

Faculty Day

23 August 2018

Research Overview



Faculty of Veterinary Science

Standial 31th

Fakulteit Veeartsenykunde Lefapha la Diseanse tša Bongakadiruiwa

Brief history of Faculty Day

Faculty Day of the amalgamated Faculty of Veterinary Science reflects a proud tradition, which had been nurtured by the original faculties of Veterinary Science of both Medunsa and the University of Pretoria, of showcasing the research activities of staff and students on a special, dedicated occasion.

Since the inception of the Faculty of Veterinary Science at Medunsa in the early 1980s, the staff, and later students, were involved in the activities of the "Academic Day", which was aimed at highlighting the research activities of the University, as well as exposing young researchers to a conference environment.

The Faculty of Veterinary Science of the University of Pretoria at Onderstepoort followed this trend shortly thereafter and the first "Faculty Day", which focused on the research activities of the Faculty, was held on 5 September 1984, sponsored by the then Dean, Prof JMW le Roux. The combined research skills of the two original institutions are today reflected in the proceedings of the Faculty Day held each year at the Onderstepoort Campus.

Sponsorships

The Faculty of Veterinary Science wishes to express its sincere thanks to the following sponsors for their very generous contribution in support of the 2018 Faculty Day.



Faculty Day

Faculty of Veterinary Science University of Pretoria

23 August 2018



Contents/Programme

08:00 – 08:25 Registration and tea (Arnold Theiler Building)

Master of Ceremonies: Dr Variadzo Mukorera

08:30 - 08:45 Welcoming address: Prof Vinny Naidoo, Acting Dean of the Faculty of Veterinary Science

08:45 – 10:00 First Session Chairperson: Dr Emma Hooijberg

- 1. Topography and splanchnology of the lungs in the African lion (*Panthera leo*) <u>Marais C.A.</u>, Crole M.R.
- 2. Comparison of thoracic radiological changes with computed tomography in dogs suffering from blunt trauma *Dancer S.C., Le Roux C., Kirberger R.M.*
- 3. Ovarian function and anti-Müllerian hormone following immunocontraceptive vaccination of mares using native and recombinant zona pellucida vaccines with non-Freund's adjuvants *Nolan M.B., Bertschinger H.J., Crampton M., Schulman M.L.*
- 4. Investigating the anti-adherence mechanism of bioactive South African Myrtaceae plants against diarrhoeagenic enterotoxigenic *E. coli* (ETEC) limiting pig production using a Caco-2 cell model *Famuyide I.M., Aro A.O., Abubakar R.H., Fasina F.O., Eloff J.N., McGaw L.J.*
- 5. Isolation of *Brucella melitensis* biovars 2 and 3 from slaughter cattle in South Africa *Kolo F.B., Adesiyun A.A., Katsande T.C., Fasina F.O., Van Heerden H.*
- 10:00 10:45 Tea (Cafeteria)

10:45 – 12:00 Second Session Chairperson: Prof Vinny Naidoo

Sir Arnold Theiler Memorial Lecture: Prof Yoshan Moodley

12:00 – 12:30 Third Session (Cafeteria)

Poster session

12:30 – 13:00 Lunch (Cafeteria)



13:00 - 14:15 Fourth Session Chairperson: Prof Gerhard Steenkamp

- 1. *Mycobacterium bovis* infection in cattle and communal resource-constrained farmers living at the wildlife-livestock-human interface in South Africa <u>Sichewo P.R.</u>, Etter E., Michel A.L.
- 2. The acute phase response in healthy and injured southern white rhinoceros (*Ceratotherium simum*) <u>Hooijberg E.H.</u>, Cray C., Steenkamp G., Buss P., Goddard A., Miller M.
- 3. Validation of an indirect immunoperoxidase test for rabies virus in domestic and wildlife species in South Africa *Janse van Rensburg D.D., Sabeta C.T., Fosgate G.T., Clift S.J.*
- 4. The effects of elevated concentrations of KCl in the feed of laying hens *Cornelius S.T., Naidoo V.*
- 5. A novel approach for antigenic vaccine matching of foot-and-mouth disease (FMD) viruses <u>Sirdar M.M.</u>, Fosgate G.T., Gummow B., Shileyi B., Mutowembwa B., Lazarus D.D., Blignaut B., Heath L.

14:15 - 15:00 Interesting research development showcase from Companion Animal Clinical Studies: Prof Amelia Goddard

15:00 – 15:15 Faculty Day Awards

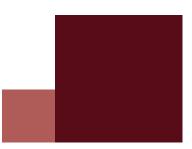
Researcher of the year

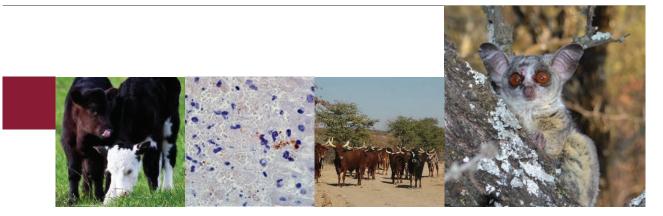
Young researcher of the year

Best oral presentation

Best poster

15:15 Cocktail (Cafeteria)





POSTER PRESENTATIONS

- 1. A flow cytometric assessment of selected splenic cell populations of dogs that died of natural *Babesia rossi* infections *Celliers A., Pazzi P., Rautenbach Y., Leisewitz A.L.*
- 2. Neutrophil myeloperoxidase index in dogs with babesiosis <u>Celliers A.</u>, Hooijberg E.H., Christopher M.M., Rautenbach Y., Goddard A.
- 3. Leptospirosis in slaughter livestock in Gauteng province, South Africa: Isolation, serological and molecular studies <u>Dogonyaro B.B.</u>, Van Heerden H., Wunder Jr E.A., Ko A.I., Casanovas-Massana A., Potts A.D., Lötter C., Katsande C., Fasina F.O., Kolo F.B., Adesiyun A.A.
- 4. Isolated flavones from *Loxostylis alata* have anti-influenza potential <u>Gado D.A.</u>, Mehrbod P., Abdalla M.A., Ahmed A.S., Ehlers M., McGaw L.J.
- 5. *In vitro* cytotoxicity induced by different cardiac glycosides <u>Henn D.</u>, Venter A., Botha C.J.
- 6. The chronicles of the captive African penguin: Bravery or desensitization towards natural and environmental stressors? *Holdstock C., Scheun J., Ganswindt A.*
- 7. Molecular characterisation of vaccine candidates from *Anaplasma marginale* strains in South Africa *Hove P., Brayton K.A., Oosthiuzen M.C., Mtshali M.S., Collins N.E.*
- 8. Epoxyscillirosidine induced cytotoxicity and ultrastructural changes in a rat embryonic cardiomyocyte (H9c2) cell line Isa H.I., Ferreira G.C.H., Crafford J.E., Botha C.J.
- 9. Raising antibodies against epoxyscillirosidine, the toxic principle contained in *Moraea pallida* Bak. (*Iridaceae*), in rabbits *Isa H.I., Ferreira G.C.H., Crafford J.E., Botha C.J.*
- 10. Tick-borne haemoparasite occurrence in eastern rock sengi (*Elephantulus myurus*) in South Africa <u>Jacobs R.</u>, Bastos A., Troskie M., Medger K., Oosthuizen M.
- 11. Soluble expression of *Bacillus anthracis* protective antigen in *Escherichia coli* and characterisation of its biological activity *Jauro S.*, *Ndumnego O.C.*, *Beyer W.*, *Van Heerden H.*
- 12. Plants used in ethnoveterinary medicine to treat prevalent livestock diseases in the Mnisi community, Mpumalanga province, South Africa
 - Khunoana E.T., Arnot L.F., McGaw L.J.
- 13. Bacterial blood microbiome of rodents captured from a human/livestock/wildlife interface in Bushbuckridge, South Africa <u>Kolo A.O.</u>, Gall C.A., Wentzel J.M., Kolo F.B., Van Heerden H., Collins N.E., Brayton K.A., Oosthuizen M.C.
- 14. *In silico* screening of the *Theileria parva* proteome for identification proteins responsible for transformation of infected host lymphocytes
 - <u>Komani N.</u>, Liebenberg J., Sibeko-Matjila K.
- 15. Spatial analysis of anthrax outbreaks and cases in Lesotho between 2005–2016 *Lepheana R.J., Oguttu J.W., Qekwana N.D.*
- 16. Prevalence of cysticercosis in cattle and pigs slaughtered in Gauteng abattoirs <u>Mabogoane N.F.</u>, Tsotetsi-Khambule A.M., Adesiyun A.A.
- 17 Tick-borne disease dynamics in calves at the wildlife-livestock interface in the Mnisi community area, Mpumalanga province, South Africa
 - Makgabo S.M., Biggs L., Brayton K.A., Oosthuizen M.C., Collins N.E.
- 18. Preliminary screening of selected South African medicinal plants for the control of root-knot nematodes, *Meloidogyne incognita* <u>Makhubu F.N.</u>, McGaw L.J., Khosa M.C.
- 19. Screening of host proteins that interact with Rift Valley fever virus glycoproteins using a yeast two hybrid system <u>Maluleke M.R.</u>, Venter E.H., Mans B.
- 20. Prevalence and characterization of *Salmonella* spp. in beef abattoirs, beef and beef products in Gauteng <u>Mangele A.</u>, Adesiyun A.A., Madoroba E., Thompson P.N.
- Prevalence of Q-fever in South Africa: A review of diagnostic laboratory data at the Agricultural Research Council Onderstepoort Veterinary Research from 2007–2009 <u>Mangena M.L.</u>, Gcebe N., Thompson P.N., Adesiyun A.A.



- 22. Species composition and the role of horse-flies in pathogen transmission in south-eastern KNP (Diptera: Tabanidae) <u>Mazibuko X.</u>, Snyman L., Smit A., Lempereur L., Neves L.
- 23. Comparison of decontamination methods for the primary isolation of *Mycobacterium* spp. from milk <u>Mazwi D.</u>, Michel A.L.
- 24. Prevalence and characterisation of *Salmonella* species from chickens sold in informal poultry markets in Gauteng, South Africa *Mokgophi M.T., Gcebe N., Thompson P.N., Adesiyun A.A.*
- 25. Prevalence and antimicrobial susceptibility of coagulase positive *Staphylococcus* species from milk samples submitted to the Onderstepoort Faculty of Veterinary Science <u>Mphahlele M.P.</u>, Petzer I.M., Oguttu J.W., Qekwana D.N.
- 26. Evaluation of the effectiveness of pulse oximetry at different attachment sites to detect hypoxaemia in immobilized impala (*Aepyceros melampus*)
 - <u>Mtetwa T.K.</u>, Zeiler G., Laubscher L., Pfitzer S., Meyer L.C.R.
- 27. Diversity of the sporozoite antigen gene p67 in *Theileria parva* field isolates from cattle and buffalo in southern and eastern Africa
 - Mukolwe L.D., Odongo D.O., Sibeko K.P.
- 28. Antimicrobial use practices and resistance in indicator bacteria in communal cattle in the Mnisi community, Mpumalanga *Mupfunya C., Naidoo V., Qekwana N.*
- 29. Pathology in dogs that died following natural infection by African horse sickness virus <u>O'Dell N.</u>, Williams J.H., Clift S.J., Steyl J.C.A.
- Pathology and tissue tropism of natural Rift Valley fever virus infection in sheep <u>Odendaal L.</u>, Clift S.J., Fosgate G.T., Davis A.S.
- 31. The *in vitro* antibacterial activity of *Ficus exasperata* leaf extracts against *Campylobacter* and *Escherichia coli* relevant in poultry infections
 - <u>Olawuwo O.S.</u>, Aro A.O., Omokhua A.G., Eloff J.N., McGaw L.J.
- 32. Antimycobacterial activity and toxicity screening of two southern Africa alien invasive plants <u>Omokhua A.G.</u>, Madikizela B., Van Staden J., McGaw L.J.
- 33. Biological activities of selected South African plants used traditionally to treat inflammation and helminth infection <u>Ondua M.</u>, Njoya E.M., Abdalla M.A., McGaw L.J.
- 34. Histology of the female reproductive tract of the cheetah (*Acinonyx jubatus*) <u>Penfold M.J.</u>, Soley J., Hartman M.J.
- 35. Prevalence of *Campylobacter* species from chickens sold in informal poultry markets in Gauteng, South Africa <u>Phosa M.</u>, Morar-Leather D., Adesiyun A.A.
- 36. In vitro activity of Psychotria zombamontana and its potential use as a poultry feed additive <u>Querl B.M.</u>, McGaw L.J., Kritzinger Q.
- A survey of selected pathogens of economic concern within the principle cultivated Tilapia (*Oreochromis* spp) producing regions of South Africa for the period 2017–2018 <u>Taylor G.D.</u>, Scarfe A.D., Huchzemeyer K.D., Steyl J.C.A.
- 38. Endemic circulation of Rift Valley fever virus in far northern KwaZulu-Natal Van den Bergh C., Venter E.H., Swanepoel R., Thompson P.N.
- 39. Evaluation of two newly developed lateral-flow immunochromatographic assays for the detection of *Mycobacterium bovis* infections in domestic cattle (*Bos taurus*) and African buffaloes (*Syncerus caffer*) <u>Van der Heijden E.M.D.L.</u>, Singh M., Rutten V.P.M.G., Michel A.L.
- 40. Adaptation of SAT2 foot-and-mouth disease viruses in cattle and goats Van der Merwe D., Van Heerden J., Heath L., Fosgate G.T., Blignaut B.
- 41. Long bone fractures in impala (*Aepyceros melampus*): A classification system and review of 62 fractures <u>Van Heerden F.</u>, Hartman M.J., Kirberger R.M.

