UNIVERSITEIT STELLENBOSCH UNIVERSITY

Stellenbosch University Animal TB Research Group

2020 ANIMAL TB RESEARCH GROUP

Welcome to the 2020 Animal TB Research Group Newsletter!

Despite the 2020 craziness, the Animal TB Research Group has had a busy 2020. The group continues to grow with the addition of two new Masters' and two doctoral students. We are proud of our 2020/2021 (March) graduates (4 MSc and 1 PhD).

Although the pandemic restrictions resulted in cancellation of our planned field projects, we have been fortunate that the group has been collecting data and samples that were available for analyses and publications. When we went to level 3, some of the students were allowed back on a limited basis to the laboratory to complete postponed experiments.

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

Meet the Team

The Animal TB Research group has continued to grow, with 14 members in 2020!

Pictured above, (left to right top row) (photo taken Jan2020): Charlene Clarke (PhD student), Dr. Tanya Kerr (post-doctoral fellow), Prof. Michele Miller (chair), Dr. Wynand Goosen (post-doctoral fellow), Dr. Leanie Kleynhans (senior scientist); middle row: Tina Meiring (PhD student), Josephine Chileshe (PhD student), Sam Goldswain (MSc student), Candice De Waal (MSc student), Pamela Ncube (MSc student); bottom row: Rebecca Dwyer (MSc student), Kat Smith (MSc student), Rachiel Gumbo (MSc student). Missing: Debbie Cooke (PhD student).

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As human populations grow, space for wildlife continues to shrink, resulting in small, fragmented populations, especially in the case of species that need large territories.

Natural mechanisms for maintaining biodiversity include the movement of animals between groups, usually through the migration of individuals outside of their home ranges. However, parks and reserves are surrounded by fences to prevent the movement of wildlife into human communities and interfaces with livestock. The result is a limited number of individual animals that can maintain the genetic pool. Inbreeding can lead to decreased fitness of the population to adapt to challenges, such as changes in the environment or to the introduction of disease that result in their local extinction.

Conservation programmes address this threat by using tools such as translocation (capture, transport and release) of animals between isolated populations to maintain genetic diversity. However, decisions are based on knowledge of the expected genetic relatedness of animals that is based on observed breeding and the identification of the offspring produced. This can be difficult in the case of elusive wildlife species. New techniques such as whole-genome sequencing, which identifies the genetic code of the animal and allows comparison between individuals, can provide definitive measures of relatedness and inform management decisions.

The African wild dog (*Lycaon pictus*) is critically endangered, with a global population of just over 6 000, and only 450 individuals in fragmented ranges in South Africa. Translocation between populations is used to maintain their genetic diversity.

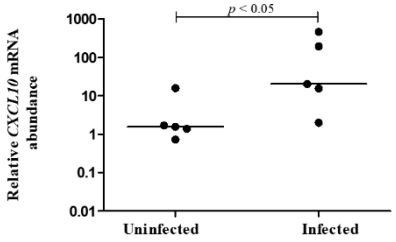
Tina Meiring, a doctoral student in SU's Animal TB Research Group and the Genetics Research Group in the Department of Biomedical Sciences, is applying whole-genome sequencing, a technique new to studying wild dogs and their genetic makeup, to blood samples taken from individual wild dogs in the Kruger National Park. In this park, the wild dog population is self-sustaining, which enables her to investigate the relatedness of animals.

Meiring's preliminary data suggest that only 13% of the wild dogs sampled were unrelated in a small-sample cohort. Ongoing studies will provide an important foundation for informing management decisions related to conservation. It will also inform decisions about translocations and management of the species by evaluating genetic factors that impact on disease susceptibility and adaptability to environmental changes.

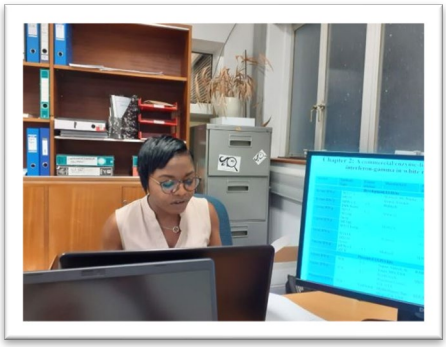
* *This article featured in the latest edition of Stellenbosch University (SU)'s multi-award winning publication* ***Research at Stellenbosch University*** .

