



2018 ANIMAL TB RESEARCH GROUP

Stellenbosch University Animal TB Research Group

IN THIS ISSUE

Welcome to the 2018 Animal TB Research Group Newsletter!

Another year has flown by with lots of changes and accomplishments by the Animal TB research team! Dr. Sven Parsons has become the Director of the Animal Unit at the Tygerberg campus but continues to mentor students and provide input to the group. Dr. Leanie Kleynhans-Cornelissen, a senior scientist with the Immunology Research Group (IRG), has joined to help lead our research in new directions.

We continue to develop new lines of research, share our work with stakeholders and the public through community engagement, publications, and presentations, and build our collaborations locally, nationally and internationally.

We are excited to share some of this news!

Meet the Team

The Animal TB Research group has continued to grow, with 12 members in 2018!

Pictured above, top row (left to right): Eduard Roos (PhD student), Wynand Goosen (post-doctoral fellow), Tanya Kerr (post-doctoral fellow), Sven Parsons (supervisor), Netanya Bernitz (PhD student). Bottom row (left to right): Sedzani Ndou (Honours student), Candice De Waal (MSc student), Leanie Kleynhans-Cornelissen (supervisor), Josephine Chileshe (PhD student), Michele Miller (Chair), Tina Meiring (MSc student), Leere Scott (Honours student).



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Hluhluwe iMfolozi Park Bovine Tuberculosis Testing Programme 2018

The Animal TB group has been involved in the annual buffalo TB testing programme held in Hluhluwe iMfolozi Park for the past seven years. This year we once again enjoyed a successful field trip with 233 buffaloes tested in total. The prevalence of TB was much higher than previous years, demonstrating the need to continue with this vital programme. The highlights of the trip included three full weeks in the abattoir where we got to see the extent of TB disease in these buffaloes.

Back at Stellenbosch University, we have cultured *M. bovis* from 60% of the test-positive buffaloes.



Animal TB group in HiP 2018 (left to right) Michele Miller, Netanya Bernitz, Tanya Kerr, and Candice De Waal.

We continue to work on developing new diagnostic algorithms which will improve control of bTB in buffaloes. We also worked on a scoring system for reporting bTB lesions with photographs taken to help with future training. Lastly, we collected formalin samples of lesions and lymph nodes for an exciting histology project to start in 2019.

The HiP buffalo testing programme is the highlight of the Animal TB Group, every year, and we owe our thanks to Dr. Dave Cooper, JP van Heerden and their teams!

Update on *Mycobacterium bovis* Infection in Rhinoceros



Animal TB team work with SANParks Veterinary Wildlife Services staff to perform diagnostic testing on rhinoceros. An endoscope is used to collect respiratory samples.

A recent publication in the journal "Emerging Infectious Diseases" describes the diagnostic findings of 6 cases of *M. bovis* infection in white rhinoceros. Infection appeared to be limited and was not the cause of death. However, these cases have resulted in quarantine of Kruger National Park.

Based on the research of the Animal TB group and researchers from the University of Pretoria, SANParks developed a TB management plan that has been accepted by DAFF.

Under this plan, rhinoceros may be moved if they pass quarantine in a biosecure facility with three negative results over a three month period, using the blood-based interferon-gamma release assay.

This demonstrates the contribution that wildlife TB research can have on policy development.

RHINOCEROS INTERFERON-GAMMA ASSAY

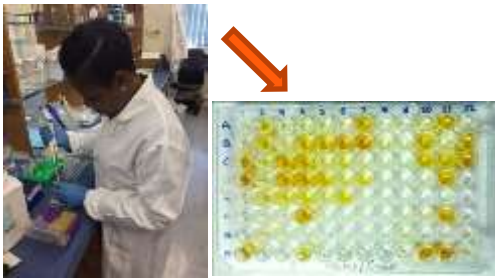
Blood stimulation

Rhinoceros heparinized blood is added to commercial QFT tubes and incubated overnight then centrifuged to separate plasma. Could be done in most clinics and frozen for shipment to lab.