Message from the Acting Dean

A great university is not one that is renowned for its ability to teach, but one that is great from its ability to teach, mentor and generate new knowledge. Realistically, it means that it is not good enough to teach the world's best ideas to young minds, but that it is of greater importance for an institution to contribute directly to the intellectual debate by teaching its own ideas.

Thus, not surprisingly, around the world, excellent universities highlight research as one of their most distinguished and competitive strengths. The ideology of our Faculty – and that of the University of Pretoria in general – is no different. We view all knowledge generated through our research initiatives as the cornerstone to sustainable development in our country's economy and the social development of all South Africans, and as a way to contribute to a globalised society, especially one built on international trade policies. Through these efforts, the life of the average South African can be improved from the benefits of better nutrition, improvements in family disposable income and, ultimately, by the growth of the South African agro-economy.

For the individual, the advantages of completing an advanced degree extends beyond an impressive degree certificate, as further training is geared towards imparting new skills and, more importantly, introduces a person to the intellectual debates and the impact of evidence-based decision making. Through detailed research, students develop critical thinking expertise, as well as effective analytical, research and communication skills that are globally sought after. Our master's students, for example, work alongside recognised study leaders during their research projects, learning side-byside as partners, while being given the opportunity to be mentored as future researchers and PhD candidates. Studying at a university with a reputable research foundation not only provides a firm platform for continued education, but the skills that are mastered also provide a real advantage. These experiences are invaluable and no doubt boost employability long after graduation. It is thus not surprising that our PhD graduates are widely sought after by other universities and research organisations, both locally and internationally.

When universities compete against each other to be among the top tertiary research institutions in the world, the process not only provides impetus to global sustainable development goals, but enables institutions to attract more researchers and students, as well as better funding opportunities. It is therefore one of the Faculty's strategic aims to improve the quality and impact of its research on a continuous basis to further its international recognition and continue to be acknowledged as such. In this regard, the Faculty is exceptionally proud of its achievement in being ranked 30th among the top 200 veterinary institutions worldwide by the Shanghai rankings in 2017, and 37th in 2018. More so, the Faculty is pleased to link these achievements to the hard work and dedication of its postgraduate students to reach their study goals amidst major changes in the current higher education climate.

To achieve both the Faculty's goal of being a top 50 veterinary school and the University of Pretoria's broader goal of being a top 500 university, the Faculty places emphasis on the quality of its research publications, as well as its master's and doctoral graduates. The success the Faculty has achieved in attaining these goals is evident by its research outputs and the postgraduate degrees it has awarded over the past decade. When the Faculty started focusing on its research outputs and postgraduate education in 2006, it had a modest research output of 55.5 units. In 2017, the Faculty had attained an all-time high research output of 107.54 subsidy units from 225 publications, which represents an average output per permanent staff member of more than one research unit. In addition, the Faculty recently recorded its highest achievement on doctoral student output, with 20 PhD degrees awarded in 2017, and all our graduates contributing to the Faculty's publication outputs. Earlier in 2018, two of our academic



Prof Vinny Naidoo Acting Dean: Faculty of Veterinary Science

staff members were honoured by the University at its annual Academic Achievers event: Prof Christo Botha (Exceptional Academic Achiever) and Prof Martina Crole (Teaching Excellence Laureate Award).

Although we are proud of the Faculty's progress and achievements, our quest remains to be perceived as a highly productive, world-class veterinary seat of excellence. It is thus imperative that we accept the local challenges of animal health, poverty and food security in southern Africa, making an impact internationally with innovative research, and continue to seek high-level collaboration and networking.

Being a proud tradition, Faculty Day provides an opportunity for our researchers to present the results of their studies and share them with their peers. It is an event that lends itself to provide further impetus to the Faculty's pursuit of excellence in support of the University's research-intensive vision.

It is a pleasure to welcome staff members, students and visitors to this year's event, in particular, Prof Yoshan Moodley from the Department of Zoology at the University of Venda, who will present this year's Sir Arnold Theiler Memorial Lecture. We look forward to listening to his lecture, entitled "The story of humanity, told by our oldest commensal: Helicobacter pylori." I am sure that this year's Faculty Day will once again serve as an inspiration to our postgraduate students and assist in pursuing the Faculty's primary research goals through sharing new knowledge, innovative concepts and scientific results. Thank you to the Faculty Day Organising Committee for making this special event possible. Last, but not least, I pass on my congratulations to the Faculty's Research Award winners of 2018, who will also be recognised during today's proceedings.

Curriculum Vitae: Prof Yoshan Moodley



Prof Yoshan Moodley BSc, MSc, PhD Department of Zoology University of Venda

Prof Yoshan Moodley is a South African scientist. He was born in Durban and was schooled in South Africa and New Zealand. After obtaining his Bachelor of Science degree at the University of Auckland, he returned to South Africa for a master's degree at the University of the Witwatersrand in 1996. He obtained his PhD in Molecular Ecology in 2003 from the University of Cape Town. For the next 12 years, Prof Moodley undertook a research career in Europe, first as a postdoctoral researcher in Cardiff (UK) and in Berlin (Germany), and more recently as a group leader in Vienna (Austria). Prof Moodley's research focus is on evolutionary genetics - particularly in inferring population history, structure and demography from DNA. These skills are particularly useful when applied in the field of conservation. The genetics and evolution of endangered species dominated his early career until he moved to Europe in 2003. This continues to be an important part of his research today.

While in Berlin, Prof Moodley distinguished himself with a series of influential studies on human evolution, but with a twist. The evolutionary history of mankind could be told from the point of view of a human pathogen – *Helicobacter pylori*. By studying the DNA sequences of this bacterium, which only lives in human stomachs, Prof Moodley and his colleagues at the Max Planck Institute for Infection Biology in Berlin were able to trace back an intimate association between humans and *H. pylori*, stemming back to long before modern humans left Africa to populate the world. In 2009, Prof Moodley settled in Vienna, Austria, as an independent group leader and Head of the molecular genetics laboratory at the Konrad Lorenz Institute. There he continued to show that, because we share the same evolutionary story, the DNA of *H. pylori* is structured very similarly to human DNA, and he has unraveled several events in human prehistory more clearly than was possible with human DNA.

Prof Moodley moved his research back home to South Africa in 2015 and is now a professor at the University of Venda, where he continues his evolutionary work on H. pylori. His Evolutionary and Conservation Genomics Group at the Department of Zoology is well known for setting up the country's first genomelevel analysis platforms dedicated to wildlife and evolutionary research. Venda and Tsonga postgraduate students, trained by Prof Moodley in evolutionary theory during their undergraduate years, are now able to carry out whole-genome research at the same level as their European counterparts.

Research topic summary: The story of humanity, told by our oldest commensal: *Helicobacter pylori*

Prof Yoshan Moodley

It may be surprising to many that the wellknown stomach bacterium, Helicobacter pylori, was only discovered in 1984. The spiral-shaped gram-negative bacterium inhabits the interface between the epithelium and the gastric mucosa, and is thereby shielded from caustic gastric lumen. Its discoverers were awarded the Nobel Prize in Medicine for identifying it as a causative agent for gastritis. The bacterium has ever since been a centre of intense medical and genetic research. We soon found out that *H*. pylori is present in 50% of human stomachs worldwide, and that, in the vast majority of infections, people remained asymptomatic.

H. pylori is a human bacterium. We are its only natural host. Children are normally infected by either family members or other children, and once contracted, the bacterium remains for the lifetime of the host. A defining feature of *H. pylori* is its high genetic diversity; higher than any other known bacterial species, and 50 times higher than human DNA. This high diversity is the result of unusually high mutation and recombination rates. The population and evolutionary genetic research I have carried out on an ever-increasing global data set over the last 12 years has demonstrated that the high genetic diversity of H. pylori is partitioned into several distinct geographic populations. These are carried by different human populations in different parts of the world. Africans have African H. pylori, Europeans have European H. pylori, Japanese have East Asian H. pylori, and so on. Thus, the structure of the genetic diversity of H. pylori faithfully mirrors that of its human host. However, because of its very high genetic diversity and fast generation time, H. pylori DNA sequences are often better able to resolve prehistoric human migrations than human DNA markers.

If all the different human populations also carry similarly differentiated *H. pylori* populations, it suggests that the association between the two species must be quite ancient. But just how ancient, and where our species was originally infected, were matters of speculation. Through population genetics, my colleagues and I have shown that humans and *H. pylori* share a co-evolutionary relationship that spans at least the last 100 000 years, and almost certainly longer. It must come as no surprise then that, as with human DNA, *H. pylori's* highest genetic diversity resides in Africa, the birthplace of both our species. Those ancestral populations of modern humans leaving Africa around 60 000 years ago must have carried *H. pylori* in their stomachs with them as they eventually became the different people we see in the world today.

Not only can we tell when H. pylori populations diverged from one another, but we can also tell when they came together and exchanged genes. In order to exchange genetic material, however, two strains must necessarily be occupying the same human stomach. This provides hard evidence of prehistoric and intimate contact between certain human populations around the world and also in South Africa. Given our long and intimate association, and the insights it has allowed us about our own prehistory, it would be sporting to regard H. pylori not as a maligned human pathogen, but rather the oldest and most faithful commensal our species has known.

Sir Arnold Theiler Memorial Lectures

1984:	T Gutsche	"Theiler – his personal significance today"
1985:	Prof HPA De Boom	"Vlammende fakkels, ou bene, ivoortorings en rooi vlae"
1986:	Prof BC Jansen	"Theiler-gedenklesing"
1987:		Opening of the Sir Arnold Theiler Building – no lecture
1988:	Dr RD Bigalke	"Important research requirements for future animal production-orientated research with particular reference to veterinary science"
1989:	Dr R Swanepoel	"The joy of research"
1990:	Dr A Schutte	"The impact of controlled breeding on the cattle industry in southern Africa"
1991:	Prof DM Joubert	"Sir Arnold Theiler-gedenklesing – Theiler en die Fakulteit Veeartsenykunde"
1992:	Dr CM Cameron	"The environment – whose responsibility?"
1993:		Opening of the Onderstepoort Veterinary Academic Hospital – no lecture
1994:	Dr W Plowright	"Rinderpest and cell-culture revolution"
1995:	Prof WL Jenkins	*
1996:	Prof PV Tobias	"Premature discoveries in science"
1997:	Prof DL Block	"Our universe: accident or design?"
1998:	Prof TW Naudé	"A stroll through the wondrous garden of South African toxicology"
1999:	*	*
2000:	Dr DW Verwoerd	"The molecular revolution in biology and its influence on veterinary science"
2001:	Prof H Huismans	"Molecular biology and its impact on the study and control of viral diseases such as bluetongue and African horse sickness"
2002:	Prof I Horak	"The joy of research"
2003:	Prof WFO Marasas	"Fumonisins: historical perspective and future objectives"
2004:	Dr RA Kock	"Wildlife domestic animal disease interface – hard or soft edge?"
2005:	Prof SS Van den Berg:	"The past, present and future of the clinical departments in the Faculty of Veterinary Science"
2006:	Dr BD Perry	"The global poverty reduction agenda: what are the implications for animal health research and development?"
2007:	Prof Dr AWCA Cornelissen	"What makes an excellent Faculty of Veterinary Medicine?"
2008:	Dr G Brückner	"New challenges for the veterinary profession in global animal disease control and the trade in animals and animal products"
2009:	Prof P Doherty	"Adventures in infection and immunity"
2010:	Dr R Moerane	"The role of the veterinary profession in the current developmental agenda in South Africa."
2011:		World Veterinary Congress in South Africa – no Faculty Day
2012:	Prof NJ MacLachlan	"Emerging viral diseases: the example of bluetongue, from Theiler to climate change"
2013:	Prof MC Horzinek	"A personal journey through coronavirus evolution"
2014:	Prof Louis J Guillette Jr	"Predisposition for health or disease: the 'new' genetics of environmental health"
2015:	Prof Graham J Louw	"Mummification – a glimpse into the sociocultural aspects of the preservation of the bodies of domesticated animals."
2016:	Prof Lucille Blumberg	"One Health: a decade of shared experiences and benefits"
2017:	Prof Robert Gilbert	"The research imperative"

* We apologise that the above list is not complete. It will be appreciated if anyone who has access to some of the missing information contacts Mr Chris van Blerk (chris.vanblerk@up.ac.za or 012 529 8436)

Research Summary: 2017-2018

The enhancement of innovative and relevant research, as well as high-quality postgraduate training, remains an integral part of the Faculty's strategic plan. In support of the University's goal of being a top research-intensive institution, the Faculty requires increased research outputs through effective postgraduate programmes, and a renewed focus on research as a primary thrust. Among others, the Faculty sees wildlife research as a major future research focus area, and is actively working on strengthening capacity in this area.