Identification of Immunological Biomarkers for Detection of Mycobacterium bovis Infection in African rhinoceros

Josephine Chileshe has been developing tests to screen rhinoceros for infection with *Mycobacterium bovis* as part of her PhD studies, and last year, validated an interferon gamma release assay that is currently being used by SANParks to test rhinoceros for movement out of the park. Since no test is 100% perfect, she has been investigating additional blood-based biomarkers for TB in rhinoceros. Using RNA extracted from stimulated rhinoceros blood cells, she created a novel PCR to determine if a cytokine gene, *CXCL10* (which has been used in other species), could distinguish between known *M. bovis* infected and uninfected white rhinoceros. Her preliminary results look promising and may provide an additional test that can be performed on the same blood sample used for the interferon gamma release assay.



Importantly, Jos has completed her doctoral thesis and will be graduating in March 2021.



Understanding the Immune Responses of African Elephants

Very little is known is known about immune responses in African elephants. This has been the focus of Candice de Waal's Master's project.

Candice used blood samples collected from elephants in Kruger National Park and stimulated them to determine what immune genes changed expression.

Candice de Waal with immobilized elephant in Kruger National Park, October 2019.

Using RNA extracted from the elephant's blood, Candice was able to sequence the cytokine genes (*CXCL9, CXCL10, IFNγ, IL4, IL10, IL12, TGFβ,* and *TNF*) for the first time in this species. These are important chemical signals in immune responses and these results will be the basis for development of immunoassays to detect disease-specific responses.

Candice has described her results and preliminary findings for immune responses to TB in her thesis "Characterization of Biomarkers of Immunological Activation in African Elephants (*Loxodonta africana*)."

Candice has a scientific paper in review in the journal "Cytokine". She will be graduating in March 2021.

Welcome back Charlene!

Charlene Clarke running inactivated buffalo tissue sample in rapid automated TB PCR machine (Xpert MTB/RIF Ultra assay, Cepheid) to detect M. bovis.

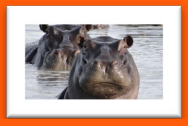
Charlene Clarke completed her BSc (Hons) and MSc in 2016 in the Animal TB research group. She rejoined the group this year to continue her PhD. Her research will investigate biomarkers in African buffaloes that can differentiate between early *Mycobacterium bovis* infection and active bovine TB disease, by evaluating responses at the local tissue level and systemically. Findings from this study will provide insight into bovine TB pathogenesis and could improve current diagnostic regimes and disease management in buffaloes.

Animal TB Research Group Student News

The Animal TB Research Group welcomed 2 new Master's students, Rachiel Gumbo and Rebecca Dwyer, and 2 new doctoral students, Charlene Clarke and Debbie Cooke. In addition, Tina Meiring upgraded her MSc project to a PhD starting in 2020.

DIAGNOSIS OF MYCOBACTERIUM BOVIS INFECTION IN FREE-RANGING COMMON HIPPOPOTAMUS

Hippopotamuses are rarely tested for bovine TB, and therefore *M. bovis* infection has not previously been confirmed in this species. As part of this collaborative study with State Veterinarians and South African National Parks (SANParks), our team (**Tanya – Postdoc; Rachiel – MSc; Wynand - Postdoc**) retrospectively screened blood and tissue samples collected from hippos in the Greater Kruger Protected Area. The first confirmed case of *M. bovis* in a free-ranging hippo was discovered following direct detection of MTBC DNA in tissue samples using GeneXpert® MTB/RIF Ultra qPCR Assay, mycobacterial culture and speciation. The strain identified (SB0121) is a strain commonly found in the GKPA. In addition, there was a bTB seroprevelance of 8% in the GKPA hippo population using a rapid serological assay (Chembio Dual Path Platform (DPP®) Vet TB Assay). These direct and indirect methods of detection show promise for screening hippos for TB.