Interferon-gamma ELISA

Commercial equine IFN-gamma ELISA has been shown to detect rhinoceros IFN-gamma and an elevated level in plasma (from QFT stimulation) indicates immune sensitization. (Research performed by Josephine Chileshe, PhD student, shown below.)



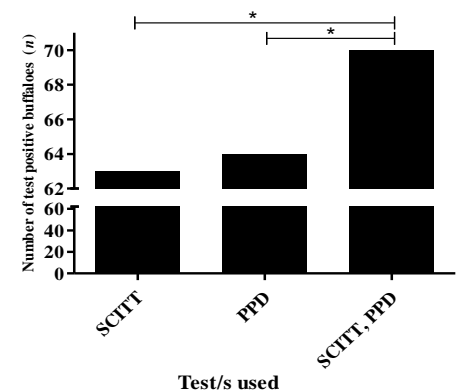
Molecular Epidemiology of *M. bovis* Isolates from Warthogs

There is a great diversity in *M. bovis* isolates from warthogs using spoligotyping, a traditional typing method. This was further supported by newer whole genome sequencing (WGS). The WGS with its greater resolution indicated that there is some diversity between the isolates from Kruger National Park (KNP) and Marloth Park (MP), which is just outside the border of KNP. This highlights that care should be taken when reporting transmission events using traditional typing methods such as spoligotyping and region of difference analysis, especially when trying to identify the source of infection. Findings from these studies will improve understanding of the spread of bovine TB in South African wildlife.



Parallel Testing Increases Detection of *Mycobacterium bovis*-Infected African Buffaloes (*Syncerus caffer*)

The diagnosis of *M. bovis* infection in African buffaloes relies on detection of the cell-mediated immune response to *M. bovis* antigens typically using the single comparative tuberculin test (SCITT) or interferon gamma release assays (IGRAs). The aim of a recent study was to determine whether parallel testing with the SCITT and an IGRA increases the number of *M. bovis*-infected buffaloes detected by these assays. The standard Bovigam® IGRA consistently demonstrated that in parallel with the SCITT, the greatest number of *M. bovis* infected buffaloes were identified. Parallel testing, using these two PPD based assays, has previously been advocated in cattle herds (Vordermeier et al., 2006; Gormley et al., 2006) and should be strategically applied when the goal of testing is to remove as many infected animals as possible. However, when testing herds with no-known history of *M. bovis* or extremely valuable animals, a different strategy focusing on increased specificity should be considered to minimize the risk of false-positive results.



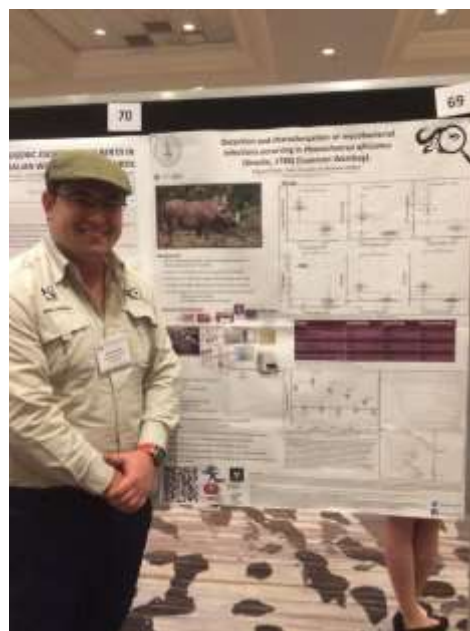
Seventy-one *M. bovis* culture-confirmed buffaloes were tested using the single comparative tuberculin skin test (SCITT) and standard Bovigam® IGRA (PPD), singly and in parallel. * p<0.05

FOCUS ON EDUARD ROOS, PHD

Eduard joined the Animal TB Research group in 2015 as a MSc student, upgraded to a PhD, and is graduating in December 2018. His research has characterized *M. bovis* infection in warthogs and resulted in 4 publications to date. He has developed serological assays to provide a rapid screening method for infection which has already been used in several private game reserves to detect bovine TB on the property. He is currently investigating the molecular epidemiology of *M. bovis* in this species.

Eduard will be starting his post-doctoral fellowship at Pirbright Institute in the UK in January 2019, and we wish him success!