The upward trend and sustained growth in research outputs, the quality of ongoing research and facilities, and the engagement of many staff members with the University's vision, suggest that the Faculty is well placed to contribute significantly to the University's strategic goals. The Faculty's research publication outputs increased from 55.3 units in 2006 to 107.54 units in 2016, which represents 25 ISI-accredited journals, and the highest number of subsidy units it has earned. The Faculty currently has 206 master's, 13 PhD and 17 postdoctorate students, and the number of postgraduate graduates continues to increase compared to previous years. Challenges to sustaining these increases include the clinical nature of academics' work in some departments, the percentage of academics with doctoral degrees or NRF ratings, and the percentage of academics supervising postgraduate students. Plans are underway to further increase these percentages over the next one to three years, and to recruit additional postdoctoral researchers and research fellows. In 2017, a total of 12 PhD candidates graduated during the University's Autumn graduation ceremony, while a further 8 PhD candidates graduated during September 2017. In 2018, 12 candidates graduated during the Autumn graduation ceremony, while six PhD candidates will graduate during the Spring graduation ceremony.

Various research and related topics deriving from the work of our researchers have featured extensively in the media and on the UP and Faculty websites over the last 12 months. Examples of these are the RhODIS® technique of the Veterinary Genetics Laboratory (VGL) to collect and catalogue DNA from rhinos and rhino horn, as well as the Faculty's Mnisi One Health platform within the context of the Transfrontier Conservation Area (TFCA) veterinary and wildlife programme, which both also featured on the University's Research Matters website.

The applicability of the Faculty's research to the needs of the country is evident in numerous showcased articles, such as features on the proper care of cheetahs in captivity, a new African horse sickness test developed by the Faculty's Equine Research Centre (ERC) that makes it possible to deliver results in only four hours compared to two weeks in the past, the current avian influenza outbreak, assistance to the South African dairy industry through focused research on unanswered questions regarding mastitis treatment failure at South African dairies, the prevention of rabies in rural areas, better understanding of the intricacies of rhino immobilisation, newer methods of minimally invasive treatment in Africa's big cats, the impact of electrical fencing on small animals such as tortoises, and innovations in new teaching methods, which are being pioneered at the Faculty's Skills Lab.

One of the highlights for the Faculty's research initiatives is the recently endorsed South African Research Chair Initiative (SARChI) Research Chair that had been awarded to the Faculty's Prof Celia Abolnik in December 2017 by the National Research Foundation (NRF) and the Department of Science and Technology (DST). This is the first Chair relevant to veterinary science to be awarded in the history of the Faculty. Prof Abolnik is the current incumbent of the South African Poultry Association's Research Chair in Poultry Health and Production in the Faculty. The Chair is designed to strengthen research and innovation capacity in the area of poultry health and production and has a term of up to 15 years, renewable every five years subject to an in-depth review. The submission process for the Chair included an extensive international peer review process, and 26 international and local projects were identified within the scope of the application.

The Faculty is training professionals who are able to protect animal health, which also impacts on human health, thereby stimulating economic growth and food security. An efficient research programme must remain relevant to the needs of South Africa, but also to a constantly changing international environment. Therefore, a strong research platform will be explicitly pursued as the basis for faculty growth and development. Its vision is thus to have strong internationally recognised research groups in wildlife, infectious diseases, One Health, epidemiology and veterinary public health in support of UP's 2025 Vision and current research clusters. At the same time, it must have the potential to generate high-impact publications, attract more postgraduate students nationally and internationally, and escalate the research status of the Faculty. Fundamental to these visionary requirements, the Faculty has also changed focus through the introduction of four new research themes, chaired by Prof Lyndy McGaw, Prof Leith Meyer, Prof Johan Schoeman and Dr Nenene Qekwana. These research themes are:

Translational Medicine

This theme will focus on the treatment of human and animal disease. For the former, this will involve the use of natural disease in animals as a model for human disease or the use of animal models of disease to study new treatment(s). For the veterinary application, it will either entail the treatment of disease in the target animals in order to optimise veterinary treatment protocols or use animal models of veterinary disease to develop new treatment modalities for the veterinary patient.

African Wildlife Health and Management

This theme focuses on the unique animals of Africa and their management. Research within this theme will be crosscutting from the basic sciences, diagnostics medicine to clinical medicine/surgery. In this theme, research will focus on physiology, farming, management, disease management, food safety and disease transmission.



Research Summary: 2017-2018 (continued)

Pathobiology of Disease

This theme will be dedicated to the study of diseases in animals, including disease epidemiology. Areas of study will include changes in the normal physiology of animals brought about by disease and disease processes. An integral component of this theme will include disease diagnostics from clinical changes observed in the patient, diagnostic imaging, clinical pathological changes, pathological changes, the molecular study of disease processes, including descriptions of new pathological agents and/or toxins, and the epidemiology of animal disease.

Sustainable Livelihoods and Wellbeing

This theme will look at the implications of animal diseases on human health and wellbeing with an emphasis on the country's wildlife interface areas. It will focus on diseases that are uniquely African and thus not under investigation in other countries. The theme also largely revolves around the One Health concept. Research in this theme will include aspects such as bacterial resistance transmission, environmental toxicity, zoonotic diseases and sustainable food production, all in an attempt to improve the livelihood of people in Africa.

Research output and growth

Measures to increase the Faculty's research output could, inter alia, be achieved by establishing a research ethos, increasing the numbers of postgraduate students and encouraging teaching staff to submit themselves to NRF rating. The Faculty's growth and progress in support of the University's strategic direction could be measured when compared to research publication outputs, growth in the number of master's and postdoctoral students over preceding years and the number of NRF-rated researchers in the Faculty.

Currently, the Faculty has 51 permanent staff members with doctorates. Since 2014, there was also a dramatic upsurge in the combined number of master's and doctoral students, and the Faculty more than doubled its postgraduate output and number of postdoctoral students. The 20 PhDs that were

awarded in 2017 represents the highest number of doctorates awarded by the Faculty in a given year. The number of NRFrated researchers in the Faculty's staff complement has shown a steady growth, reaching 29 in 2017 and 32 in 2018. The Faculty now has nine B-rated, 19 C-rated and four Y-rated staff members, with Prof Christo Botha, Head of the Department of Paraclinical Sciences, rated at B1 level, which makes him the highest NRF-rated researcher in the Faculty.

Faculty Day 2017 and research awards

The annual Faculty Day on 25 August 2017 provided an opportunity for our researchers to showcase the research activities in the Faculty to colleagues and peers, and was well attended by staff members, visitors and sponsor companies alike. The Sir Arnold Theiler Memorial Lecture, entitled "The research imperative" was delivered by Prof Robert Gilbert, Head of the Department of Clinical Sciences in the School of Veterinary Medicine at the Ross University in St. Kitts. Excellence in research performance was recognised at the event with the identification of the Faculty's top 10 researchers and the allocation of the following research awards:

Researcher of the Year Prof Vinny Naidoo

Nine top researchers in the Faculty

Prof Estelle Venter Prof Geoff Fosgate Prof Peter Thompson Prof Anita Michel Prof Johan Schoeman Prof Koos Coetzer Prof Andre Ganswindt Prof Marinda Oosthuizen Prof Christo Botha Supplier of veterinary instrumentation, diagnostic equipment and farm related products to the Veterinary and Agricultural market in Southern Africa



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Research Programme: Oral Presentations



Topography and splanchnology of the lungs in the African lion (*Panthera leo*)

<u>CA Marais</u>, MR Crole

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Anatomy of the domestic cat (*Felis catus*) is well documented. Although the African lion (*Panthera leo*) is of the same family as the domestic cat, some key differences and adaptations do exist. The African lion is an iconic African wildlife species and one of the 'Big 5'. Although classified as Vulnerable (IUCN), lions are intensively bred in captivity and a large captive population exists. Lions are important in South Africa for both tourism and trophy hunting. Veterinarians need to increase their knowledge of African lion anatomy to accommodate the increasing demand for veterinary intervention in these animals. Direct comparison to the domestic cat is inadequate and this study aims to provide a detailed description of the lungs of the African lion.

Five (± 3 years old) captive-bred African lions, embalmed after being culled on a private game farm, were used in this study. Specimens were rinsed in water for 7 days prior to dissecting. Both frontlimbs were removed and the thoracic muscles stripped. Topography was noted with and without the ribs. Ribs were removed with shears. The lungs were either removed individually or together with the heart. Two lungs were dissected to reveal the internal features. Standard anatomical dissection and descriptive techniques were employed.

The left and right lungs span ribs 2-13, however, minimal lung tissue is present cranial to rib 4. The *Cupula pleura* is free from lung tissue and projects 1-1.5 cm cranial to rib 1. The basal

edge of the left lung runs from the 2nd costochondral junction (CCJ), to just ventral to the 7th CCJ and then dorso-caudally to the angle of rib 13. The right lung is similar except for the basal border which is dorsal to the 5th-6th CCJ. The *Incisura* cardiaca is more prominent on the right and is formed by the notched space between the ventral margins of the cranial and middle right lung lobes. The site for intracardiac injection is recommended in intercostal spaces 4 or 5 either side of the 5th CCJ. Thoracocentesis can be safely performed just ventral to the 7th–8th CCJ's. The lungs are not lobulated but well lobated with complete and deep fissures on the Facies costalis and no fissures on the Facies vertebralis/mediastinalis. The left lung displays cranial and caudal lobes whereas the right lung is comprised of cranial, middle, caudal and accessory lobes. The trachea and primary bronchi are massive and display a large, flimsy Paries membranaceus.

The functional lung lobes in the African lion are more caudal in comparison to the domestic cat. The massive front limbs may limit thoracic expansion with the result that the bulk of the lung tissue is present caudal to the tricipital line. The very wide, thin *Paries membranaceus* may pose a risk for indiscriminate intubation. The large lung volume restricted to the caudal thorax may decrease respiratory function if the stomach is full. The large trachea and bronchi show adaptation for rapid airflow, however, the restricted lung volume would not support sustained activity for the size of the animal.





