UNIVERSITEIT STELLENBOSCH UNIVERSITY

Stellenbosch University Animal TB Research Group

2021 ANIMAL TB RESEARCH GROUP

Welcome to the 2021 Animal TB Research Group Newsletter!

As the pandemic has continued to affect plans for travel, gatherings, and field work, the Animal TB Research Group adapted. Although we did not have our usual group photo, we hope to share photos of all our members throughout the newsletter to introduce you to new members and celebrate those continuing or completing their studies.

We are excited to share some of our findings and news with you!

Meet the Team

Our group continues to grow with the addition of a new Honours, a new Masters, and two continuing Master's students that have upgraded their projects in 2021 to PhD studies. **We are proud of our March 2021 graduates (Josephine Chileshe, PhD; Candice de Waal, MSc; Pamela Ncube, MSc; Samantha Goldswain, MSc).**

The group members are:

Prof. Michele Miller (NRF South African Research Chair in Animal TB)

Dr. Leanie Kleynhans (senior scientist)

Dr. Tanya Kerr (post-doctoral fellow)
Dr. Wynand Goosen (post-doctoral fellow)

Charlene Clarke (PhD student)
Tina Meiring (PhD student)
Debbie Cooke (PhD student)
Pamela Ncube (new PhD student)

Rebecca Dwyer (MSc student)
Rachiel Gumbo (MSc student)
Maureen Kamau (new MSc student)

Jana van Heerden (Hons student)

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Detection of elephant endotheliotropic herpesvirus (EEHV) in free-ranging African elephants

Honours student Jana van Heerden analyzing PCR data to detect EEHV in African elephant samples.

African elephants are threatened by poaching, habitat loss and human-elephant conflict, however little is known about the role of infectious diseases, such as tuberculosis (TB). Elephant endotheliotropic herpesvirus (EEHV) is a novel herpesvirus present in elephants, and causes acute, often fatal, EEHV haemorrhagic disease (EEHV-HD). The disease typically affects young human-managed Asian elephants between the ages of 1-8 years and death generally occurs within 1-7 days after the first appearance of clinical signs. In human-managed Asian elephants, EEHV-HD is the most common cause of juvenile death and mortality rates of up to 85% have been reported worldwide. However, disease has only recently been reported in African elephants in zoos, and there is limited information on EEHV free-ranging African elephants.

This project was a collaborative study with the National Elephant Herpesvirus Laboratory (NEHL), Smithsonian's National Zoo, Washington D.C., USA and South African National Parks (SANParks). Our team (**Jana – BSc Hons; Tanya – Postdoc**) retrospectively screened blood and respiratory samples (trunk wash and bronchealveolar lavage fluid) previously collected from free-ranging African elephants in the Kruger National Park (KNP) to determine if EEHV is present in this populaton, and identify the strains present. This was achieved using PCR techniques previously developed for zoo elephants.

Findings from this study confirmed the presence of strains EEHV2 and EEHV3 in blood and/or respiratory samples of eight free-ranging African elephants from the KNP, with an estimated EEHV prevalence of 8%. In addition, another five elephants were suspected of being infected, based on PCR results. Although skin nodules suspected to be due to herpesvirus were found in African elephants in 1971 during culling in KNP, **this is the first evidence that EEHV can be shed in respiratory secretions of free-ranging KNP elephants and detected using real-time qPCR in a range country**. We hope that this study will help facilitate the diagnosis and improve the detection of EEHV in the region, as well as provide foundation for future surveillance in other populations.

Bovine TB found in Kruger elephants

A 2021 publication by the Animal TB Research Group, along with Kruger wildlife and state veterinary staff, described **infection with *Mycobacterium bovis* in two young bull elephants** that died of other causes. The report was published in the CDC's scientific journal Emerging Infectious Diseases.

The significance of this finding is that elephants that share a habitat with species that can harbour and maintain bovine TB, such as African buffaloes, may be at risk for being infected. A previous study by Dr. Tanya Kerr showed that 6-9% of Kruger elephants tested had immune responses that suggested infection with either *Mycobacterium bovis* or the human strain, *Mycobacterium tuberculosis*.

Since human TB has caused the death of a bull elephant in the park, this discovery suggests that further surveillance and identification of TB strains in elephants are important for determining potential sources of infection and understanding the risk that this disease presents.