TB DETECTION IN CHEETAHS AND LEOPARDS

Following discovery of *M. bovis* generalized disease in a cheetah and promising preliminary diagnostic results with immunoassays used in lions (Kerr et al., 2020), we decided to expand this project in 2020. Therefore, our new MSc student, Rachiel Gumbo's current MSc study focuses on identifying host and pathogen biomarkers that can be used to detect *M. bovis* infection by investigating cell-mediated immune responses in leopards and cheetahs. For this study she will be screening additional biomarkers (*CXCL10, IFNg*) for use in GEA (as previously done in African lions) as well as looking at the use of commercially available ELISA's for diagnosing TB in these species. The accumulated preliminary data shows that the commercially available feline antibodies (IL-1β, IFN-γ, TNF-α) can be used for measurement of cell-mediated immune activation in mitogen stimulated blood from cheetahs and leopards using ELISA, and will now be further investigated to determine if these candidate biomarkers are able to distinguish between *M. bovis* infected and uninfected individuals. Watch this space in 2021!

Rachiel Gumbo joined the Animal TB as a Hons student and has continued her project on cheetah and leopard diagnostic TB test development.

Rachiel preparing to run a cheetah interferon gamma ELISA.

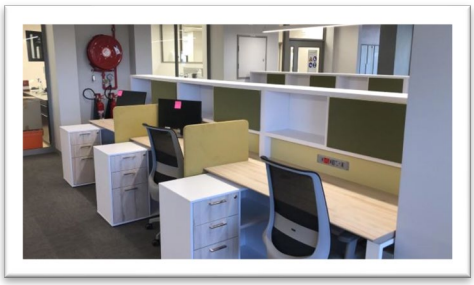
THANK YOU TO OUR COLLABORATORS AND SUPPORTERS

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

PHINDA
PRIVATE GAME RESERVE

A New Home for the Animal TB Research Group

In November 2020, the Animal TB Research Group moved to the new Biomedical Research Institute (BMRI), at the Faculty of Medicine and Health Sciences (Tygerberg Campus). This state-of-the-art facility will provide additional laboratory, offices, and student workspace. We have already started work, although we are still following pandemic restrictions at reduced capacity.



ENDANGERED WILDLIFE TRUST

EZEMVELO KZN WILDLIFE
Conservation, Partnerships & Ecotourism

South African NATIONAL PARKS

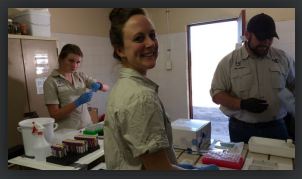
HESC HOEDSPRUIT ENDANGERED SPECIES CENTRE

RECOGNITION

Congratulations to Dr. Wynand Goosen for being one of the top 20 post-doctoral fellows at Stellenbosch University in 2020!

CONGRATULATIONS TO KATRIN SMITH!

Kat successfully completed her MSc, entitled "The evaluation and validation of cell-mediated immunological responses for the improved detection of *Mycobacterium bovis* infection in African buffaloes (*Syncerus caffer*)". Her research investigated new assays and buffalo-specific cutoff values to improve detection of bovine TB in buffaloes. Kat has graduated in December. We wish her success in pursuing her PhD!

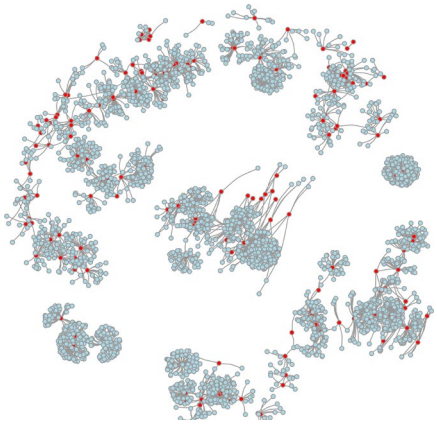


IMPACT OF COVID

Kat was in her 2nd year of MSc this year and was lucky enough to finish her practical work before lockdown commenced. Thus, those hours stuck in the house served her well as evident by the three publications achieved from her thesis research, with a possible fourth (submitted just last week for a special Frontiers edition on TB biomarkers).

Epidemiology of *Mycobacterium bovis* in Greater Kruger Protected Area (GKPA) – a Multi-Host Ecosystem

Mycobacterium bovis the causative agent of bovine TB is endemic in the wildlife population in Greater Kruger Protected Area (GKPA). To date, more than 15 wildlife species have been reported to have confirmed infection, including iconic and threatened/endangered species, such as African elephants, black and white rhinoceros, leopards, African lions, and African wild dogs. However, the routes and risk of *M. bovis* transmission are largely unknown due to the logistical difficulties associated with studies in free-ranging wildlife in a complex ecosystem.



