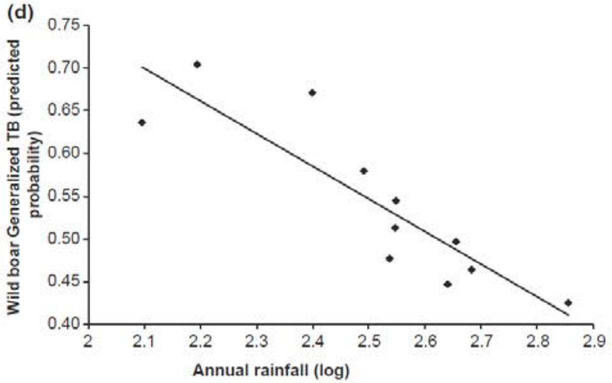

Figure 11. Relationship between the annual predicted probability of wild boar having generalized TB lesions and previous annual rainfall (mm). From Vicente-Serrano, Sergio M., et al. 2014. Temporal evolution of surface humidity in Spain: recent trends and possible physical mechanisms. Climate dynamics 42:2655-2674.

Information on animal movement and home ranges have been gathered using drones. These data are combined with material collected in farmer interviews and through GIS. Detection of gross TB lesions in wild boar has been facilitated by using hunter harvests. These studies have allowed detection of trends associated with TB in wildlife.

The Doñana National Park in southwestern Spain is considered a biodiversity hotpot. There has been an increase in cattle skin test reactors in the area, despite culling. Strains isolated from cattle are the same as those found in wildlife. More than 1000 cattle share the park with wild boar (estimated population 1700), fallow deer (800), red deer (600), and the endangered Iberian lynx (<50) (EBD-CSIC). Similar to the prevalence of BTB in buffalo populations in Kruger National Park, there is a significant geographical gradient in *M. bovis* prevalence in wildlife. Using GPS collars, the spatial overlap of cattle and wild boar was shown to be highest in summer in the marsh-shrub ecotone and at permanent water sources. The spatial gradient in potential overlap between the two species across the park corresponded well with the spatial variation in the observed incidence of TB in cattle and prevalence of TB in wild boar. Further studies have demonstrated that there is a significant risk of indirect transmission at shared waterholes. *M. tuberculosis* complex organisms were detected at 24/42 (57%) waterholes tested.

Some of the BTB risk factors for cattle farms in Spain are similar to those found globally. These include larger herd size, history of BTB cases on the farm, breed of cattle (bullfighting>beef>dairy), and contact with goats and domestic pigs. However, there are also factors associated with wildlife TB, such as wild boar and red deer abundance and BTB prevalence, proximity of farm to wildlife areas (parks or hunting estates), and water availability (less sites, more BTB)(figure 11). In most industrialized countries, animal BTB control is based on cattle test and slaughter and movement control policies. However, farm biosecurity is an important component of disease control. In addition, strategic vaccination may play a role. Vaccine trials in wild boar have shown promising results. Therefore, it is possible to significantly reduce BTB risks in cattle and wildlife, through strategic actions.

Comments by organizers:

The presentation by Prof. Gortazar demonstrates the similarities and challenges experienced when *Mycobacterium bovis* is introduced to a multi-host system, especially when there is the opportunity for livestock-wildlife interactions. The research performed in Spain may serve as a model for South Africa in understanding bovine TB in complex environments.

Additional Reading

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