Photo credit: Dr. Peter Buss

New developments for TB diagnosis in Africa's big cats

Rachiel Gumbo joined the Animal TB Research Group in 2019 as a BSc Honours student. Since then, she has been hard at work investigating new tools for the detection of *M. bovis* immune responses in cheetahs and leopards for her MSc project. Despite COVID-19, 2021 has been an exciting and productive year for Rachiel as she successfully upgraded her MSc to a PhD starting in 2022. She will focus on developing novel methods that can diagnose bovine TB in three African big cats (leopards, cheetahs, and lions). Over the last year, Rachiel has screened commercially available feline cytokine assays using whole blood samples stimulated using QuantiFERON®-TB Gold Plus (QFT) tubes (used for human TB diagnosis). Using a panel of tests and samples, she found that the Mabtech Cat IFN-γ ELISA^{Basic} could distinguish between *M. bovis*-infected and uninfected individuals. A manuscript detailing the cytokine release assay (CRA) development in cheetah has already been accepted for publication by the Journal of Zoo and Wildlife Medicine. The validation of this assay for use in African lions is currently underway. The development and validation of species-specific diagnostic immunoassays is critical as they will provide the basis for TB management plans and for screening translocation candidates and facilitate conservation programs in southern Africa. **The highlight of this work is the development of a single diagnostic assay which can be used in lions, leopards, and cheetahs to diagnosis *M. bovis* infection for all three species!**

Left: Rachiel preparing leopard samples for testing.

*Below: Rebecca Dwyer (left), Rachiel Gumbo (center), and Josephine Chileshe (right) working together to ensure that assays are performed consistently by all group members. *Note: group picture taken pre-COVID*

THE ANIMAL TB RESEARCH TEAM IS INDEBTED TO THE KRUGER VETERINARY WILDLIFE SERVICES TEAM FOR THEIR SUPPORT OF OUR RESEARCH!

Dr. Peter Buss, Dr. Lufuno Netshitavhadulu, Johan Malan, Guy Hausler, Leanna Rossouw, Tebogo Manemela, & capture team

Understanding risk factors for TB in Kruger's rhinoceros

Rebecca Dwyer joined the group in 2020 and has spent most her MSc project under the cloud of COVID. However, she took advantage to learn a statistical program to model the epidemiology of tuberculosis in rhinoceros in the Kruger National Park by assessing the risks that may predispose to infection in this population. This year she completed work on a first-of-its-kind study that included information for over 400 individual rhinoceros. Based on the analyses, a cluster of cases was found near the southwestern park border, although infection was widespread across KNP (Figure A, next page). When factors were evaluated, there was an **increased risk of *M. bovis* infection when the rhino was exposed to higher numbers of African buffalo herds near the rhinoceros sampling location**. This suggests that spill over of infection may be occurring from African buffaloes (which are important maintenance hosts of bovine TB in KNP) to rhinoceros sharing the same environment.
It also appeared that there was a significantly higher chance of infection during a period of drought. The findings in this study provide a foundation for further investigation of bovine TB in this complex ecosystem.
Rebecca has successfully upgraded her MSc to a PhD and will continue further work on development of *M. bovis* diagnostic techniques for rhinoceros.

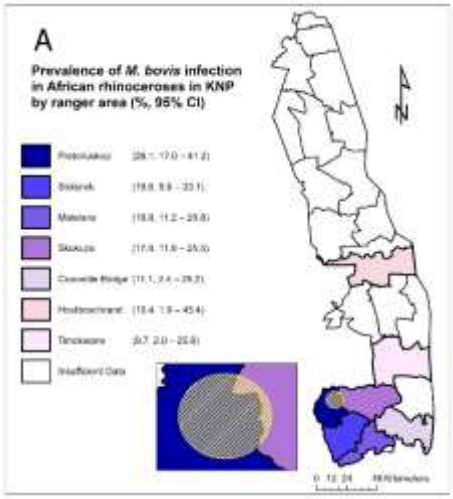


Figure A. Prevalence (%) of *M. bovis* infection in rhinoceros in Kruger National Park, South Africa, 2016-2020 (n = 420). No significant differences in *M. bovis* prevalence according to ranger area were identified (Fisher's exact p > 0.05 for all comparisons).

Below: Rebecca in KNP helicopter (mask removed for photo).

Dr. Carmel Witte, an epidemiologist with San Diego Zoo Wildlife Alliance, has joined the Animal TB Research group as a Stellenbosch University Research Fellow. Her expertise in spatial network analyses in disease investigation brings a new approach to our application of the tools we are developing.

"If you want to go fast, go alone. If you want to go far, go together."
African proverb

Carmel Witte, PhD – Research Fellow
Stellenbosch University

TUBERCULOSIS IN GOATS – UNDERRECOGNIZED HOST?