Eduard Roos presenting at the international Wildlife Disease Association conference 2018 in Florida, USA.



RECOGNITION

Congratulations to Dr. Dave Cooper, a friend, teacher and collaborator of the Animal TB Group, who was awarded the Umvikeli Wildlife Protector Award in New York in November this year. Dr. Cooper received this award for his lifetime of dedication to the care, management and conservation of African rhino and wildlife. The Animal TB group thanks you for being an amazing role model!



NEW PROJECT 2018... the year of the antelope

In popular culture, antelope species often get overlooked in favour of the more “charismatic” wildlife species. This is all about to change with post-doctoral fellow Dr. Tanya Kerr turning her attention to these to bovine tuberculosis in these species. Tanya will be evaluating the QuantiFERON®-TB Gold Plus (QFT) tubes and the Qiagen cattletype® IFN-gamma ELISA as a TB diagnostic test for antelope. She has already successfully screened samples from bushbuck, kudu, nyala, roan antelope, sable antelope, waterbuck, and Impala. This work will continue in 2019 in order to advance our understanding of bTB prevalence and epidemiology in antelope, with aim of developing blood-based tests for use in bTB surveillance and control programs.

Preliminary Results - Bushbuck



All - Negative Control <small>(Blank to background 0%)</small>	OD (450-630): 0.051
TB - Optimized for detection of C24 and C29T self response	OD (450-630): 2.971
Antigen - Positive Control <small>(comparable to other wildlife species 0%)</small>	OD (450-630): 1.374
10µg/ml PWM	OD (450-630): 2.251



Positive
SP% ≥ 35

Bushbuck 15/299
SP% = 349.2823

Culture Confirmed
Mycobacterium bovis

NEW PROJECT

Investigating genome variation in a South African wild dog population: Towards understanding their susceptibility to *Mycobacterium bovis* infection

African wild dogs (*Lycaon pictus*) are key role-players in the ecosystem and biodiversity in Africa, however, they are now considered critically endangered. Recently, fatal cases of bovine tuberculosis have been documented in this species. This apparent susceptibility to *M. bovis* infection could be due to the lack of genetic diversity, therefore we aim to investigate the genome variation in a wild dog population from the Kruger National Park to understand their susceptibility or resistance to diseases and other threats; and to develop tools to identify genetic factors conferring adaptive advantages. Genomic information generated from this study will aid in the identification of conservation units for recovery, management and protection of wild dog populations.

Tina Meiring is a MSc student that joined the Animal TB group in 2018. She completed a BSc (hons) in Genetics at Stellenbosch University in 2017. Her research will investigate the genetic diversity, relatedness, population structure and to identify patterns of selection in certain genomic regions of wild dogs using whole genome sequencing.



Tina is indicated by the left arrow and Tanya by right arrow, along with other Animal TB team members with a hyena during a visits to KNP in May 2018.

NEW PROJECTS

Elephant TB Diagnostic Test Development

Candice De Waal started her MSc project, "Characterization of Biomarkers of Immunological Activation in African Elephants (*Loxodonta africana*)", in 2018, after completing her Honours with the Animal TB Research Group in 2017.

She has been screening cytokine and reference genes to identify potential biomarkers for measuring immune activation blood from African elephants. Her initial findings will form the basis for the development of an antigen-specific cytokine gene expression assay for the detection of tuberculosis in African elephants. As part of the project she also had the opportunity to collect samples from African elephants in Kruger National Park and the National Zoological Gardens, Pretoria.

ELEPHANT SAMPLE COLLECTION IN KNP



Candice (right) is pictured with Leana Rossouw, a veterinary technician with SANParks Veterinary Wildlife Services, observing the collection of blood samples from an immobilized elephant bull in Kruger National Park in 2018.

NEW PROJECT

Direct Detection of Mycobacteria in Clinical Samples from Rhinoceros & Elephants



Dr. Goosen lifts the trunk of immobilized elephant to collect fluid for detection of mycobacteria.