Comparison of thoracic radiological changes with computed tomography in dogs suffering from blunt trauma

<u>SC Dancer¹</u>, C le Roux¹, RM Kirberger¹

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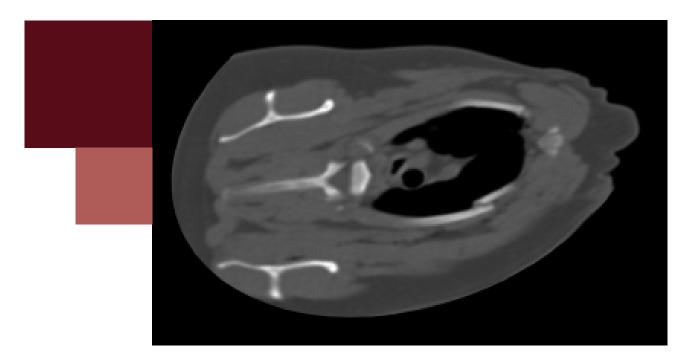
Blunt trauma patients presenting with thoracic injuries are common emergencies in veterinary medicine. To date, no literature exists comparing radiology to computed tomography (CT) in canine blunt thoracic trauma patients. The aim of this prospective case series was to compare the sensitivity and specificity of thoracic radiology to CT in detecting common trauma related injuries such as lung contusions, pneumothorax and pleural effusion. The hypothesis was that CT is more sensitive than radiography at detecting injuries related to blunt thoracic trauma. Fifty-nine patients met the inclusion criteria and standard as well as horizontal beam thoracic radiographs were compared to thoracic CT-findings. Results indicated that radiology underestimated the presence of lung contusions and overestimated the severity of the contusions detected

when compared to CT. There was also a high Interobserver disagreement of lung contusion severity between observers. Both standard as well as horizontal beam radiographs elicited poorer sensitivities for the detection of pneumothorax, pleural effusions and rib fractures when compared to CT. This study proved that CT has a higher sensitivity for common thoracic trauma pathology such as lung contusions, pneumothorax, pleural effusion and rib fractures. It therefore should be considered as a first line diagnostic modality in patients presenting with polytrauma, since CT has the benefit of evaluating both soft tissue and osseous structures in multiple planes, which allows for detection and quantification of more trauma-associated pathology. These findings will help the clinician to better prognosticate and manage patients.

Lesion	n	Карра (95% СІ)	Sensitivity* (95% Cl)	Specificity* (95% Cl)		
Lung contusion	35	0.64 (0.49, 0.79)	0.69 (0.52, 0.82)	0.83 (0.65, 0.94)		
Pneumothorax	16	0.47 (0.32, 0.62)	0.19 (0.05, 0.43)	1.0 (0.93, 1.0)		
Pleural effusion	7	0.38 (0.23, 0.53)	0.43 (0.12, 0.78)	0.96 (0.88, 0.99)		
Rib fractures (left)	5	0.33 (0.18, 0.47)	0.60 (0.18, 0.93)	0.96 (0.88, 0.99)		
Rib fractures (right)	7	0.22 (0.07, 0.37)	0.43 (0.12, 0.78)	1.0 (0.94, 1.0)		
Rib fractures (any)	9	0.30 (0.16, 0.45)	0.56 (0.24, 0.84)	0.96 (0.87, 0.99)		

CI = confidence interval. LL = left lateral view. RL = right lateral view. VD = ventral-dorsal view

* Estimated for the consensus radiographic diagnosis relative to CT as the gold standard.





Ovarian function and anti-Müllerian hormone following immunocontraceptive vaccination of mares using native and recombinant zona pellucida vaccines with non-Freund's adjuvants

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An important determinant in the selection of any contraceptive agent is the impact on ovarian function.

The aims of this study were to describe ovarian function (clinically assessed) in mares following treatment with native porcine ZP (pZP; University of Pretoria, South Africa), recombinant ZP (reZP; CSIR, Biosciences, Pretoria, South Africa) or pZP and reZP combination vaccines with non-Freund's adjuvants, and to assess the utility of measuring anti-Müllerian hormone (AMH) concentrations, with minimal sampling frequency, for monitoring ovarian function.

Recruited subjects (n=31) were stratified by body condition scores, parity and age and randomly assigned to one of four treatment groups for a repeated measures study. Treatments and measurements (blood sampling for AMH and serum progesterone (ELISA AMH Gen II,Beckman Coulter, USA; Immulite® 1000, Siemens, Germany) and transrectal palpation and ultrasound of the reproductive tract), were initiated in December (d=0) and repeated in January (d=35), February (d=70), March (d=105) and May (d=175). Treatments were administered into the gluteal muscles, as follows:

Adjuvant control: (n=8) combined adjuvant 6% polymeric adjuvant (Montanide[™] Pet Gel A, Seppic, France) and 500 µg Polyinosinic-polycytidylic acid - TLR3-agonist (Poly(I:C) HMW VacciGrade[™], Invivogen, USA) at d=35 and a second identical treatment at d=70;

<u>pZP only</u> (n=7) 100 μ g native pZP proteins, containing the combined adjuvant at d=35 followed by an identical booster vaccination at d=70;

<u>reZP only</u> (n=8) 500 μ g recombinant ZP3 and ZP4 proteins containing the combined adjuvant at d=0, followed by an identical booster vaccination at d=35 and a second booster with the same formulation at d=70;

<u>pZP and reZP</u> (n=8) 100 μ g pZP containing the combined adjuvant at d=35, followed by a booster vaccination, of 500 μ g reZP containing the combined adjuvant at d=70.

Data were analysed using general and mixed linear models. Treatment, time and treatment by time interaction had an effect on ovarian activity (P<0.001, P<0.001, P<0.001, respectively). Five weeks after the final booster treatment (d=105) 3/8 control mares, 3/7 pZP only mares, 5/8 pZP & reZP mares and 7/8 reZP only mares had displayed anoestrus (P<0.05). A significant difference in AMH concentrations between treatments was seen at d=175 (P<0.05), however no differences were evident between individual treatments following pairwise comparisons. Mares treated with a reZP vaccine experienced changes in AMH concentrations over time, with higher concentrations at d=70 than at d=105 (P<0.05). Serum AMH concentrations were positively correlated with ovarian volume (r=0.171, P<0.05), and mare age (lower concentrations observed in mares \leq 3y) (p=0.269, P<0.001) and negatively so with serum progesterone (r=-.373, P<0.05). In conclusion, reZP with non Freund's adjuvant is highly associated with ovarian suppression. The measurement of AMH at greater than monthly intervals is associated with important suggestive parameters of ovarian activity including ovarian volume and serum progesterone. Age effects on AMH concentration warrants additional study.





Investigating the anti-adherence mechanism of bioactive South African Myrtaceae plants against diarrhoeagenic enterotoxigenic *E. coli* (ETEC) limiting pig production using a Caco-2 cell model

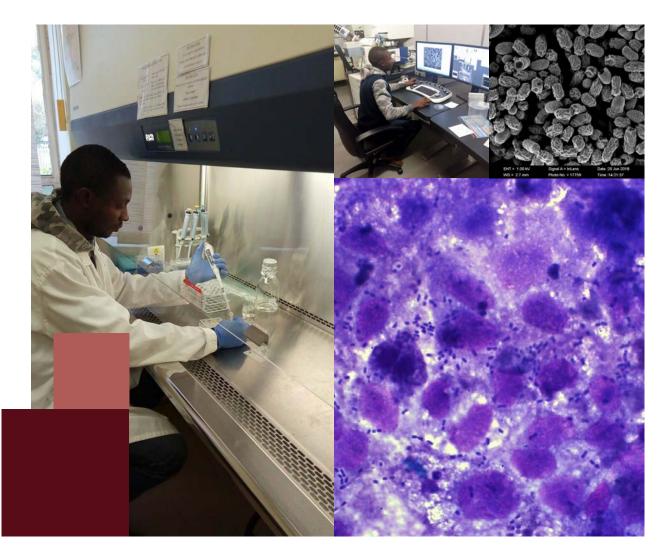
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Diarrhoea is one of the most serious impediments to the swine industry globally, causing huge annual economic losses, reduced growth rates and high treatment costs. Enterotoxigenic E. coli (ETEC) accounts mostly for neonatal and post-weaning diarrhoea in piglets, and the onset of its pathogenesis is mostly via adherence and colonization of enterocytes. Antibiotics are commonly added to livestock feed to promote growth and for prophylaxis against microbial infections. The widespread occurrence of antimicrobial resistance and subsequent bans against antibiotic use in feeds in many countries calls for further research on non-antibiotic feed additive alternatives. Empirical results show that phytogenic products/botanicals are promising replacements for antibiotics in animal feed. We selected eight under-explored South African plants from the Myrtaceae family based on promising antibacterial activity previously observed from this family. A two-fold serial microdilution

assay was used to determine the minimum inhibitory concentration (MIC) of the extracts against five pathogenic ETEC with different virulence genes isolated from diarrhoeic piglets and a reference E. coli strain. Cytotoxicity was determined using a tetrazolium-based colorimetric assay against Caco-2 intestinal epithelial cells. Caco-2 cells were used as an intestinal model to explore the anti-adhesion potential of the plants in a cell culture anti-adhesion assay against an ETEC possessing a fimbriae virulence gene. Most (80%) of the plants had excellent mean MIC (<100 µg/ml) against the E. coli strains with Syzygium legatii having the best mean MIC of 39 µg/ml against the *E. coli* serotypes. The plants (62.5%) significantly reduced ETEC adhesion to Caco-2 cells compared to the untreated control and were not cytotoxic with good selectivity indices. Many of the plants may be useful for development as phytogenic feed additives but animal feed trials need to be conducted.





Isolation of *Brucella melitensis* biovars 2 and 3 from slaughter cattle in South Africa

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Brucella melitensis is primarily a pathogen found in goats and sheep, but occasionally it can be found in cattle. In South Africa, this organism has been responsible for outbreaks of brucellosis in goats and has also been reported to be the cause of brucellosis in humans. This study reports for the first time in South Africa, the detection and isolation of *B. melitensis* from tissue samples of slaughter cattle from abattoirs in the Gauteng province of the country.

Two hundred serum and corresponding tissue samples (lymph nodes, spleen and liver) were collected from slaughter cattle between September 2016 and April 2017. Serological tests using the Rose Bengal Test (RBT) and indirect enzymelinked immunosorbent assay (iELISA) were conducted on the serum samples. The genus-specific 16S–23SrDNA internal transcribed spacer (ITS) PCR assay detected *Brucella* DNA in the tissues of the iELISA positives. All ITS-positive tissues were inoculated on both standard Farrell's and modified CITA media. Morphologically identified *Brucella* colonies were biotyped. All isolates of *Brucella* spp. were subjected to the ITS PCR assay to confirm *Brucella* DNA, as well as the AMOS multiplex PCR assay that differentiates *B. abortus*, *B. melitensis*, *B. ovis* and *B. suis*.

Of the 200 sera tested, 22 (11.0%) and 11 (5.5%) were positive for antibodies to *Brucella* spp. by RBT and iELISA, respectively. The ITS PCR assay detected *Brucella* DNA in 9 of 11 tissues of iELISA-positive samples, while *Brucella* spp. were isolated from seven of nine PCR-positives. AMOS PCR identified four of seven isolates as *B. melitensis* and biotyping classified two isolates as *B. melitensis* biovar 3 and one isolate as *B. melitensis* biovar 2. The implication of these findings underscores the fact that humans can become infected with *B. melitensis* from infected cattle, either through drinking of unpasteurized milk or consumption of undercooked or uncooked meat products from these infected animals.





Mycobacterium bovis infection in cattle and communal resource-constrained farmers living at the wildlife-livestock-human interface in South Africa

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Cattle are the reservoir of Mycobacterium bovis (M. bovis) but many other domestic and wild animals including humans can be affected by bovine tuberculosis (BTB). In South Africa, BTB in cattle is only partly controlled and the African buffalo (Syncerus caffer) acts as a wildlife reservoir of M. bovis and gives rise to a complex wildlife-livestock-human interface. A One Health strategy was designed to assess the role of *M. bovis* at the interface of cattle, humans and wildlife in a resource-constrained rural community in the northern region of KwaZulu-Natal province in South Africa. In the first phase of this multi-disciplinary study, a cross-sectional study was done to determine the prevalence of *M. bovis* infection in 659 cattle from a total of 192 herds using a modified BOVIGAM[®] interferon gamma assay. Infection was confirmed by post mortem examination and *M. bovis* isolation from three test positive cattle. In phase two, a collaborative study with the Department of Health was conducted to investigate the occurrence of M. bovis in members of 75 households associated with BTB infected herds. The initial screening was done using the GeneXpert for the *Mycobacterium tuberculosis* complex (MTC) followed by speciation using molecular techniques. In phase three, a socio-anthropological study

was conducted to investigate the communal farmers' risk practices to BTB transmission among cattle and to humans as well as to assess their awareness of BTB. A structured questionnaire was administered and focus group discussions were facilitated in the study community. Opportunistic sampling of wildlife in game reserves in the study area is ongoing for the isolation and genotyping of *M. bovis*. The apparent *M. bovis* prevalence rate in cattle at animal level was 13.5% (95% CI: 11.0-16.4) with a true prevalence rate of 8.5% (95% CI: 3.9-13.8). Overall, 27.6% of the farmers had at least one test positive animal. Out of 71 respondents, 63 (89%) of the individuals did not know about bovine tuberculosis in wildlife and, although 61 (86%) of the respondents were aware of BTB in cattle, 70% of these were not aware of its transmission to people. The confirmation of a high prevalence of *M. bovis* in cattle poses a risk to human health. The study demonstrated poor knowledge of most cattle owners concerning BTB and its transmission pathways among people, livestock and wildlife. The One Health approach is hence a suitable approach to investigate *M. bovis* infection and its risk factors in cattle, humans and wildlife at the interface.