Debbie Cooke, a full-time animal health technician, performing a tuberculin skin test on a goat (pre-COVID).

Debbie Cooke is a full-time animal health technician, mother, and PhD student. Her years of experience with TB testing domestic livestock and wildlife, as well as training these techniques, provide her first-hand knowledge of the importance of accurate diagnostic tests for identifying infection in different species.

In rural communities, goats, cattle, and people mix, which provided an interface for disease spread. Debbie, with support from Dr. Wynand Goosen, Dr. Carmel Witte, and Prof. Michele Miller, is evaluating the tuberculin skin test as well as blood-based assays to improve diagnosis of TB in goats. With limited information regarding TB in this species in South Africa, **her data will improve understanding of this disease and provide information to goat owners and animal health regulatory agencies for surveillance and control of bovine TB in this species.**

TUBERCULIN SKIN TEST

The tuberculin skin test is used to screen animals for TB. This is based on measuring an immune response to mycobacterial proteins injected into the skin by detecting swelling usually 2-3 days later.
Although commonly used, validation and interpretation guidelines are usually based on extrapolation from cattle. Therefore, to improve the accuracy of this test, it needs to be evaluated in each species, and even different geographical regions.

Buffalo TB diagnostic tests

Charlene Clarke rejoined the Animal TB Research group in 2020 to pursue her PhD, after completing her Honours and Masters degree with our group. Her project investigates environmental mycobacteria and their influence in causing false positive TB tests in buffalo. In addition, she is evaluating novel techniques for field friendly sample storage and direct detection of mycobacteria in samples such as oronasal swabs.

Dr. Wynand Goosen (left) and Charlene Clarke taking swab sample from immobilized buffalo. Note: masks removed for picture.

Use of PrimeStore® Molecular Transport Medium (MTM) and Xpert MTB/RIF Ultra for rapid detection of *Mycobacterium bovis* in African buffaloes was published in Nature Scientific Reports. MTM stabilizes DNA/RNA in samples while inactivating any viruses/bacteria and allows safe transport at room temperature. This may allow samples to move from areas under restrictions for disease, such as Foot-and-Mouth Disease. Samples then were tested in a rapid automated PCR used for human TB diagnosis (Xpert MTB/RIF Ultra), with results available in 2 hours.

Using these techniques, Charlene has found in a pilot study that *M. bovis* DNA could be detected in 5/12 swabs from known infected buffaloes. Oronasal swabs from uninfected buffaloes (n = 20) were negative on Ultra, indicating the high specificity of this test. **These tools will provide a new way to evaluate risks of transmission by identifying animals that are shedding *M. bovis*.**

Improving detection of *M. bovis* in animal samples

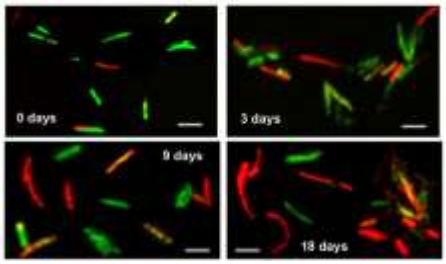
Dr. Wynand Goosen is a post-doctoral fellow, and his work focuses on rapid antemortem and postmortem detection (DNA detection and enhanced culture) of tuberculosis in African elephants and rhinoceros using unconventional difficult to culture samples such as trunk wash and bronchial alveolar lavage (BAL) samples. In partnerships with Cepheid and TiKa Diagnostics, Wynand has been evaluating use of the human GeneXpert MTB/RIF Ultra assay (Ultra) and the new TiKa-MGIT culture test, respectively. To date, **we have shown that Ultra is able to detect TB bacilli as low as 2 CFU/ml and the new TiKa-MGIT system to significantly enhance mycobacterial growth and isolation from culture, which is important for samples that have low numbers of bacteria present**. This novel culture method improves diagnosis of TB, and provides live mycobacteria which is important for molecular genetic analyses and epidemiological studies. In recognition of his contributions, Wynand has been awarded a spot in the top 10 young emerging scientists in South Africa selected by the National Science and Technology Forum in 2019, 2020 and 2021.

Dr. Wynand Goosen at 2021 NSTF awards ceremony (fourth from left). Note: masks removed for picture.

Understanding Mycobacterium bovis "behavior" – can infection become latent in wildlife hosts?

TB in humans can exist as a latent infection which may not cause any signs or symptoms for years. However, it is unknown if *M. bovis* remain in this "persister" state in animals.

Pam Ncube is trying to answer this question. Pam is a joint PhD student in the Animal TB and Mycobactomics Research groups. Her research entails using a novel fluorescence detection system (developed in the Mycobactomics group) to evaluate *M. bovis* culture growth and viability under different conditions, assumed to occur in the host during infection.



