UNIVERSITEIT STELLENBOSCH UNIVERSITY

Stellenbosch University Animal TB Research Group

2022 ANIMAL TB RESEARCH GROUP

Welcome to the 2022 Animal TB Research Group Newsletter!

The end of pandemic restrictions in 2022 has allowed the Animal TB Research Group to return to the lab, travel, and field work. We look forward to sharing our exciting new research and team members in this newsletter.

Meet the Team

Our group continues to grow with the addition of a new postdoctoral fellow, and continuing Masters and PhD students. **We are proud of our March 2022 graduate (Tina Meiring).**

The group members shown in the picture above are:

First row – left to right

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

Rachiel Gumbo (PhD student)

Charlene Clarke (PhD student)

Rebecca Dwyer (PhD student)

Back row – left to right

Dr. Tanya Kerr (post-doctoral fellow)

Pamela Ncube (PhD student)

Dr. Leanie Kleynhans (senior scientist)

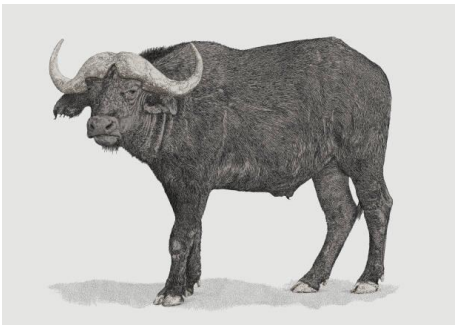
Dr. Abisola Okunola (new post-doctoral fellow)

Dr. Wynand Goosen (Wellcome Trust Fellow)

Absent from photo:

Maureen Kamau (MSc student)

Debbie Cooke (PhD student)



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"Spotting" TB in South Africa's Big Cats

Doctoral student Rachiel Gumbo analyzing blood samples from lions to detect TB.

Rachiel is a PhD student who joined the Animal TB Research Group in 2019. Her work focuses on developing immunological assays for the detection of *Mycobacterium bovis* infection in African big cats (lions, cheetahs, and leopards). Accurate diagnosis of *Mycobacterium bovis* infection in African big cats minimizes the risk of disease spread during translocation of animals between fragmented populations, a crucial component of conservation programs in South Africa and globally. Therefore, ongoing research is needed to develop and optimize species-specific diagnostic tests for African big cats as part of the management plan for these species.

After developing a cytokine release assay (CRA) for use in cheetahs during 2021, Rachiel went on to validate this CRA for use in African lions and her work was published in a special issue of the journal "Pathogens" in 2022. Given the similarity between felid species, she has also tested leopard samples with this assay. The results indicated immune mediators (cytokine) were produced upon blood cell stimulation and provided evidence that interferon-gamma concentrations could be used to identify *M. bovis*-infected leopards. We are very excited about the prospect of having a single blood-based assay which can be used for the detection of *M. bovis* infection in lions, leopards, and cheetahs. Rachiel is currently evaluating the performance of cytokine gene expression assays, previously described for use in African lions, using leopard samples as well as assessing a rapid PCR assay detect presence of *M. bovis* DNA directly in leopard tissues. It has been an exciting and productive year for Rachiel as she managed to write and publish two articles and we are really looking forward to 2023 as she takes her final steps towards completing her PhD.

Immobilized leopard (left); Dr. Peter Buss (upper right); Dr. Lufuno Netshitavhadulu and capture team (lower right)

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 Freese, Tebogo Manemela, & capture team

In addition, we wish to thank the skilled pilots and staff of Kruger's airwing.

Dr. Wynand Goosen (Wellcome Trust Fellow) and Charlene Clarke (PhD student) obtain samples from private buffalo herds to advance TB research in this species.

Advancing our Understanding of TB in African Buffaloes

African buffaloes are important species for tourism and ecosystem balance. However, they are also recognized maintenance hosts of *Mycobacterium bovis*, the cause of bovine TB. Charlene Clarke's doctoral research is contributing to development of improved techniques to detect mycobacteria and understand factors that may confound interpretation of TB diagnostic tests in this species.

During 2022, Charlene's findings were published in 3 scientific papers. She was able to show that she could detect *M. bovis* in oronasal swabs placed in a stabilizing medium that could be safely transported to a lab for further testing. A rapid PCR assay (used for testing human sputum for TB) could provide same-day results to diagnose infected buffaloes (instead of the 3+ months needed for culture results). This is a significant advance that can identify infectious animals rapidly and accurately to prevent further disease spread.