The acute phase response in healthy and injured southern white rhinoceros (*Ceratotherium simum*)

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The acute phase response is part of the innate immune system and is triggered by a variety of inflammatory stimuli. Acute phase reactants (APRs) are useful as diagnostic, prognostic and therapeutic markers. The acute phase response has not been investigated to the authors' knowledge in the southern white rhinoceros (*Ceratotherium simum simum*).

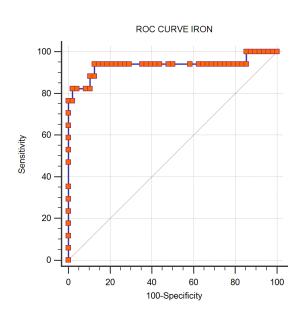
The objectives of this study were to:

- 1) Generate reference intervals (RIs) for the positive APRs fibrinogen, haptoglobin and serum amyloid A (SAA), and the negative APRs serum albumin and iron in healthy adult white rhinoceros
- 2) Evaluate the diagnostic utility of these APRs in animals with acute and chronic tissue injuries, using tissue injury as an example of an inflammatory condition.

Serum samples collected from the auricular vein were used for analysis. SAA was measured with a sandwich ELISA, fibrinogen with the modified Clauss method and iron, albumin and haptoglobin with automated colorimetric methods. Reference intervals were generated from a group of 48 clinically normal free-ranging adults of both sexes for all analytes except SAA where the sample group included only 23 individuals. Differences in APR concentrations between healthy animals and those with acute (n=13) and chronic injury (n=17) were assessed using the Kruskal-Wallis test. Receiver-operator characteristic (ROC) curve and stepwise logistic regression analyses were used to evaluate the diagnostic performance of the various APRs (fibrinogen and SAA excluded from the latter due to smaller data sets). P< 0.05 was considered significant.

RIs were: albumin 18-31 g/L, fibrinogen 1.7-2.9 g/L, haptoglobin 1.0-4.3 g/L, iron 9.7-35.0 µmol/L, SAA <20 mg/L. Iron and albumin were significantly lower and fibrinogen, haptoglobin and SAA higher in both acute and chronic tissue injury groups, compared to healthy animals. Iron showed the best diagnostic accuracy with an area under the curve (AUC) of 0.91 followed by fibrinogen (0.89), albumin (0.76), haptoglobin (0.72) and SAA (0.67). Iron \leq 15.1 µmol/L and haptoglobin >4.7 g/L were significant predictors of inflammatory status and together correctly predicted the clinical status of 91% of cases. SAA >20 mg/L had a specificity of 100%.

Based on these results, albumin and iron are negative APRs and fibrinogen, haptoglobin and SAA positive APRs in the white rhinoceros, similar to domestic animals such as horses and cows. The combination of iron and haptoglobin had an excellent diagnostic accuracy for detecting inflammation in this study. The acute phase reactants studied here have the potential to detect inflammation in other clinical scenarios in the white rhinoceros and their diagnostic performance in other diseases should be studied further.







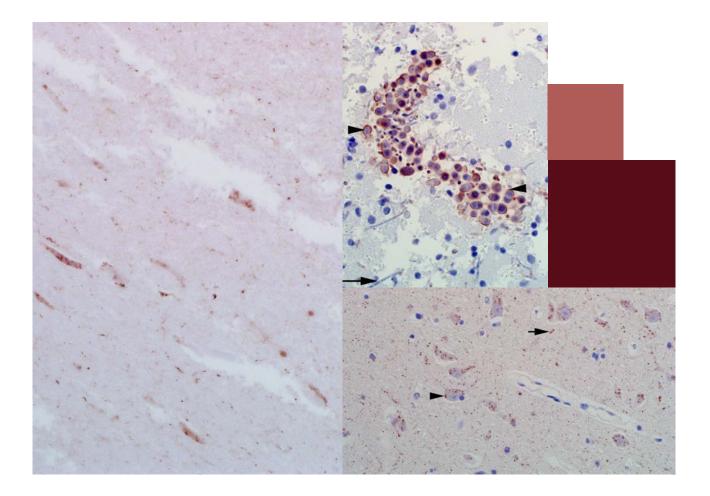
Validation of an indirect immunoperoxidase test for rabies virus in domestic and wildlife species in South Africa

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Rabies virus is a member of the genus Lyssavirus, in the family Rhabdoviridae. This fatal but preventable disease is of significant public health and veterinary importance in the developing world. The only reliable method of detection is to determine the presence of the virus via immunodetection of viral antigen in the central nervous system (CNS) tissue following death of the animal. Rapid diagnosis is key in preventing human deaths due to rabies and to implement effective control measures in the field. The purpose of this study was to validate the rabies indirect chromogenimmunohistochemistry test (IC-IHC), and to estimate its diagnostic sensitivity and specificity on routine specimens submitted for the rabies direct fluorescent antibody test (FAT) collected in South Africa. The IC-IHC test was performed on CNS tissues obtained from a variety of mammalian host species commonly submitted for rabies diagnostic testing in South Africa, samples from domestic dogs and cattle were submitted in the greatest number to the two accredited FAT rabies laboratories in South Africa during the study period from 2013 - 2017. Specimens from wild animal species that included, but were not limited to, jackal, wild dog, hyena, aardwolf, leopard, lion and mongoose were also tested. One

hundred ninety-nine cases were evaluated, of which 99 were positive and 100 were negative for rabies on FAT. The IC-IHC test results were compared to those of the gold standard to determine its sensitivity (Se) and specificity (Sp). The overall Se and Sp for the rabies IC-IHC test was 98% (95% confidence interval (CI): 93% - 100%) and 99% (95% CI: 95% - 100%) respectively. As part of the validation study, we investigated the effects of autolysis (30.7% of test cases were severely autolysed and 43.7% moderately autolysed), extended time in formalin (samples fixed in formalin for up to a year) and freezing on the diagnostic Se and Sp of the IC-IHC. It was determined that the brainstem and the thalamus were the best samples to collect for rabies virus detection using the IC-IHC test in all species. The IC-IHC test used in the current study is an excellent diagnostic test for rabies in South Africa and compares well with the FAT. The test has the added benefit of working on formalin fixed tissues and since formalin inactivates the virus, the safety of field and laboratory staff and couriers is assured. Also, if the sample examined with the IC-IHC is negative, there is the added benefit of histopathologic examination of the brain tissue to look for other differential diagnoses.





The effects of elevated concentrations of KCl in the feed of laying hens

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In formulating feeds, commercial or home grown, one of the constraints is ensuring the correct level of salt inclusion. Excess sodium and potassium chloride derived from fishmeal, soya, and molasses, which may have variable potassium content, can cause episodes of wet droppings in laying hens and broilers. A small-scale farmer reported that Hyline hens in lay produced brownish liquid manure, accompanied by a drop in egg production within three to four days after consuming a new batch of feed formulated with molasses.

The feed sampled from the affected farm had elevated potassium levels (1.5%) which were 200% higher than expected. It was decided to ascertain if the higher potassium percentage could have been the cause behind the decrease in egg production from an associated electrolyte and acid base disturbances. For the first study the influence of three different levels of potassium at 0.5% (control), moderate (0.9%) and high (2%) in the presence of ad lib water intake were ascertained on egg production and faecal moisture

content. For the second study, the influence of a single day restriction on water access (66.66% of normal) after a 7-day acclimatisation period was determined in the presence of normal (0.5%) or moderate increases in potassium (0.9%). In all cases, the birds were at 85-90% in lay and 23–24 months old and maintained on a standard commercial laying mash (16% protein). The birds were otherwise maintained under commercial conditions at a temperature of 20 to 25°C in standard wire-mesh cages in groups of three sharing a single cup drinker.

For study 1, no significant differences were evident for plasma sodium, potassium, chloride, glucose concentrations, or egg production. For study 2, both groups experience a 60% drop in egg production and concurrent increase in plasma sodium, chloride and osmolority.

It would thus appear that a greater determinant in egg production is water intake rather than the level of potassium salts in the diet.





A novel approach for antigenic vaccine matching of foot-and-mouth disease (FMD) viruses

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Foot-and-mouth-disease (FMD) is a highly contagious transboundary animal disease that has negative consequences on regional and international trade. Vaccination is one of the most important approaches for FMD control because it reduces the number of susceptible animals in a population. The essential component of vaccination is the degree of cross-protection provided by the vaccine against currently circulating field viruses. Thus, the FMD virus used to produce the vaccine must share antigenic characteristics with potential outbreak strains for vaccination to be effective. The control of FMD in Southern Africa is complicated by the genetic and antigenic variability of the Southern African Territories (SAT) FMD viruses and the uncertainty surrounding protection by currently used FMD vaccines. The objective of this study was to develop a new vaccine matching technique that does not require the live homologous vaccine virus in the laboratory performing the vaccine matching. As a proof of concept, we assessed the vaccine-match of 40 FMD field viruses isolated from southern Africa over the previous 25 years.

A diverse group of 20 SAT1 and 20 SAT2 isolates collected from 1990–2015 were selected for the study.

Virus neutralization tests (VNT) were performed following the method described in the OIE Manual (2012).

Two sets of pooled sera were used for each serotype; vaccinated bovine sera (4-16 weeks post-vaccination) and convalescent bovine sera (3 weeks post-experimental challenge). Novel r1-values were calculated for the vaccine titre compared to a standardized positive control. The validation r1-value was calculated based on the assumed homologous vaccine virus. There was a strong positive correlation between the novel r1-value and the validation method (Spearman's rho = 0.84, P< 0.01 and Spearman's rho = 0.90, P< 0.01 for SAT1 and SAT2 viruses respectively). In addition, there was good agreement between the novel and validation methods for both serotypes based on a r_1 -value cut-off of 0.3, which is assumed to represent a good vaccine-match (kappa = 0.70; 95% Cl, 0.47-0.95). The prevalence-adjusted and bias-adjusted kappa (PABAK) estimated the agreement between the two methods to be 0.67 and 0.84 for SAT1 and SAT2, respectively.

The new method provides a feasible and reliable vaccine matching approach that will contribute to the control of FMD in southern Africa.



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Research Programme: Poster Presentations

A flow cytometric assessment of selected splenic cell populations of dogs that died of natural *Babesia rossi* infections

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Babesia rossi occurs predominantly in South Africa and this canine tick-borne haemoprotozoan parasite is considered the most virulent of the Babesia parasite species. The spleen is essential in mounting an adequate immunological response against babesiosis and requires that both the innate and adaptive (humoral and cellular response) immune systems work in concert to limit the extent of parasitaemia. The main objectives of this study were to (a) to quantify the proportions of CD3⁺CD4⁺ (helper) T-lymphocytes, CD3⁺CD8⁺ (cytotoxic) T-lymphocytes, CD14⁺ cells and CD21⁺ B-lymphocytes present in the spleens of dogs terminally infected with B. rossi and in Babesia-uninfected controls, using flow cytometry; (b) to compare the proportions of these selected immune phenotypes present in spleens of terminally infected dogs to spleens collected from Babesia-uninfected control dogs; and (c) to compare the various proportions of the above mentioned selected immune phenotypes in the spleen, with the peripheral blood of dogs infected with *B. rossi* and in Babesia-uninfected controls.

Splenic samples (2x2cm) from 10 dogs that died or were euthanased due to naturally acquired *B. rossi* infections and six *Babesia*-uninfected control dogs that were euthanased due to various other non-infectious reasons, were collected within one hour after death. The spleens were macerated, a single cell suspension obtained and used to perform flow cytometry after incubation with canine-specific, fluorochrome conjugated, anti-CD3, CD4, CD8, CD14 and CD21 cell markers. Blood samples were collected from the jugular vein immediately prior to death or euthanasia and incubated using the same methods as for the spleen, to prepare the sample for flow cytometric analysis. Flow cytometry was completed within six hours of death or euthanasia.