Dr. Wynand Goosen, a post-doctoral fellow, has a long history with the Animal TB group. He started with a Honours degree, then continued onto a MSc which was upgraded to a PhD, which he completed in 2017. After a post-doctoral year at the University of Cape Town's Lung Infection and Immunity Unit, he returned to the Animal TB group. Wynand has initiated various projects and collaborations with companies with the focus on the *ante mortem* diagnosis of tuberculosis in African elephants and rhinoceros using unconventional, difficult to culture samples such as trunk wash and bronchial alveolar lavage (BAL) samples. Collaborations with Cepheid and Thermofisher Scientific led to the evaluation of a human GeneXpert MTB/RIF Ultra qPCR assay and the VetMAX™ *M. tuberculosis* Complex test, respectively. He has been able to use the GeneXpert MTB/RIF Ultra assay to detect TB bacilli in elephant respiratory fluid at concentrations as low as 2 CFU/ml. He is also collaborating with a new UK based company called TiKa-Diagnostics to increase the speed and sensitivity of culture of *M. bovis* clinical samples. This innovative research will improve accuracy and speed of diagnosis to inform decisions on animal tuberculosis management in South Africa.

Outreach 2018



Animal TB Research team members provided various interactive components to promote TB awareness in school children at the "TB under the spotlight" Winelands public engagement event.

Outreach to stakeholders and the public are a crucial part of sharing research findings to promote science awareness and communication. The Animal TB research group regularly shares "in progress" research results with their collaborators to elicit input and strengthen relationships through transparency.

During 2018, members of the Animal TB research team increased TB awareness by participating in public events such as the "TB under the microscope" art event, "TB under the spotlight" Winelands public engagement project for school groups sponsored through the Western Cape Department of Education, radio interviews for a SABC series on TB research at Stellenbosch University, as well as through several press releases.

Eight members of the Animal TB group visited Kruger in May 2018 to take part in various project field work and conduct an information session for the SANParks Veterinary Wildlife Services staff and local provincial animal health regulatory staff. This was an opportunity for the research team to receive feedback on research priorities and challenges in managing TB in local livestock and wildlife.

Prof. Miller participated in a TB information session for the South African Wildlife Ranchers Association to discuss the current status of diagnostic tests for wildlife, detection and management. Over the last few years, the private wildlife industry has become active partners in the group's research, which is crucial for obtaining needed samples.





Animal TB Research Honours Students 2018

Congratulations to our Honours students, Sedzani Ndou (left) and Leere Scott (right) who will be graduating in December! Both students have received distinctions for their work during the year.

Sedzani's project was entitled investigation of RNA stabilization techniques in wildlife and domestic animal species. She determined the optimal methods of preserving and extracting RNA from blood samples from cattle, elephants and warthogs, which is important for performing gene expression assays.

Leere characterised cytokine gene expression of CXCL10 and production of IP-10 in cattle in order to investigate possible pathways to explain spontaneous release of this protein in whole blood incubated for diagnostic assays. This will aid our understanding of the immunobiology of cattle.

Students

Focus on Netanya Bernitz



Netanya Bernitz joined the Animal TB group in 2016 as a PhD student. Her PhD project is focused on characterization of the diagnostic immunology of *Mycobacterium bovis* infection in African buffaloes and cattle. She has written two manuscripts proposing improved testing algorithms for TB detection in buffaloes. She is finishing her labwork this year and will be moving to the UK to finish her thesis in 2019. We wish Netanya a smooth transition!



Focus on Josephine Chileshe

Josephine Chileshe was trained as an animal health technician and completed her MSc before joining the Animal TB group in 2017. As a Zambian, she serves as the international representative on the post-graduate student council. The aim of her PhD research is to identify immunological biomarkers that can be used for development of diagnostic tests for detection of *M. bovis* infection in African rhinoceros.

WELCOME NEW STUDENTS

The Animal TB Research group will have 3 new MSc students joining in 2019.

Pamela Ncube is from Zimbabwe and will be shared with the Mycobacteriomics group. She will be developing an in vitro model to determine if *M. bovis* isolates from wildlife can become "persisters", which is involved in latency.

Katrin Smith is completing her BSc Hons in Microbiology at Stellenbosch University. Her MSc research will continue our focus on buffalo TB diagnostics by identifying novel biomarkers that can help distinguish infection from disease.