For her MSc project, Pam isolated *M. bovis* from several wildlife hosts (warthogs, buffaloes, lions, wild dogs) and compared the bacteria's ability to form "persisters" in culture. In a pilot study, she found that the **animal strains apeared less likely to form persisters compared to laboratory adapted *M. tuberculosis* and *M. bovis* (BCG) strains.**

Building on this work, she will evaluate other conditions and more strains for her PhD to determine if M. bovis from certain hosts or with genetic differences can contribute to persister formation. This will provide a better understanding of whether animals can develop latent infections.

THANK YOU TO OUR COLLABORATORS AND SUPPORTERS

Photo credit: Bence Mate

Research is a team effort – we could not accomplish our work without interested veterinarians, wildlife managers, conservation organizations, companies producing scientific products, and fellow researchers. Thank you!

Mycobacterium bovis infection and population genomics of African wild dogs

Tina Meiring, a final year PhD student in the Animal TB and Genetics Research groups, concentrated her research on understanding more about the bovine TB in African wild dogs in the Kruger National Park (KNP) area (KNP)and Hluhluwe iMfolozi Park (HiP), and the genetic diversity of the KNP population. In a recent publication, she described pathological features of bTB in 12 wild dogs and found infection in multiple organs (including in 2 pups), indicating that this disease can be severe in this species.

Genetic diversity is considered a key feature associated with long-term survival of a population and adaptability to changing conditions and disease. To evaluate the role of genetics in the apparent susceptibility to bTB, Tina assessed levels of genetic variation in the KNP wild dog population (71 individuals) using whole genome sequencing, producing the largest database of genetic information for this species to date. By comparing the genetic codes between wild dogs, it appeared that the variation in the gene pool was low, although there was also a low level of inbreeding, which suggests a previous bottleneck. Although the genomic features observed in the KNP wild dog population may not impact short-term viability, the low levels of genomic variation may compromise population recovery.

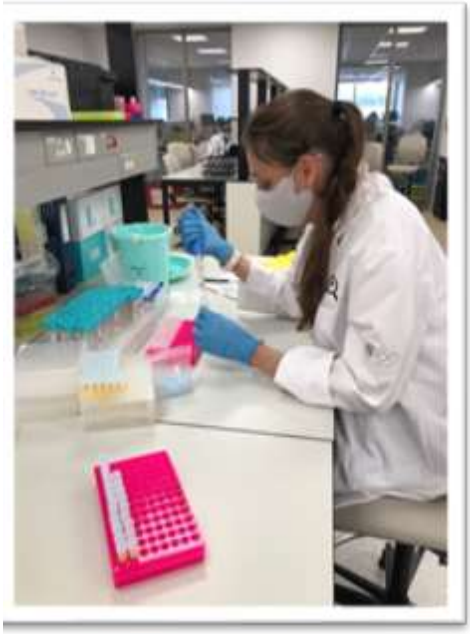
Tina has submitted her PhD thesis and plans to perform further analyses to look at disease susceptibility and develop a genetic panel for screening translocation candidates to improve population management of this species.

Dr. Lufuno Netshitavhadulu and Tina Meiring examining a rhino in Kruger National Park. Note: masks removed for picture

Using whole genome sequencing (WGS) to evaluate the epidemiology of *Mycobacterium bovis* isolates in multi-host ecosystems across South Africa

In South Africa, *Mycobacterium bovis* (*M. bovis*) is the most common cause of wildlife tuberculosis (TB), with two of the country's largest wildlife reserves, namely Kruger National Park (KNP) and Hluhluwe iMfolozi Park (HiP), declared endemic for *M. bovis*. To date, *M. bovis* infection has been confirmed in 24 wildlife species in South Africa. Spoligotyping is commonly used to identify the genotype of *M. bovis* isolates and determine the relationships between organisms from different geographical regions. Historically, data suggested that a single dominant strain of *M. bovis* circulated within wildlife in specific geographical locations in South Africa, with SB0121 widely considered the KNP strain, while SB0140 was considered the KZN strain. However, this dogma has been challenged in the last decade with the detection of several *M. bovis* strains circulating within these locations**. The genetic diversity of *M. bovis* in South African wildlife may be indicative of genetic divergence, ongoing transmission, or the introduction of new strains into regions through movement of infected animals, but further epidemiological studies are required.** Therefore, the goal of this ongoing study is to use whole genome sequencing (WGS) of *M. bovis* isolates from wildlife in multi-host ecosystems across to South Africa to investigate intra- and inter-species transmission.