In the control dogs, the proportions of CD4⁺ T lymphocytes were significantly lower in the spleen (26.600; IQR: 21.575 – 27.900) than in the blood (36.550; IQR: 29.225 – 40.450), P = 0.037. The same was found in control dogs when looking at CD14⁺ cell proportions in the spleen (40.750; IQR: 30.025 – 43.575) and the blood (89.850; IQR: 67.700 – 96.950), P = 0.037. Only the proportion of splenic CD21⁺ lymphocytes were significantly lower in *Babesia*infected dogs (Median: 11.200; Interquartile range (IQR): 8.800 – 18.700) than in *Babesia*-uninfected control dogs (22.600; IQR: 16.475 – 31.475), P = 0.021. No significant differences were found in other splenic immune phenotypes of *Babesia*-infected dogs and uninfected control dogs. There were also no other significant differences between phenotypes in the spleen and the blood.

These results could indicate that the humoral immune system is not significantly upregulated or that suppression of the humoral immune system takes place in terminal *B. rossi* infected patients. Further studies are necessary to determine the pathogenesis of altered splenic cell populations as well as the clinical implications.

Neutrophil myeloperoxidase index in dogs with babesiosis

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Babesiosis caused by the more virulent tick-borne hemoprotozoan, *Babesia rossi*, leads to a marked systemic inflammatory host response in dogs. Neutrophils are part innate immune system and myeloperoxidase is the predominant component of the neutrophil lysosomal protein in azurophilic granules. This enzyme plays a crucial role in the process of destruction of microbes by neutrophils. Neutrophil myeloperoxidase index (MPXI) is a reflection of the intracellular myeloperoxidase content and a recognized marker of neutrophil activation. The aims of this study were to (a) determine whether MPXI is correlated with outcome in dogs with babesiosis caused by *B. rossi*; and (b) determine correlation with the severity of the host response using cytokine concentrations.

Data for 140 dogs, naturally infected with *B. rossi*, and 20 healthy control dogs were retrospectively evaluated. Owner consent was obtained for enrolment of each case, together with approval from the University's Animal Ethics committee. MPXI was generated on an automated cell counter, ADVIA 2120, and various cytokine concentrations (interleukin-2

(IL-2), IL-6, IL-8, IL-10, IL-18, granulocytic-macrophage colony stimulating factor (GM-CSF) and monocyte chemo-attractant protein-1 (MCP-1)) were determined using a canine-specific multiplex assay.

Fifteen/140 (14%) of the *Babesia*-infected dogs died. MPXI was significantly higher in the *Babesia*-infected survivors (P = 0.033), and in the non-survivors (P = 0.009), compared to the controls. For the dogs that died, significant correlations were found between MPXI and IL-2 (r = 0.616, P = 0.033), IL-6 (r = 0.615, P = 0.033), IL-18 (r = 0.613, P = 0.034), GM-CSF (r = 0.630, P = 0.028) and MCP-1 (r = 0.713, P = 0.009).

These findings suggest that significant neutrophil activation is present in dogs with *B. rossi* infection. In addition, increased MPXI was associated with disease outcome and was correlated with the severity of the cytokine-driven proinflammatory host response. Further investigation is required to confirm the prognostication value of MPXI in canine babesiosis.

Leptospirosis in slaughter livestock in Gauteng province, South Africa: Isolation, serological and molecular studies.

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Leptospirosis, caused by pathogenic species of the genus *Leptospira*, is a re-emerging zoonosis of global importance. Approximately 500,000 annual cases are reported in humans worldwide, with a mortality rate of between 5–20%. In animals, the disease causes economic losses. In South Africa, human and animal leptospirosis have been reported to range between 6.5% to 12.5% and 1.6% to 62.1%, respectively. Abattoir facilities can serve as invaluable resources for both active and passive disease surveillance. Little information is available regarding *Leptospira* spp. in South Africa, with the last isolation reported in 1999. The aim of the study was to determine the prevalence of *Leptospira* spp. in slaughter livestock by isolation, serology and quantitative real-time PCR (qPCR), and to type these isolates genetically.

A total of 646 samples (341 sera and 305 kidneys) were collected aseptically, from cattle, pigs and sheep slaughtered in 14 randomly selected abattoirs. Sera were analyzed by the microscopic agglutination test (MAT), using a panel of 26 serovars. Kidney samples (n=237) were subjected to qPCR for quantification of leptospires. Isolation of *Leptospira* spp. was achieved in semi-solid EMJH medium incubated at 29°C for a period of 3 to 6 months using standard procedures.

The seroprevalence of leptospirosis was 23.8% (81/341). Isolates and sequencing of kidney samples showed pathogenic *Leptospira* spp. Twelve (3.9%) of the 305 kidneys cultured were positive for pathogenic *Leptospira* spp., including the first isolate from slaughtered cattle in South Africa. The qPCR revealed 29.1% (69/237) of pathogenic *Leptospira* spp. with the highest positive rate of 54.3% (19/35) in ovine samples. This is also the first report of qPCR as a diagnostic tool for leptospirosis in South Africa.

High prevalence of pathogenic *Leptospira* spp. in slaughtered animals is revealed, with great potential for risk of spread to humans through occupational and environmental routes especially in abattoir workers, veterinarians, farmers and possibly the general public. There are no human vaccines available for use in South Africa. There are vaccines available for livestock, but they may not contain all the currently circulating serovars in the country.

Finally, combination of the qPCR, serological and isolation techniques in conjunction with active and passive surveillance for zoonotic diseases at abattoirs, would be important to comprehensively evaluate the epidemiology of leptospirosis in South Africa for effective prevention and control of the disease.

Isolated flavones from Loxostylis alata have anti-influenza potential

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Influenza A infection remains an important cause of acute respiratory disease and a major health threat for animals and humans requiring effective antiviral remedies. One of the primary defence responses to infection by both humans and animals is inflammation. However, an over-mobilization of inflammatory defence cells could damage tissues; hence the role of anti-inflammatory agents is important against infectious diseases. The use of phytomedicines as alternatives to conventional medicines has been advocated over the years. Preliminary screening of the extracts of Loxostylis alata for both antibacterial and anti-influenza potential led to the isolation of compounds from the ethyl acetate fraction of the methanolic crude extract. Hence, this study investigated the anti-influenza and anti-inflammatory potential of some of the compounds (DGLA 1, DGLA 11, and DGLA 111) isolated from the plant. The 50% cytotoxic concentration (CC_{50}) of the compounds was determined using MDCK cells, revealing low toxicity against MDCK cells for all the compounds. Cells were

subsequently treated with effective concentration (EC_{50}) of the compounds with approximately 100 tissue culture infectious dose (100 TCID₅₀) of the virus (PR/8/34, H1N1) at varying exposure types: simultaneous, pre-penetration and postpenetration combined treatments with an incubation time of one hour. The antiviral potential of the compounds was tested by standard haemagglutination (HA) and haemagglutination inhibition (HI) virological assays. Anti-inflammatory activities of the compounds were determined using the 15-lipoxygenase enzyme inhibitory assay. Dose-dependent HI patterns of the compounds was observed. However, from the cell viability (MTT) assay, all compounds caused a significant increase in cell viability in all combination treatments compared to the virus sample. Overall, compound DGLA 11 $(EC_{co} = 0.417 \pm 0.045)$ had the most significant cell viability and anti-inflammatory value (IC_{50} : 3.26 ±0.44 ug/mL). This study reports *L* alata as a potential source of new anti-IAV or antiinflammatory preparations.

In vitro cytotoxicity induced by different cardiac glycosides

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Cardiac glycoside poisoning is one of the six most important plant poisonings affecting livestock in South Africa. Based on their chemical structure, cardiac glycosides are classified as either cardenolides or bufadienolides. Plants containing cardenolides are seldom eaten by livestock and thus of little veterinary importance compared to plants containing bufadienolides. Bufadienolides can be divided into two groups i.e. non-cumulative bufadienolides, which cause acute bufadienolide poisoning, and cumulative bufadienolides, that besides acute poisoning, can also induce a chronic, paretic condition known as krimpsiekte. Research regarding the mechanism by which cumulative bufadienolides induce krimpsiekte is important since the chronic intoxication of livestock has a significant impact on small stock production and economic development in South Africa. The study objectives were to confirm the neurotoxicity of the cumulative bufadienolides in vitro and compare the effects of the different cardiac glycosides on myocardial and neuroblastoma cell lines. The cytotoxic effect of three different cardiac glycosides on rat myocardial (H9c2) and mouse

neuroblastoma (Neuro-2a) cells were examined using the MTT assay. Cells were incubated with digoxin (cardenolide), epoxyscillirosidine (non-cumulative bufadienolide) or lanceotoxin B (cumulative bufadienolide) for 24 h, 48 h and 72 h. The data obtained was used to calculate the IC_{50} by constructing non-linear regression curves in GraphPad Prism 6.0. All assays were performed in triplicate with three biological repeats. Transmission electron microscopy (TEM) was used to compare the ultrastructural changes of the cells exposed to the different cardiac glycosides. Selected samples were also examined using scanning electron microscopy (SEM). The cumulative bufadienolide, lanceotoxin B induced the greatest degree of cytotoxicity when exposed to Neuro-2a cells, followed by epoxyscillirosidine, while digoxin induced the least cytotoxic effect. When exposed to the H9c2 cells, the cytotoxicity induced by epoxyscillirosidine was the greatest with both digoxin and lanceotoxin B showing less cytotoxicity. From the results obtained the cumulative, neurotoxic bufadienolide had the greatest effect on the neuroblastoma cells in vitro compared to the other cardiac glycosides.

The chronicles of the captive African penguin: Bravery or desensitization towards natural and environmental stressors?

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Anthropogenic activities have led to the unprecedented decline of various seabird species, such as the endangered African penguin (Spheniscus demersus). Conservation practices for S. demersus include the housing of individuals within the captive environment. Although captive breeding occurs freely, no information of the physiological stress experienced by captive individuals due to anthropogenic activities or across various life history stages exist. Non-invasive hormone monitoring offers a robust tool for determining alterations in glucocorticoid metabolites, as a proxy of physiological stress experienced, in avian species. Additionally, non-invasive hormone monitoring tools applied to and validated in captive populations can be used with a high level of confidence in the free-ranging environment. The collection of urofaecal samples from birds for glucocorticoid hormone metabolite monitoring, allows for minimal researcher-animal interactions, while allowing for longitudinal sample collection.

At the National Zoological Gardens of South Africa (NZG) uro-faecal samples were collected from seven male and seven female individuals in order to monitor uro-faecal glucocorticoid metabolite (UrGCM) and uro-faecal thyroid hormone metabolite (UrTM) activity during important life history stages; this included the breeding, non-breeding and moult periods of *S. demersus*. A thyroid stimulating hormone (TSH) challenge was also conducted to determine

the suitability of the appropriate enzyme immunoassay (EIA) for monitoring UrTM patterns in the *S. demersus*. Throughout the entire study behavioural observations were collected from all fourteen study individuals for three days per week in the morning and afternoon to determine whether significant changes in behaviour, if any, do occur as a result of changes in endocrine patterns.

The results for the UrGCM concentrations showed no significant difference across the different life history stages, the TSH challenge has yielded inconclusive results, and the behavioural results show small differences across the different periods.

Spheniscus demersus in captivity could be displaying desensitization to the captive environment possibly due to the absence of predators as well as having a stable diet which does not seem to influence their stress levels. The results for this study are also important because it can assist zoo curators in establishing more efficient enclosure designs to minimise stressors experienced by animals in a captive environment and can also offer insight into the stressors that could be experienced by free-ranging individuals. This information can also help with the rehabilitation and subsequent release of animals from captive facilities back to the wild, thereby aiding in the conservation of species.

Molecular characterisation of vaccine candidates from Anaplasma marginale strains in South Africa

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Bovine anaplasmosis is an economically important tick-borne disease caused by the obligate, intraerythrocytic rickettsia, Anaplasma marginale. We have estimated losses due to mortality arising from the disease in South Africa to be R115 million per year. Despite the economic impact, there are few studies on prevalence and control of bovine anaplasmosis in the country. A blood vaccine containing the closely related rickettsia, Anaplasma centrale, is available, but it does not protect against all field strains and may transmit other blood-borne pathogens. Outer membrane protein (OMP) preparations are known to induce immune protection in nearly all animals tested, thus demonstrating the potential efficacy of a subunit vaccine. Five potential OMP vaccine candidates from North American A. marginale strains have been identified and are well-characterised in A. marginale strains from the United States of America (USA). However, their levels of conservation in other countries must be ascertained in order to inform their use in making a vaccine with regional or global efficacy. This study therefore aimed to evaluate the presence and genetic diversity of A. marginale in South Africa, and to characterise the OMP vaccine candidates in South African A. marginale strains.