Charlene has also worked with private buffalo herds to understand why some animals may have a suspect tuberculin skin test reaction, even when they are not infected with *M. bovis*. She was able to identify a large diversity (55 species) of environmental mycobacteria found colonizing buffalo respiratory tracts. These may lead to cross reactions, and false positive test results, which can result in quarantine of the premises. In addition, Charlene was able to develop a blood-based test algorithm to minimize false positive test results and provide improved options when screening buffalo herds for this controlled disease.

Charlene has submitted her PhD thesis and already passed her examinations. We wish to congratulate her on her graduation in March 2023 and plans for the future!

Dr. Wynand Goosen prepares samples for DNA detection assay to improve rapid diagnosis of TB.

Prestigious Wellcome Trust International Training Fellowship Awarded to Dr. Wynand Goosen

In 2022, Wynand was awarded a 3-year training fellowship for his project titled "**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 ground-breaking research will use a One Health approach to develop improved mycobacterial culture and culture-independent molecular techniques to identify pathogenic mycobacteria in human, livestock, wildlife, and environmental samples. These novel tools will then be applied to identify locations and sources of potential infection for humans, especially in rural communities that have close contact with animals. Zoonotic TB is a global concern and the presence of TB in animals hinders TB eradication programs for humans. Therefore, this research will have a wide global impact on strategies for animal and human TB management.

wellcome

Tuberculosis in goats - should we be worried?

Debbie Cooke joined the Animal TB research group in 2020 as a PhD student. She, along with Dr. Wynand Goosen, Charlene Clarke, Dr. Carmel Witte, Asst. Prof. Tristen Burgess, and Prof. Michele Miller, have been investigating TB in goats in communal areas of Northern KwaZulu Natal.

TB testing and surveillance in domestic goats is not widely applied in South Africa, as TB control programmes are mostly directed at cattle. The traditional approach to keeping livestock in South African communities involves goats sharing communal pastures and water sources with cattle and other livestock. Since *M. bovis* is considered endemic in communal cattle and wildlife in South Africa, goats may be a potential source of *M. bovis* spread to cattle or other livestock as well as humans and wildlife.

Since DALRRD's publication of bTB testing guidelines in 2018, prescribing the use of the tuberculin skin test (TST) for goats in South Africa, there have been no published reports of any field application of the prescribed test criteria in goat herds. In a recent publication, Debbie evaluated the performance of these guidelines in almost 800 goats. Building on this, she is further investigating the use of other novel TB diagnostic assays in goats.

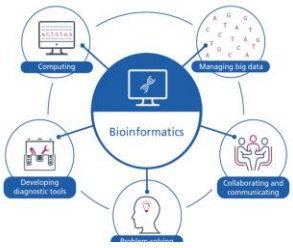
She also aims to identify and characterise any mycobacteria detected in the nasal swabs of these goats. It is important to understand the extent of nontuberculous mycobacterial (NTM) exposure in these populations as some can induce a cross-reactive immune response resulting in false positive test results, while others may potentially infect people and other livestock.

Her studies will help us understand TB in goats, identify whether goats are potentially an unrecognized sources of intra- and inter-species transmission of TB and whether they present a potential threat to bovine TB eradication in South Africa.

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

WELCOME DR. ABISOLA OKUNOLA

We are excited to welcome a new post-doctoral fellow to the Animal TB Research Group in 2022! Abisola completed his BSc in Medical Laboratory Science and MSc in Biomedical Science from Nigeria and UK in 2010 and 2014 respectively. He obtained his PhD from Stellenbosch University in 2020. He joined the TB diagnostic group for his postdoctoral research in 2021, and moved to the Animal TB research group in July 2022. His research within the group focuses on investigating the intra- and inter species transmission of *M. bovis* in wildlife. He will also expand our group's expertise in bioinformatics. Abisola enjoys listening to music and spending time out with friends and family.