Social network graph of a subset the San Diego Zoo Global bird network, 1992–2014 (Avian TB+ birds in red). C. Witte et al., 2020. PLoS ONE

In order to understand the epidemiology of TB in this multi-host system, the Animal TB Research Group have begun a collaboration with an expert epidemiologist, Dr. Carmel Witte from San Diego Zoo Global (USA). With her expertise in spatial/social network analyses of infectious diseases like avian tuberculosis, we have begun to investigate and model risk factors and interspecies *M. bovis* transmission in a multi-host ecosystem using whole genome sequences (WGS) generated from *M. bovis* isolates cultured from GKPA wildlife hosts. Dr. Tanya Kerr, post-doctoral fellow, is leading this effort.

INVESTIGATION OF TB IN DOMESTIC GOATS (*CAPRA AEGAGRUS HIRCUS*) IN KWAZULU-NATAL

The goat (*Capra hircus*), which has been with humans for hundreds of years, is considered the smallest domesticated ruminant and has even been termed the 'poor man's cow' in some developing countries. In South Africa, the goat population among the nine provinces, is estimated at approximately 5.62 million, with just over 700 000 occurring in KwaZulu-Natal. It is estimated that approximately 63% of South African goats are made up of indigenous goats in non-commercialized sectors. In South Africa, despite bTB being endemic, TB in goats has not been extensively investigated. Reporting, testing, and surveillance are significantly lacking and for these reasons, there is insufficient information available on the TB status of the South African goat population.

Goats are considered to be spill over hosts for *M. bovis,* since it appears that they are most susceptible to infection when the infection pressure is high as in the case of TB positive cattle or wildlife occurring in the same locality. However, goat herds infected with TB can act as a reservoir, increasing the risk to cattle, other animals and humans.

The study will investigate the presence and prevalence of TB in goats in KwaZulu-Natal (KZN). This will be achieved through in vivo skin testing, in vitro humoral and cell mediated immune response assays and direct mycobacteria detection methods. Comparisons will be made to determine which test or combination of tests can be used to optimally distinguish between MTBC sensitized and uninfected goats. The identification of possible demographic risk factors associated with MTBC infection in goats in KZN will also be evaluated.

Debbie Cooke, who a full-time senior animal health technician with the State Veterinary Epidemiology unit in Pietermaritzburg, KZN, has had an interest in TB for all her professional career. She has been assisting the Hluhluwe-Imfolozi Buffalo TB control program since it commenced in 1997 and became introduced to the Animal TB Research group during our research as part of this program. Debbie formally joined the group in January 2020 to pursue her PhD.

Despite restrictions associated with the pandemic restrictions, Debbie was able to organize with provincial animal health staff to administer skin tests and sample more than 500 goats in KZN during November. Debbie shared some photos of the challenges and fun associated with her field work.

Above: Debbie performing intradermal tuberculin test on goats.
Below: left – goats being brought for testing; right – volunteer helpers from the community.

Importantly, community involvement was a key component of this project and local animal health technicians solicited participation and gained consent from owners for the voluntary testing. Free antiparasitic treatment for goats was provided as an incentive.

INVESTIGATING THE EPIDEMIOLOGY OF TUBERCULOSIS IN BLACK AND WHITE RHINOCEROS

Since joining the Animal TB group at the start of 2020, Rebecca Dwyer (MSc student) has focused on characterising the epidemiology of tuberculosis in rhinoceros in the Kruger National Park, in order to assess the risk posed by TB to these populations in the Kruger National Park. This year a review was published in Frontiers in Veterinary Science, which described current knowledge and information gaps regarding the epidemiology of TB in African rhinoceros (https://doi.org/10.3389/fvets.2020.580476). This is a collaborative study between the Animal TB Research group and San Diego Zoo Global collaborators, Dr. Carmel Witte and Mr. Simon Kedward (a GIS specialist); this study will use univariable and multivariable analyses to identify risk factors associated with having a positive TB test, using data collected from rhinoceros in Kruger National Park from 2016-present. The goal of this research is to provide information that can be used to assess the risk of infection in the population and improve strategies for testing and managing animals for translocation.