Blood samples were collected from 517 cattle from all provinces of South Africa, and tested for the presence of *A. marginale* and *A. centrale* using a duplex quantitative real-time PCR (qPCR). Genetic diversity of *A. marginale* strains was assessed using *msp1a* genotyping. The OMP genes encoding the five vaccine candidates (Am779, Am854, Omp7, Omp8 and Omp9) were amplified from a total of 85 *A. marginale* positive samples and the amplicons were sequenced. Nine samples representing the major variants were selected, the respective

genes were amplified and cloned into the pET SUMO expression vector. Recombinant OMPs were expressed in *E. coli* and immunoblotted against sera derived from bovines vaccinated with OMP preparations from North American *A. marginale* strains, as well as sera from bovines either vaccinated with *A. centrale* or naturally infected with South African *A. marginale* strains.

Using qPCR, A. marginale and A. centrale were detected in 57% and 17% of samples respectively, with 15% being co-infected. An analysis of A. marginale strains present in the samples revealed high genetic diversity, as reflected by the 190 *msp1a* genotypes derived from 99 Msp1a amino acid repeat sequences. This genetic diversity is attributable to a high rate of evolution. Our data reveal that while 22% of the 99 repeat sequences were detected in other countries, only two of the genotypes found in South Africa have been identified elsewhere in the world. The OMPs Am854 and Am779 were found to be highly conserved, with 99-100% amino acid identity. Omp7, Omp8 and Omp9 were also conserved with 79–100% identity with US strains. As has been shown previously, Omp7-9 possess conserved N- and C- termini, along with a central hypervariable region. A previously identified, highly conserved T-cell epitope, FLLVDDAI/V, was also found in the conserved N-terminus of these three OMPs. Western analysis of recombinant OMPs revealed antigenic relationships between South African and US strains of A. marginale. Our results provide evidence to suggest that these five A. marginale OMPs are good vaccine candidates for use in a global vaccine cocktail, although further work on the best formulation and delivery methods will be necessary.

Epoxyscillirosidine induced cytotoxicity and ultrastructural changes in a rat embryonic cardiomyocyte (H9c2) cell line

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Moraea pallida Bak. (yellow tulp) poisoning is economically the most important cardiac glycoside-induced intoxication in ruminants in South Africa. The toxic principle is a bufadienolide, 1 α , 2 α -epoxyscillirosidine. The aim of this study was to evaluate the cytotoxic effect of epoxyscillirosidine on rat embryonic cardiomyocytes (H9c2 cells) by evaluating cell viability, cell membrane leakage as well as characterizing ultrastructural changes induced in H9c2 cells. Rat embryonic cardiomyocytes (10, 000 per well) were exposed for 24, 48 and 72 h to varying doses of epoxyscillirosidine (10, 20, 40, 60, 80, 120, 160, 200 μ M) and evaluated using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) and the lactate dehydrogenase (LDH) assays. H9c2 cells (100, 000 per well) were exposed to epoxyscillirosidine (40, 80, 120, 160 and

200 μ M, over similar exposure times), processed and viewed with a transmission electron microscope (TEM). Cell viability indicated a hormetic dose/concentration-response which was characterized by a biphasic low dose stimulation and high dose inhibition. The cytotoxic effect was dose and time dependent. The LC₅₀ was 382.68, 132.28 and 289.23 μ M for 24, 48 and 72 h, respectively. Ultra-structural changes observed were dose and time dependent. Appearance of numerous cytoplasmic vacuoles, karyolysis and damage to the cell membrane, indicative of necrosis, were observed at higher doses. The study showed that the toxic effect of epoxyscillirosidine to H9c2 is necrosis as evidenced by LDH leakage and confirmation by TEM and is consistent with myocardial necrosis observed microscopically in poisoned livestock.

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Raising antibodies against epoxyscillirosidine, the toxic principle contained in Moraea pallida Bak. (Iridaceae), in rabbits

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Moraea pallida Bak. (yellow tulp) poisoning is the most important plant-induced cardiac glycoside toxicosis in South Africa. Cardiac glycoside poisonings collectively account for about 33 and 10 % mortalities due to plants, in large and small stock respectively, in South Africa. The toxic principle, a bufadienolide, is 1α , 2α -epoxyscillirosidine. The aim was to investigate the potential to develop a vaccine against epoxyscillirosidine. Epoxyscillirosidine and the commercially available proscillaridin and bufalin, were conjugated to the carrier proteins [Hen ovalbumin (OVA, bovine serum albumin (BSA) and Keyhole limpet haemocyanin (KLH)], rendering them immunogenic. Adult male New Zealand White rabbits were immunized. In Trials 1 and 2, rabbits (n=6) were, each assigned in two groups. Experimental animals (n=3; n=4) were vaccinated with epoxyscillirosidine-OVA, while the control (n=3; n=2) were vaccinated with OVA, using Freund's adjuvant and Montanide, respectively, for Trials 1 and 2. In Trial 3, rabbits (n=15), allocated into 5 equal groups

(I, II, III, IV and V), were vaccinated with proscillaridin-BSA, bufalin-BSA, epoxyscillirosidine-KLH, epoxyscillirosidine-BSA conjugates, and BSA respectively, using Montanide as adjuvant. Vaccination was done on Days 0, 21 and 42 by intradermal injection of 0.4ml of the immunogen. Blood was collected pre-vaccination and at 3 week intervals thereafter. Antibody response was determined using an indirect ELISA. There was poor immune response associated with the dose and adjuvant used in Trial 1. For Trial 2, antibodies against the immunogen were successfully raised. In Trial 3, epoxyscillirosidine-KLH induced the highest immune response. Proscillaridin and bufalin antibodies cross-reacted with epoxyscillirosidine-OVA as antigen, in an ELISA, albeit with low activity. The study successfully demonstrated synthesis of antibodies against the bufadienolides. The cross-reactivity of proscillaridin and bufalin with epoxyscillirosidine could be explored in future studies to prevent yellow tulp poisoning by vaccination.

Tick-borne haemoparasite occurrence in eastern rock sengi (*Elephantulus myurus*) in South Africa

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Sengis, or elephant shrews, of the order Macroscelidea, are small insectivorous mammals endemic to Africa. Several studies have shown that sengis are parasitised by large numbers of ixodid ticks. Previous studies also provided strong evidence that the eastern rock sengi (*Elephantulus myurus*) may be a natural reservoir host of *Anaplasma bovis*, a rickettsial pathogen of cattle. Despite the importance of sengis as hosts of immature ticks and the association of these tick species to known pathogens, limited information is available on the role of *E. myurus* as a reservoir of tick-borne pathogens. The aim of the study was, therefore, to determine the tick-borne haemoparasite diversity in eastern rock sengi of South Africa by screening blood samples for the presence of *Theileria, Babesia*, *Ehrlichia* and *Anaplasma* spp. using the reverse line blot (RLB) hybridization assay. A total of 47% of the DNA extracts from eastern rock sengi blood samples tested negative or below the level of detection of the assay. PCR products hybridized with the *Theileria/Babesia* catch-all probe in 1.5% of the samples and 33.3% hybridized with the *Ehrlichia/Anaplasma* catch-all probe. The PCR products failed to hybridize with any Anaplasmataceae species-specific probes. This could suggest the presence of a novel species or variant of a species. The parasite 16S rDNA of selected positive samples was subsequently amplified and sequenced. The results confirmed the presence of *A. bovis*-like DNA and enable design of an RLB probe specific for the detection of the sengi *A. bovis*-like strain.

Soluble expression of Bacillus anthracis protective antigen in Escherichia coli and characterisation of its biological activity

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Bacillus anthracis, a Gram-positive, spore-forming bacterium, is the causal agent of anthrax. The Sterne live spore vaccine (SLSV) is the most widely used vaccine for the prevention of anthrax in animals. Though mostly effective in the control of the disease, the SLSV has several shortcomings. These problems could be addressed with the advent of non-living recombinant protein alternatives. The principal virulence factors of *B. anthracis* are coded on two plasmids, pXO1 and pXO2. The pXO1 encodes the toxic factors, protective antigen (PA), lethal factor and edema factors (LF and EF), while pXO2 encodes the poorly immunogenic poly-y-D-glutamic acid capsule. The exotoxins are basically regulated by PA which form half of the toxins. When LF binds to PA lethal toxin (LT) is formed and when EF binds to PA edema toxin (ET) is formed. It also facilitates the competitive intracellular translocation of LT and ET. In addition, it is the basis for the immune response in anthrax infection, anthrax vaccine and serological diagnosis. Therefore, this study focused on soluble and cost-effective expression of the recombinant protective antigen in E. coli host cells.

The *B. anthracis* PA with N-terminus polyhistadine-tagged was expressed in pStaby1.2 vector and two recombinants PA83-1-pStaby1.2 and PA83-2-pStaby1.2 were selected.

The two recombinants were transformed into *E. coli* SE strain (Delphin Genetics). Individual clones were subject to expression using different Isopropyl *B*-D-1- thiogalactopyranoside (IPTG) concentration (1 mM, 0.5 mM and 0.3 mM) to induce expression when culture absorbance reached OD_{600} 0.6-08 nm at different temperatures (37°C, 29°C, 25°C, 20°C and 15°C) and harvesting time post-induction. The protein was purified using two purification methods: crude extraction and Protino® Ni-TED packed column method under native condition. The biological activity was analysed using indirect enzyme-linked immunosorbent assay (*i*ELISA).

PA sequences of both recombinants, PA83-1 and PA83-2, were confirmed. The expression profile of the recombinants was analysed using Western blot. The optimum expression of PA on immunoblot was observed in cultures grown for 3 hours post-induction with 1 mM IPTG and overnight with 0.3 mM IPTG at 25°C as the optimal expression temperature. The anti-PA *i*ELISA results confirmed that the biological features of the recombinant PA (rPA), such as antibody binding epitopes, are intact. The soluble rPA expressed in this study which is biologically active can therefore be used as a non-living and cost-effective vaccine candidate for anthrax.

Plants used in ethnoveterinary medicine to treat prevalent livestock diseases in the Mnisi community, Mpumalanga province, South Africa

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Traditional medicine used for both animal and human health care plays a vital role in the development of new pharmaceuticals. In South Africa it is estimated that over 60% of people use plants as medicine. However, only a few plants have been documented for use in ethnoveterinary medicine (EVM). EVM is used mostly in developing countries like South Africa where there are many rural areas consisting of small scale farmers that have limited access to Western veterinary services or expensive pharmaceutical drugs. EVM serves as a low-cost and readily available alternative. Since EVM knowledge is passed on from generation to generation orally, there is a concern that with time the information may be lost, or inadequate information may be passed on to future generations, thus there is an urgent need to document the available knowledge of EVM. Following the Rapid Rural Appraisal (RRA) methods, a survey was conducted in the Mnisi Community at Bushbuckridge in Mpumalanga Province, South Africa with the aim of documenting the plant species that are used as EVM. 50 people between the age of 18 and 83 were interviewed. Eleven plant species belonging to seven families were reported by the farmers for use as EVM. Fresh plants from the wild were used to prepare the remedies as decoctions, infusions, pastes and extracted sap. The farmers of Mnisi community have been using traditional practices to sustain their livestock's health, production, disease prevention and control. More research will be conducted to scientifically validate the biological activities of the plants.

Bacterial blood microbiome of rodents captured from a human/livestock/ wildlife interface in Bushbuckridge, South Africa

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The Mnisi community is in Mpumalanga province, South Africa and is nestled in the heart of a human/livestock/ wildlife interface. Recent research in the area found rodents to be abundant with 76% of sampled households reporting rodents in and around their homes. Although it is well known that wild rodents serve as reservoir hosts for many human pathogens and that they play a key role in the natural circulation of viral, bacterial and parasitic infections, the role of rodents in Mnisi in the transmission of zoonotic pathogens to humans is unknown. The aim of this project was to provide a comprehensive insight into bacterial pathogens in the blood of wild rodents captured from different habitat areas in Mnisi using next generation sequencing approaches.

The 16S rRNA gene was amplified from genomic DNA extracted from the blood of 25 wild *Mastomys* species rodents using barcoded primers. Purified PCR amplicons were submitted for circular consensus sequencing on the Pacific Biosciences platform at the genomic sequencing core at Washington State University, Pullman, USA.

