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

2 March 2015

**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-Mar de Klerk-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**

The authors would like to thank the Wildlife Disease Association Small Grants Committee for funding to support the production and publication of these presentations. We would also like to acknowledge the input of all previous members of the TB Wildlife Study Group for their contributions and input over the years, as well as the University of Pretoria and the WOTRO Integrated Programme 2008 for sponsoring the Outreach Day. We recognize the expertise of the Stellenbosch University Media Centre for producing a professional product. Finally, we thank all our collaborators, researchers, veterinarians, wildlife managers, and regulatory officials for their commitment to advancing our understanding of TB in wildlife.

---

**Current Members of the TB in Wildlife Study Group**

- Prof. Michele Miller, Stellenbosch University
- Prof. Anita Michel, University of Pretoria
- Dr Peter Buss, South African National Parks
- Dr. Lin-Mar de Klerk-Lorist, Office of the State Veterinarian (Kruger)
- Dr. Louis van Schalkwyk, Office of the State Veterinarian (Kruger)
- Dr Markus Hofmeyr, South African National Parks
- Dr Tiny Hlokwe, ARC-Onderstepoort Veterinary Institute
- Dr Emily Lane Mitchell, National Zoological Gardens
- Prof. Paul van Helden, Stellenbosch University
- Dr. Sven Parsons, Stellenbosch University
- Dr. Dave Cooper, KZN Wildlife
- Prof. Eileen Hoal van Helden, Stellenbosch University
- Drashama Maro Leach, University of Pretoria
The Origin of Bovine TB in South Africa
Indigenous or Foreign?
Prof. R. J. Michel, Department of Veterinary Tropical Diseases, University of Pretoria

Bovine tuberculosis (TB) 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 M. bovis. These include African buffalo, greater kudu, warthog, and lechwe in Africa; European badger (BTB. These include African buffalo, greater kudu, warthog, and lechwe in Africa; European badger, and leopard, cheetah, baboon to name but a few. 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 Hutcheson 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 at a genetic level. While the African-1 M. bovis strain is found predominantly in west African countries, and the Africa-1 strain in east Africa, the European-1 strain is of European origin (European-1). This strain has been traced back to the UK, as well as Auvergne. This supports the theory that BTB is a foreign disease in South Africa. Wildlife species are often resistant to endemic infections. However, M. bovis is capable of causing pulmonary pathology in certain wildlife species in South African wildlife. These species are native 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

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 and southern Africa at a genetic level. While the African-1 M. bovis strain is found predominantly in west African countries, and the Africa-1 strain in east Africa, the European-1 strain is of European origin (European-1). This strain has been traced back to the UK, as well as Auvergne. This supports the theory that BTB is a foreign disease in South Africa. Wildlife species are often resistant to endemic infections. However, M. bovis is capable of causing pulmonary pathology in certain wildlife species in South African wildlife. These species are native 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.

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 and southern Africa at a genetic level. While the African-1 M. bovis strain is found predominantly in west African countries, and the Africa-1 strain in east Africa, the European-1 strain is of European origin (European-1). This strain has been traced back to the UK, as well as Auvergne. This supports the theory that BTB is a foreign disease in South Africa. Wildlife species are often resistant to endemic infections. However, M. bovis is capable of causing pulmonary pathology in certain wildlife species in South African wildlife. These species are native 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

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 and southern Africa at a genetic level. While the African-1 M. bovis strain is found predominantly in west African countries, and the Africa-1 strain in east Africa, the European-1 strain is of European origin (European-1). This strain has been traced back to the UK, as well as Auvergne. This supports the theory that BTB is a foreign disease in South Africa. Wildlife species are often resistant to endemic infections. However, M. bovis is capable of causing pulmonary pathology in certain wildlife species in South African wildlife. These species are native 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.
infected animals, spread from adjacent property, communal grazing and movement of wildlife.