Photo credit Dr. Peter Buss

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 that will be graduating in 2020/2021.

Katrin Smith – MSc, December 2020

Josephine Chileshe - PhD, March 2021

Candice de Waal – MSc, March 2021

Samantha Goldswain – MSc, March 2021

Pamela Ncube – MSc, March 2021

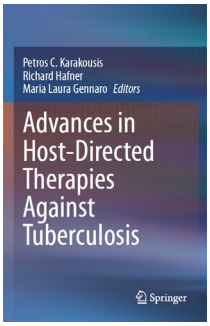
Continuing Students

Rebecca Dwyer and Rachiel Gumbo will be continuing their MSc studies with the group in 2021.

Tina Meiring, Charlene Clarke, and Debbie Cooke will be further working on their PhD projects. Pam Ncube will be starting her PhD in 2021.

Leadership

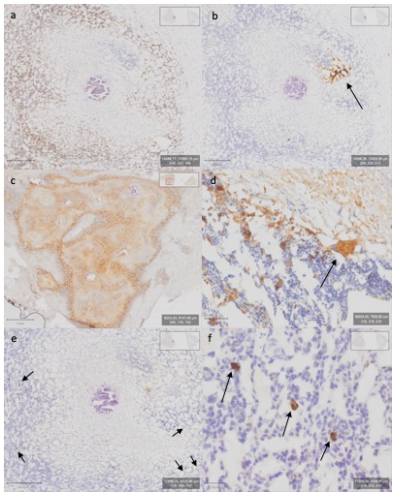
Drs. Wynand Goosen and Tanya Kerr will be providing supervision, administrative support, research projects, and all manner of leadership to the Animal TB Research Group as our post-doctoral fellows and members of the senior team.



Dr. Leanie Kleynhans continues to supervise and lead the daily operations of the Animal TB Research Group in Cape Town. We congratulate her on the recent publication of a book chapter on "Targeting Suppressor T Cells.

CHARACTERISATION OF LESIONS AND ASSOCIATED IMMUNE CELL POPULATIONS IN THE LUNG OF AFRICAN BUFFALO (SYNCERUS CAFFER) INFECTED WITH MYCOBACTERIUM BOVIS

Understanding the development of disease associated with *M. bovis* infection provides important insights as to the host's immune responses and provides information on the interpreting diagnostic test results. Samantha Goldswain (MSc student) has focused on characterisation of the microscopic changes and associated immune cell populations in lung granulomas from *M. bovis* infected African buffalo for her project. She developed a scoring system to compare numbers and distributions of different immune cells, as well as other pathological changes in different lesions. In addition, an immunohistochemistry (IHC) staining technique was optimized for immune cell surface marker detection in buffalo lung tissues. The findings showed that lymphocytes and macrophage lineage cells appeared to be the predominant immune cell types present although their distribution and relative numbers appeared to change as pulmonary granulomas develop. As expected, many characteristics of buffalo lesions were similar to granulomas in cattle. However, mineralisation may not be a consistent feature, suggesting some species-specific differences that should be further investigated. This study also demonstrated that immunohistochemistry is a practical method for further characterisation of the local immune responses to bTB in buffalo. Sam has submitted her thesis and will be graduating in March 2021.



Photomicrographs of the different antibody staining patterns observed in buffalo lung tissue (a) CD3+ (T) cells; (b) CD21+ (B) cells; (c) CD163+ (macrophage lineage) cells; (d) example of an individual CD163+ cell (e) NCR1+ (NK) cells; (f) example of individual NCR1+ cell.

Collaborations with Industry

Research funding to purchase state-of-the-art equipment and reagents is often difficult to acquire, especially for wildlife. The Animal TB Research group has been fortunate to develop a number of partnerships with industry to provide in-kind donations or lease of equipment that facilitates research projects, such as the evaluation of Cepheid's GeneXpert platform for animal samples. Opportunities to get first-hand knowledge of the challenges experienced in wildlife research by collaborators often provide a common ground to discuss strategies to overcome these obstacles. We are grateful to all our partners, especially Cepheid, Chembio Diagnostics, TiKa, MabTech, Qiagen, Thermofischer Scientific, Longhorn Vaccines and Diagnostics, and others.

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

Publications by Animal TB Research Group 2020

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