To date, 30 *M. bovis* isolates from 8 wildlife species have been successfully spoligotyped (SB0121; SB0140; SB1681; SB1947; SB2681; SB2723) and sequenced (Figure). Preliminary analysis of 14 whole genome sequences has been performed. These 14 isolates all originate from wildlife in the Greater KNP and primarily represent SB0121 and share a common ancestor with isolates previously reported for South Africa. Our current focus is to continue generating WGS data before continuing with more detailed analysis on the larger, more complete dataset. We hope to have some exciting results during 2022.



Rapid detection of *Coxiella burnetii* DNA in ticks in Kenya using in situ DNA extraction and a handheld portable thermocycler

Dr. Maureen Kamau is a Kenyan veterinarian with an interest in zoonotic diseases and One Health. She joined the Animal TB group in 2021 as a MSc student. Her work will be evaluating a field friendly PCR to determine the presence of *Coxiella burnetti* in ticks to assess transmission risks.

Q fever, a zoonotic disease caused by the bacterium *Coxiella burnetii*, affects humans associated with livestock, and can be transmitted between livestock and wildlife by ticks. Q fever prevalence data, critical for guiding public health actions and resource allocation, are often limited and may not be available, especially in resource-poor settings due to limited diagnostic capability. This study aims to fill this gap by validating a field-friendly diagnostic technique, that requires minimal laboratory training and uses battery-powered, portable equipment to test for *Coxiella burnetii* DNA in ticks collected at watering holes used by livestock and wildlife in Laikipia County in Central Kenya as a method for disease surveillance.

RECOGNITION

CONGRATULATIONS TO DR. WYNAND GOOSEN ON RECEIVING A WELLCOME TRUST INTERNATIONAL TRAINING FFELLOWSHIP

Wynand who has been awarded a prestigious three-year training fellowship from Wellcome Trust (UK). His research will work towards 'Improved surveillance of zoonotic Mycobacteria through rapid direct detection and genotyping in livestock, wildlife, and their environment from low-resource areas in South Africa. This project will build on his direct detection method development and collaborations with researchers at the African Health Research Institute (Dr. Emily Wong's research team), One Health researchers at the Schulich School of Medicine and Dentistry in Canada (Prof. Francisco Olea-Popelka) and Wellcome Clinical Research Career Development Fellow at Universitiy of Abderdeen (Dr. Mark Moseley), Dr. Sandra Telfer (University of Aberdeen), and TB researchers at Stellenbosch University School of Medicine and Health Sciences.

Congratulations to Rachiel Gumbo and Pam Ncube for being awarded DAAD PhD Scholarships

DAAD is a German academic scholarship program which supports post-graduate students in collaborating African universities. This highly competitive scholarship provides financial support for tuition, research allowance, travel to a German research institution, health insurance, and yearly stipend. This year, both

Rachiel and Pam have been awarded one of only four doctoral awards. The scholarship will start in 2022.

Congratulations to Rebecca Dwyer and Rachiel Gumbo for upgrading their MSc projects to PhD studies!

ANIMAL TB RESEARCH TEAM MEMBER SUCCESSES

Despite the uncertainty, cancelled field projects, restricted access to the lab, virtual meetings, and intermittent internet access due to load shedding, the Animal TB Research Team faced the challenges and were able to complete data analyses, manuscript drafting, and thesis writing. We proudly recognize all the students for another successful year!

Congratulations Tina on your thesis submission and completing your PhD defense!

Tina will be graduating in March 2022.

THANK YOU TO ALL THAT SUPPORT OUR STUDENTS

The success of a student depends on the investment of their supervisors, proposal committee members, and thesis examiners, as well as the administrative staff of the university, co-workers, family, and friends. We would like to thank all these individuals for helping the Animal TB Research Group's students throughout the years.

"Success isn't just about what you accomplish in your life; it's about what you inspire others to do."
-- Unknown

"Success is walking from failure to failure with no loss of enthusiasm."
-- Winston Churchill

Ig Nobel Prize

Prof. Michele Miller was one of a team of wildlife researchers to win the 2021 Ig Nobel Prize, which honors curious and "imaginative" discoveries, for a study which investigated whether rhinos could be safely transported by suspending them upside down under a helicopter. The award is a spoof of the real Nobel Prizes for science but attracts an international following including coverage in print and broadcast media.

(Top) Michele takes blood sample from suspended immobilized rhino in Namibia. (Bottom) Airlifted rhino (photo credit Dr. Pete Morkel).

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