Animal TB Research Group Promotes TB Awareness

Charlene, Tanya, and Rebecca at Division Open Day November 2022

Poster for World TB Day 2022

Science Communication

With easing of pandemic restrictions on travel and in-person gatherings, the Animal TB Research Group used the opportunity to share information at conferences, workshops, and through public media. Here are a few examples:

Tanya, Wynand, and Michele presented new findings at the International *M. bovis* conference in Galway, Ireland.

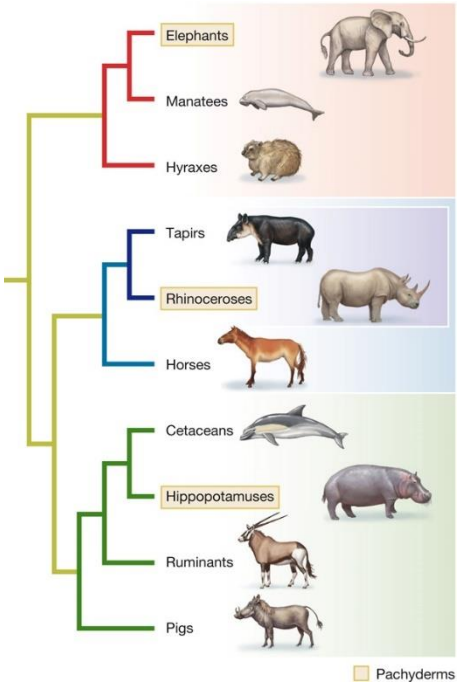
Animal TB group members reunite at a South African Veterinary Immunology Workshop (left to right – Dr. Eduard Roos, Prof. Michele Miller, Dr. Sven Parsons, Dr. Wyand Goosen, Dr. Tanya Kerr)

A Mammoth Task – Studying Immune Responses in Elephants

Tanya, Michele, and Leanie with immobilized elephant in Kruger National Park 2022.

One of the many interesting aspects of research on animal health is discovering and comparing how each species "works". In order to understand how and why a disease occurs, it is crucial to investigate the unique features of the immune system in different taxonomic groups. Elephants are unique, being more closely related to some marine mammals then other African mammals.

A team including a human immunologist (Dr. Leanie Kleynhans), conservation ecologist/molecular biologist (Dr. Tanya Kerr), and wildlife veterinarian/researcher (Prof. Michele Miller), along with collaborators, are tackling the task of understanding how elephants' immune responses function in health and disease in this long-lived species.



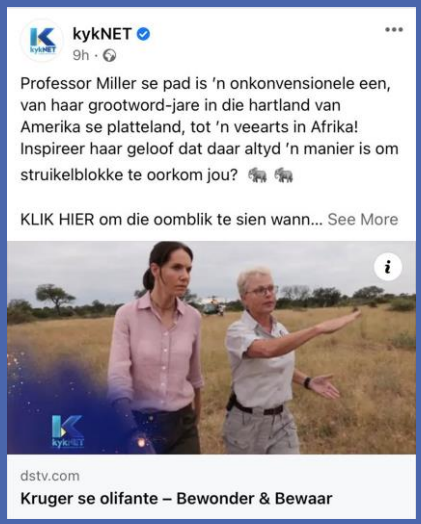
Our research will provide insight into health management of this iconic species, including diagnosis and monitoring of different diseases.

THANK YOU TO OUR COLLABORATORS AND SUPPORTERS

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.

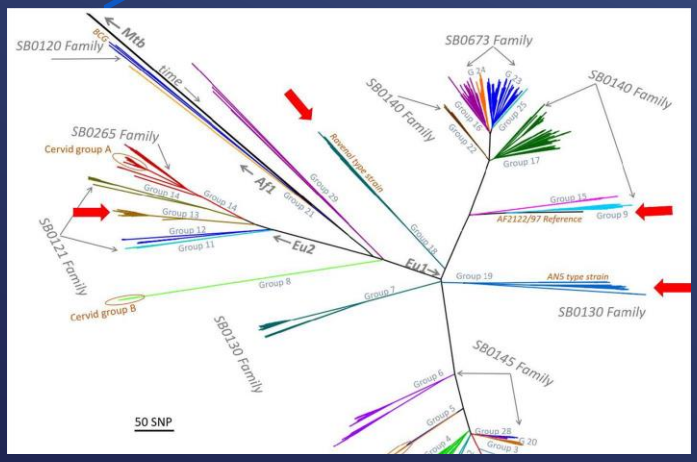
HIGHLIGHTING ELEPHANT RESEARCH

An episode of a popular series on kykNET TV featured Prof. Miller and her career path to becoming the leader of a success wildlife research group.