Recurrence of cases on a property may reflect residual infection, or re-infestation 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 well as co-existing with livestock, wildlife research on each reservoir species will be required to explain the role of wildlife. Some common risk factors for transmission of TB in wildlife include contact with infected cattle and wildlife supplemented feeding and action of wildlife on 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, pathogens, and environment. Species may have differing roles as spill-over or maintenance hosts for BTB. The movement of a wildlife species between cattle and wildlife. Beside cattle, at least 21 different wildlife species in South Africa have been implicated 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

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 serological tests for the latter scheme were carried out by various laboratories. There were considerable variations in approach regarding BTB eradication. The emphasis changed regarding cattle participating in each scheme. The BTB eradication program was expanded and diagnostic schemes increased at the expense of accreditation. In 1986-87, a new system was implemented which included a 5 year period. Herds were reallocated from the annual diagnostic scheme to herd diagnostic tests. The general approach was to manage BTB 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 culling of BTB reactors was uneconomic and fiscally difficult. RAFF farmers were unwilling to test because they now had no incentive to test for BTB. There was a change in approach regarding BTB eradication. The emphasis changed regarding cattle participating in each scheme.
Changes were also occurring in the animal health sector. National and provincial veterinary structures of veterinary services were implemented during 1995-97 in which the National Directorate of 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 1990s. 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 of Animal Health, communal cattle herds were tested starting in 1997-98.

Game farming was emerging as a major economy in South Africa during the 1990s. Game farmers were reluctant to have their animals tested on a voluntary basis because of the consequences were reluctant to have their animals tested on a voluntary basis because of the consequences. This should there be BTB found on the farm. However, a voluntary basis because of the consequences were reluctant to have their animals tested on a voluntary basis because of the consequences.

Game farming was emerging as a major economy in South Africa during the 1990s. Game farmers were reluctant to have their animals tested on a voluntary basis because of the consequences were reluctant to have their animals tested on a voluntary basis because of the consequences. This should there be BTB found on the farm. However, a voluntary basis because of the consequences were reluctant to have their animals tested on a voluntary basis because of the consequences.

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 1990s. 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 of Animal Health, communal cattle herds were tested starting in 1997-98.

What can we learn from this? We are facing similar problems today in South Africa, such as manpower shortages, budget cuts, 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 be 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 Tuberculosis Control Scheme. The current BTB Control Program, which was introduced in 1981-82, has undergone several changes since its initial introduction. The Bovine Tuberculosis Control Scheme (R.1953 of 30 Sept 1986) 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 diagnostic program (old annual diagnostic) will be accommodated in this program at their own expense. Farmers that do not need certification can be accommodated in this program at their own 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 3 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 reactor animals 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.
Between 2000 and 2014, 16,681 cattle were destroyed due to BTB. This caused an economic loss of R1.26 billion, based on average market value for beef cattle 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 BTB 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 common duiker. More recently, BTB has been reported in multiple wildlife species in Kruger National Park, South African National Parks.  More recently, BTB has been reported in kudu, springbok, bushbuck, hares and common duiker.  In 1940, additional cases were discovered in kudu, springbok, bushbuck, hares and common duiker.

In 1928, BTB was found in a greater kudu and common duiker. In 1940, additional cases were discovered in kudu, springbok, bushbuck, hares and common duiker. More recently, BTB has been reported in multiple wildlife species in Kruger National Park, South African National Parks.

In 1970, 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.

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 1960s.

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).

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 confirmed. BTB cases in animals other than cattle were infrequent until the late 1990’s. The index buffalo case was detected in July 1990 in the south-western region of the park. By 1997, based on analysis of State Veterinary records, BTB was present in the late 1950’s or early 1960’s in a dairy herd south of the Sabie River. Molecular typing of 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 Hi5 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, garrick, blue wildebeest, and wild dog.

Bovine tuberculosis 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. 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 Carne-Whitchetty 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 4/1467 (0.28%) 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 (S0121) as Kruger wildlife, confirming the first spillback 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 occurred. One event occurred in Madikwe Game Reserve in North-West Province. In 2012, 3 buffalo that had tested negative in test and free of interferon assay were slaughtered and found to have TB lesions. M. bovis was isolated and genotyped as strain S01140, which is the strain found in cattle.

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 increases with their proximity to humans and their domesticated cattle” (Griffith 1928); although the risk is greater in wildlife 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.
and wildlife in KwaZulu Natal (KZN). The VNTR genotypes exactly matched that of a BTB case near Muze (Hokwe et al. 2016). A myela 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 (380140).