Samathan Goldswain is currently finishing her BSc Hons in Genetics at the University of Pretoria. For her MSc, she will be characterizing the immune responses to *M. bovis* infection in buffalo tissues using immunohistochemistry. These include samples collected during our annual testing program in Hluhluwe-iMfolozi Park.

Candice De Waal and Tina Meiring will be entering their second year of their MSc studies with the group.

It takes a team to make the dream!



Publications by Animal TB Research Group 2018

1. **Bernitz, N., C. Clarke, E.O. Roos, W.J. Goosen, D. Cooper, P.D. van Helden, S.D.C. Parsons, and M.A. Miller.** 2018. Detection of *Mycobacterium bovis* infection in African buffaloes (*Syncerus caffer*) using QuantiFERON-TB Gold (QFT) tubes and the Qiagen cattletype IFN-gamma ELISA. *Vet. Immun. Immunopath.* 196:48-52.
2. Lyashchenko, K.P., C. Gortazar, **M.A. Miller**, and W.R. Waters. 2018. Spectrum of antibody profiles in tuberculous elephants, cervids and cattle. *Vet. Microbiol.* 214:89-92.
3. **Roos, E.O.,** F. Olea-Popelka, P. Buss, L.-M. de Klerk-Lorist, D. Cooper, **P.D. van Helden, S.D.C. Parsons, and M.A. Miller.** 2018. Seroprevalence of *Mycobacterium bovis* infection in warthogs (*Phacochoerus africanus*) in bovine tuberculosis-endemic regions of South Africa. *Transb. Emerg. Dis.* 65:1182-1189. DOI:10.1111/tbed.12856
4. **Roos, E.O.,** F. Olea-Popelka, P. Buss, L.-M. de Klerk-Lorist, D. Cooper, R.M. Warren, **P.D. van Helden, S.D.C. Parsons, and M.A. Miller.** 2018. IP-10: A potential biomarker for detection of *Mycobacterium bovis* infection in warthogs (*Phacochoerus africanus*). *Vet. Immun. Immunopath.* 201:43-48. doi: 10.1016/j.vetimm.2018.05.007
5. **Clarke, C.,** D. Cooper, **W.J. Goosen, R. McFadyen,** R.M. Warren, **P.D. van Helden, S.D.C. Parsons, and M.A. Miller.** 2018. Antigen-specific interferon-gamma release is decreased following the single intradermal comparative cervical skin test in African buffaloes (*Syncerus caffer*). *Vet. Immun. Immunopath.* 201:12-15. doi: 10.1016/j.vetimm.2018.05.002
6. Hooijberg, E.H., **M. Miller,** C. Cray, P. Buss, G. Steenkamp, and A. Goddard. 2018. Serum protein electrophoresis in healthy and injured southern white rhinoceros (*Ceratotherium simum simum*). *PLoS ONE* 13(7): e0200347. <https://doi.org/10.1371/journal.pone.0200347>
7. **Miller, M.A.,** M. Finnegan, T. Storms, M. Garner, and K.P. Lyashchenko. 2018. Outbreak of *Mycobacterium tuberculosis* in a herd of captive Asian elephants (*Elephas maximus*): antemortem diagnosis, treatment and lessons learned. *J. Zoo Wildl. Med.* 49(3):748-754. <https://doi.org/10.1638/2017-0200.1>
8. Meyer, L., A. Fuller, M. Hofmeyr, P. Buss, **M. Miller,** and A. Haw. 2018. Use of butorphanol and diprenorphine to counter respiratory impairment in immobilized white rhinoceros (*Ceratotherium simum*). *J. S. Afr. Vet. Assoc.* 89(0): a1683. <https://doi.org/10.4102/jsava.v89i0.1683>
9. **Roos, E.O,** F. Olea-Popelka, P. Buss, **G.A. Hausler,** R. Warren, **P.D. van Helden, S.D.C. Parsons,** L.-M. deKlerk-Lorist, and **M.A. Miller.** 2018. Measuring antigen-specific responses in *Mycobacterium bovis*-infected warthogs (*Phacochoerus africanus*) using the intradermal tuberculin test. *BMC Vet. Res.* 14:360. <https://doi.org/10.1186/s12917-018-1685-8>.