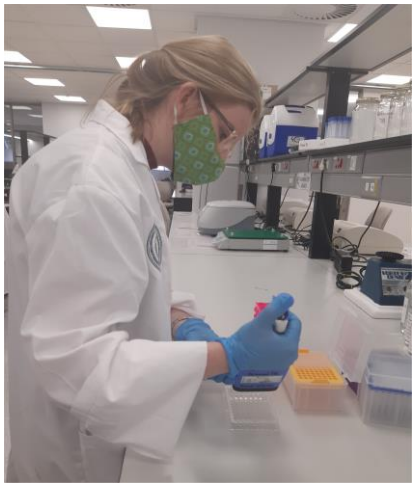


Using whole genome sequencing (WGS) to evaluate the epidemiology of *Mycobacterium bovis* isolates in multi-host ecosystems across South Africa – Project Update

In 2021, we launched an exciting project which uses whole genome sequencing (WGS) of *M. bovis* isolates from wildlife in multi-host ecosystems across South Africa to investigate intra- and inter-species transmission. This project has been continued through 2022 and to date, 104 *M. bovis* isolates from 12 wildlife species have been successfully spoligotyped and sequenced with the majority of the samples originating form Kruger National Park (KNP) and Hluhluwe iMfolozi Park (HiP). Additional samples are being added as they become available. These sequences are contributing to a worldwide database on all the strains of *M. bovis* found in livestock, wildlife, and even humans. This information facilitates epidemiological investigation of TB outbreaks. Our newest team member, Dr Abisola Okunola, is currently performing phylogenetic analysis on these sequences to evaluate the genetic diversity of *M. bovis* in South African wildlife and we hope to have some exciting results to share during 2023.



Exploring TB Epidemiology in African Rhinoceros



In 2022, Rebecca continued work on her PhD project, which is focused on the development of diagnostic techniques for detecting bovine tuberculosis in African rhinoceros, and the use of these tools to investigate epidemiology and potential inter- and intra-species transmission. Highlights of Rebecca's work in 2022 include a research publication in Proceedings of the National Academy of Sciences (PNAS) (IF = 12.78) that was focused on a study of the epidemiology of *Mycobacterium bovis* infection in rhinoceros in Kruger National Park, and a short research publication in Veterinary Immunology and Immunopathology, which reported the reduced capability of refrigerated white rhinoceros whole blood to produce interferon gamma upon mitogen stimulation. And in other news – Rebecca got married this year to Brydon Leonard and intends to graduate with her PhD under her new name, Rebecca Leonard!

RECOGNITION

CONGRATULATIONS TO DR. WYNAND GOOSEN ON RECEIVING THE TW KAMBULE-NSTF BEST EMERGING RESEARCHER AWARD IN 2022

Congratulations to Prof. Miller for being awarded the Inaugural Eugene Cloete Outstanding Postdoc Mentor Award and 2022 Research Excellence Award

Congratulations to Dr. Tina Meiring on receiving her doctoral degree in March 2022

Building TB Bioinformatics Capacity – A Collaboration between South Africa, Madagascar, and the French Embassy

A grant from the French Embassy has supported training and collaboration to build capacity in TB next generation sequencing and bioinformatics. This included a scientific exchange in which Prof. Miller visited the Institute Pasteur de Madagascar to increase awareness of One Health research including *M. tuberculosis* infection in animals and *M. bovis* infection in people and animals. In addition, a young Madagascan scientist visited Stellenbosch University to receive hands-on training in mycobacterial culture, sequencing, and analyses. We look forward to continued exchanges and collaborations!

Dr. Niaina Rakotosamimanana, Prof. Miller, Tojo Andrianarivo (veterinary student), Dr. Paulo Ranaivomanana at Institute Pasteur de Magagascar

"Education is the most powerful weapon which you can use to change the world." — Nelson Mandela

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Animal TB Research Group enjoying sunset in Kruger National Park

For Information

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http://www.sun.ac.za/english/faculty/healthsciences/Molecular_Biology_Human_Genetics/animaltb/Pages/default.aspx

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Thank you to all our collaborators, funding organizations, and supporting partners!