A black rhinoceros died on a private game farm in North-West Province and the M bovis was isolated was a unique strain (380121). A previously undetected strain (382200) of M bovis was isolated from a blue wildebeest that was translocated from another area in Mpumalanga to a private reserve adjacent to KNP (Hokwe 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 which 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. 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 20.5% per year. In 2014 alone, 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. Tracking of wild animal movements can be implemented through tracking of permits. The integrated electronic permit administration system (EPAS) 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 Clerk-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 big game 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 (Hokwe 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 an infection in 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 under review. 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.
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 acquire wildlife from reputable translocation operators.

Wildlife in South Africa has enormous economic importance. Recreational and trophy hunting industry (along with taxidermy) generate 4.2 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 buffaloes, 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). Why is Diagnosis of Bouine 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 \textit{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 positives are actually false positives (30/38).

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

<table>
<thead>
<tr>
<th>TST Result</th>
<th>Infected</th>
<th>Uninfected</th>
<th>Totals</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>8</td>
<td>27</td>
<td>35</td>
</tr>
<tr>
<td>Negative</td>
<td>20</td>
<td>872</td>
<td>892</td>
</tr>
<tr>
<td>Total</td>
<td>100</td>
<td>900</td>
<td>1000</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>TST Result</th>
<th>Infected</th>
<th>Uninfected</th>
<th>Totals</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>80</td>
<td>27</td>
<td>107</td>
</tr>
<tr>
<td>Negative</td>
<td>20</td>
<td>872</td>
<td>892</td>
</tr>
<tr>
<td>Total</td>
<td>100</td>
<td>900</td>
<td>1000</td>
</tr>
</tbody>
</table>

Another challenge in diagnosing TB has to do with the organism itself. TB is caused by infection with a member of the \textit{Mycobacterium tuberculosis} complex (MTBC). However the genus \textit{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 pathovar of 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 strict identification of the isolate using molecular techniques. This is especially important since there are animal- specific MTBC such as M. avium, M. mungi, and M. argis that belong to the complex but may have different pathogeneses than M. bovis.

In conclusion, when dealing with TB diagnosis, all is not what it at first seems. Make no assumptions, any positive result is used from results (or use multiple labs). Detection sensitivity is improved when any positive result is used from labs. Perform multiple tests to increase confidence to minimize variability which may occur in different labs. Ideally, use the same laboratory for repeat samples.

Implications of BTB in Wildlife in Spain
Prof. Christian Gortazar–Schmidt, Head, SaBis (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 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. As such, the Eurasian badger, deer, European bison, and Eurasian wild boar may serve as reservoir hosts for BTB. The distinctiveness of complex-infected wild boar has been shown to be consistent with distribution of outbreaks of BTB in cattle in France. Prior to the start of the cattle TB test and slaughter program in the 1980s, the prevalence of BTB in cattle was constant with evidence of spillover to wildlife in the 1950s (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 in the last 15 years. Some of the factors contributing to these changes include increased wild ungulate protection and re-introduction and more intensive wildlife management practices such as culling and feeding.

In conclusion, when dealing with TB diagnosis, all is not what it at first seems. Make no assumptions, any positive result is used from results (or use multiple labs). Detection specificity is improved when any positive result is used from results (or use multiple labs). Perform multiple tests to increase confidence to minimize variability which may occur in different labs. Ideally, use the same laboratory for repeat samples.

There is a need for ongoing research to further understand the role of MTB in wildlife in Spain, including the impact on wildlife and the potential for spillover to domestic animals.

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 Donana National Park in south-western Spain is considered a biodiversity hotspot. 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 buffalo (estimated population 1700), fallow deer (900), red deer (600), and the endangered Iberian lynx (<50). The park is an important geographical gradient in M. bovis prevalence and variation in the observed incidence of TB in cattle across the park corresponded well with the spatial availability (less sites, more BTB) of shared waterholes. It is introduced to a multi-host complex endemic to the southern African subregion. There has been the opportunity to significantly reduce BTB risks in cattle and wildlife, through strategic actions. The 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.