10. **Miller, M.A.,** P. Buss, **S.D.C. Parsons, E. Roos, J. Chileshe, W.J. Goosen,** L. van Schalkwyk, L.-M. de Klerk-Lorist, M. Hofmeyr, **G. Hausler,** L. Rossouw, T. Manamela, E.P. Mitchell, R. Warren, and **P. van Helden.** 2018. Conservation of white rhinoceros threatened by bovine tuberculosis, South Africa, 2016-2017. *Emerg. Inf. Dis.* 24(12):2373-2375. <https://doi.org/10.3201/eid2412.180293>.
11. Buss, P. and **M. Miller.** Update on field anesthesia protocols for free-ranging African lions. 2018. In Miller, R.E., N. Lamberski, and P.P. Calle (eds.), *Zoo and Wild Animal Medicine, Current Therapy 9th ed.*, Elsevier, St. Louis, Missouri. Pp. 536-538.
12. **Meiring, C., P.D. van Helden, and W.J. Goosen.** 2018. TB control in humans and animals in South Africa: A perspective on problems and successes. *Front. Vet. Sci.* 5:298. Doi: 10.3389/fvets.2018.00298
13. **Sylvester, T.T., Parsons, S.D.C, van Helden, P.D., Miller, M.A.,** Loxton, A.G. 2018. A pilot study evaluating the utility of commercially available antibodies for flow cytometric analysis of *Panthera* species lymphocytes. *BMC Vet Research.* doi.org/10.1186/s12917-018-1717-4
14. Brites, D., C. Loiseau, F. Menardo, S. Borrell, M.B. Boniotti, R. Warren, A. Dippenaar, **S.D.C. Parsons,** C. Beiseln M.A. Behr, and J.A. Fyfe. 2018. A new phylogenetic framework for the animal-adapted *Mycobacterium tuberculosis* complex. *bioRxiv*, p.384263.
15. **Bernitz, N., W.J. Goosen, C. Clarke, T.J. Kerr, R. Higgitt, E.O. Roos,** D.V. Cooper, R.M. Warren, **P.D. van Helden, S.D.C. Parsons, and M.A. Miller.** 2018. Parallel testing increases detection of *Mycobacterium bovis*-infected African buffaloes (*Syncerus caffer*). *Vet. Immun. Immunopath.* In press.
16. **Miller, M.A.,** P. Buss, **T.T. Sylvester,** K.P. Lyashchenko, L.-M. deKlerk-Lorist, R. Bengis, M. Hofmeyr, J. Hofmeyr, N. Mathebula, **G. Hausler, P. van Helden,** E. Stout, **S.D.C. Parsons,** and F. Olea-Popelka. 2018. *Mycobacterium bovis* in free-ranging lions (*Panthera leo*) – Evaluation of serological and tuberculin skin tests for detection of infection and disease. *J. Zoo Wildl. Med.* In press.
17. Rosen, L.E., F. Olea-Popelka, S.L. Deem, R. Isaza, D. Schmitt, and **M. Miller.** 2018. Survey of anti-tuberculosis drug administration and adverse effects in elephants in North America. *J. Zoo Wildl. Med.* In press.
18. Viljoen, I., **T. Sylvester, S. Parsons,** R. Millar, **P. van Helden, and M. Miller.** 2018. Performance of the tuberculin skin tests in *Mycobacterium bovis*-exposed and unexposed Africa lions (*Panthera leo*). *J. Wildl. Dis.* In press.
19. **Higgitt, R.L.,** O.L. van Schalkwyk, L.-M. deKlerk-Lorist, P.E. Buss, P. Caldwell, L. Rossouw, T. Manamela, **G.A. Hausler, P.D. van Helden, S.D.C. Parsons, and M.A. Miller.** 2018. An interferon gamma release assay for the detection of immune sensitization to *Mycobacterium bovis* in African wild dogs (*Lycaon pictus*). *J. Wild. Dis.* In press.

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Thank you to all our collaborators and supporting partners!



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