A total of 65,062 bacterial sequences were obtained, with the average number of reads per sample being 2602, which was

sufficient to satisfy a rarefaction curve indicating that all operational taxonomic units (OTUs) were captured. Notable organisms of zoonotic interest included members of the genus Bartonella, B. grahamii and B. tribocorum which were the dominant organisms in the rodent blood microbiome. Overall, rodents from Hlalakahle (urban/periurban) and Thlavekisa (communal rangeland) had higher proportions of Bartonella species (~85%), while Gottenberg (urban/periurban) and Manyelethi (protected area) (~45%) had lower Bartonella loads. Other organisms of zoonotic and veterinary significance detected included Ehrlichia chaffeensis (~0.03%), Anaplasma phagocytophilum (~0.5%), Anaplasma centrale (~0.01%) and members of the genus Brucella (~1%): Brucella abortus, B. melitensis and B. ceti. The results of the microbiome analysis were validated for zoonotic organisms of interest using conventional PCR techniques.

This study serves as the first report of the detection of zoonotic agents such as *Bartonella* species, *Ehrlichia chaffeensis* and *Brucella* species in rodents in the Mnisi community and highlights the possible risk they pose to human health within the community.

In silico screening of the Theileria parva proteome for identification proteins responsible for transformation of infected host lymphocytes

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Theileria parasites can be grouped into schizont transforming and non-transforming species and the former are the leading cause for mortality and morbidity in livestock worldwide. Transforming Theileria species infecting cattle include T. parva and T. annulata and very little is known about the genetic basis of the virulence. The availability of the genome sequences of these parasites has provided an opportunity to study their biology and pathogenic processes. Thus, the aim of this study is to investigate the T. parva proteome for identification of host cell phenotype modulators (HCPMs). Possible HCPMs were predicted using a combination of bioinformatics tools targeting secreted, membrane and cytoplasmic proteins. Tools for signal peptide, subcellular localization, homology, proteinprotein interactions and domain/motif prediction were among those employed, namely SIGNALP, CELLO2GO, KEGG, BLASTp, STRING, SMART and Pfam. Signal peptide and subcellular localization prediction analyses resulted in identification of 1188 proteins from the proteome of 4035 proteins. Subsequently, 977 proteins with homologs, orthologs and paralogs, in non-transforming parasites were negatively

selected. The remaining 211 proteins were further analysed for protein-protein interactions (PPI) and domain prediction. The PPI analysis revealed 20 proteins with ten interacting partners in the T. parva proteome, with some of the interacting partners associated with up to 18 proteins. Among interactors were heat shock (HSP90) and proteinase protein families which are associated with oncogenic pathways playing a significant role in replication, activation of the NF-kB complex and regulation of apoptosis and metastasis. Further analysis of domains/motifs of proteins without homologs/orthologs/ paralogs revealed three dominating domains/motifs, FAINT also known as DUF529 (n=118), PEST motif (typical of Tash protein) (n=37) and DUF1430 (n=9). Proteins possessing a DUF1430 domain are reportedly involved in immunity and proteins with a PEST motif, typical of Tash AT-hook proteins, are associated with pathogenesis, while those with a FAINT domain are predicted to play a role in host cell modification. Considering the predicted functions of these proteins in the parasite and the host, they are worth investigating further as possible host cell phenotype modulators.

Spatial analysis of anthrax outbreaks and cases in Lesotho between 2005-2016

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Limited information is available on the epidemiology of anthrax disease among livestock animals in Lesotho. Therefore, this study investigated the spatial pattern of anthrax outbreaks and cases in Lesotho. Descriptive and spatial analyses were performed on anthrax outbreaks and cases reported to the Department of Livestock Services between 2005 and 2016. Anthrax outbreaks and cases were geo-coded at village level and aggregated at the district level. Moran's I and spatial scan statistics were used to investigate spatial clusters of outbreaks and cases at village spatial scale. A total of 38 outbreaks in the Lowlands districts were reported. The highest proportion of outbreaks and cases were in Maseru district, 52.6% and 70.2% respectively. Leribe district reported the least proportion of outbreaks (5.3%) and cases (0.6%). At village level, 18.4% proportion of outbreaks were in Maseru urban followed by Mofoka (13.2%) and Ratau (10.5%). However, Mofoka reported the highest proportion of cases (40.1%), followed by Ratau (24.5%). There was a significant clustering of anthrax outbreak cases at village spatial scale. Three significant clusters were identified. The first cluster involved five villages (Relative Risk [RR]: 6.1, P=0.001). The second and the third clusters were in Thaba-Chitja (RR: 9.8, P=0.001) and Kolo (RR: 13.1, P=0.001), respectively. Three significant clusters of high-risk of anthrax were detected in the Lowlands. Therefore, anthrax prevention and control strategies should be targeted towards lowlands areas of Lesotho.

Prevalence of cysticercosis in cattle and pigs slaughtered in Gauteng abattoirs

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Cysticercosis is a muscular infection caused by metacestodes of the zoonotic parasites, *Taenia saginata* and *Taenia solium* in cattle and pigs respectively. Humans are final host of these tapeworms whereas cattle and pigs are intermediate hosts. Meat inspection has been the only test used at abattoirs as a diagnostic tool for cysticercosis; however, serological and molecular assays have been developed and are more sensitive and specific. This study was conducted to determine prevalence of cysticercosis in cattle and pigs slaughtered around Gauteng Province using meat inspection, serology and molecular tools. Demographic information on slaughter animals was obtained and records (4-5 years) of visual diagnosis of cysticercosis in Gauteng province were reviewed. Blood and tissue samples (masseter, diaphragm, heart and tongue) were collected from 323 cattle and 106 pigs in 18 Gauteng abattoirs. Visual inspection was conducted to detect cysts. Commercially available B158C11A10/B60H8A4 Ag-ELISA (apDIA Cysticercosis) with manufacturer's instructions (ApDia n.v, 2004) was used to detect antigens of *Taenia* infections and a real-time polymerase chain reaction (qPCR) was used to detect *T. saginata* and *T. solium* DNA in tissue samples. Records revealed that the prevalence of bovine and porcine cysticercosis between the year 2013 and 2017 ranged from 0.0002 to 0.7% in cattle and 0.0002 to 0.0006% in pigs. No cysts were observed during meat inspection; seroprevalence of cysticercosis in cattle and pigs was 2.1% (7/323) and 0.99% (1/106) respectively. Analyses of the data from PCR assays are nearing completion.

Tick-borne disease dynamics in calves at the wildlife-livestock interface in the Mnisi community area, Mpumalanga province, South Africa

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Anaplasmosis, babesiosis, and heartwater are three most important tick-borne diseases of cattle in South Africa resulting in a large number of mortalities, while theileriosis remains controlled. Endemic stability contributes to disease control, but little is known about the conditions required for maintenance of endemic stability. Through the ongoing Health and Demographic Surveillance System in Livestock in the study area of the Mnisi One Health Platform, Mpumalanga, a great deal of information is being collected on the cattle in the area. Numerous cattle have been identified for tick burden assessment, serological analysis and parasite identification in blood samples. However, little is known about the timing of infection of cattle with various tick-borne hemoparasites. Therefore, this study aimed to investigate the time-course of infection in calves (n=10) during a period of 1 year using pathogen-specific reverse line blot (RLB) hybridization and quantitative polymerase chain reaction (qPCR) assays.

Blood samples were collected monthly from calves in two areas of the Mnisi community: five located in a peri-urban area and five at the wildlife interface. A total of 119 blood samples were collected. The RLB hybridization assay revealed the presence of both pathogenic and non-pathogenic hemoparasites (Theileria, Babesia, Anaplasma and/or Ehrlichia species) in all 10 calves in both areas. All calves tested RLBpositive for one or more hemoparasite from the age of 1-2 months. The qPCR assays confirmed the presence of A. marginale, E. ruminantium and B. bigemina in calves from 0-1 months. The levels of parasitemia as determined by the qPCR assays varied from the time of infection to one year old, and at some time points dropped below the detection limit. A higher number of calves had detectable pathogenic hemoparasite infections throughout the year in the periurban area than calves at the wildlife-livestock interface. In two calves at the wildlife-livestock interface, A. marginale was not detected in their first year of life.

		Number of calves testing qPCR-positive at each month of age											
		1	2	3	4	5	6	7	8	9	10	11	12
Anaplasma marginale	Peri-urban	3	5	5	5	5	5	1	2	4	5	5	4
	Interface	0	0	0	0	0	0	1	2	1	2	2	2
Ehrlichia ruminantium	Peri-urban	2	4	3	1	0	2	1	1	2	1	1	0
	Interface	2	2	3	1	0	0	3	2	1	3	0	0
Babesia bigemina	Peri-urban	2	3	2	4	4	3	5	5	5	5	5	4
	Interface	0	1	0	1	1	1	3	3	4	4	4	4

The results confirm the exposure of calves in the Mnisi Community to non-pathogenic and pathogenic tick-borne hemoparasites from the age of 0-1 month. However, some calves were either infected at levels below the detection limit of our assays, or they were not infected at all. If the latter, it is possible that exposure to related non-pathogenic hemoparasites might help to establish and maintain endemic stability. Other factors such as cattle density and dipping methods may also play a role.

Preliminary screening of selected South African medicinal plants for the control of root-knot nematodes, *Meloidogyne incognita*

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The root-knot nematode (RKN), Meloidogyne incognita Kofoid and White (Chitwood), is considered one of the most important plant-parasitic nematode (PPN) species affecting the quantity and quality of many annual and perennial agricultural crops worldwide. Symptoms on infected plants include stunting, root galling and nutrient deficiency, particularly nitrogen deficiency. In South Africa many rural and peri-urban communities and households depend on crops that are highly susceptible to RKN, such as vegetables for food and dietary requirements, and the impact of this pathogen on food security is, therefore, real and substantial. Resource-poor rural and peri-urban tomato growers do not have the financial means to buy nematicides, which are usually effective in reducing PPN population densities below damage threshold levels in the short term. This creates the need to find alternative, affordable, but also effective practices to control RKN on this

crop. The main objective of the study was to examine and select botanical plants through extensive literature survey to control Meloidogyne incognita. Plants were selected based on published nematocidal activity reported against free-living and animal parasitic nematodes. Eleven plant species were selected for preliminary screening against J2 nematode larvae of Meloidogyne incognita. Fresh leaves from these plants were extracted in water, acetone and methanol:dichloromethane (1:1) to obtain a total of 33 crude extracts. The extracts were incubated with 100±120 J2 nematodes for 24, 48 and 72 h at the highest concentration of 1 mg/mL. Lantana rugosa, Leonutus leonurus and Clausena anisata extracts showed nematicidal activity after 48 hours of exposure. This report correlates with activity reported on free-living and animal parasitic nematodes and further studies are undergoing to determine LC₅₀ values and the mechanism of action.

Screening of host proteins that interact with Rift Valley fever virus glycoproteins using a yeast two hybrid system

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Rift Valley fever (RVF) is an acute mosquito-borne viral disease affecting humans and a wide range of animals including sheep, cattle, goats and some wildlife species. The causative agent, Rift Valley fever virus (RVFV) belongs to the genus *Phlebovirus* in the family *Bunyaviridae*. The viral particle has three genome segments, termed large, medium and small. The lipid bilayer anchors the glycoproteins (Gn also termed G1, and Gc also termed G2). These form spike-like projections and are the outer parts of the viral particle. The glycoproteins play an important role in penetration of the virus during infection, and entry of the virus into the host cell is mediated by these glycoproteins through an unidentified receptor/s. The study aims to identify host receptors for the Gn and Gc capsid proteins of RVFV.

The objectives of the study were a) Construction of cDNA from baby hamster kidney cells (BHK21), b) Evaluation of cDNA library, c) Construction of bait plasmids and d) Use of the expressed glycoproteins (bait plasmids) to screen the library for possible interacting proteins using a yeast two hybrid (Y2H) system.

The cDNA from BHK cells was prepared and cloned into pGADT7-Rec shuttle vector. For the bait, the ectodomains of Gn and Gc were constructed in the pGBKT7 shuttle vector.

The constructs were expressed in the Y2HGold yeast strain. The bait protein expressions were validated by Western blot analysis, autoactivation and toxicity tests in comparison with the controls (empty vector pGBKT7).

The BHK cells were chosen to act as host cells in the laboratory because hamsters are susceptible to RVF. From the constructed cDNA library, 51 clones revealed genes from the Syrian/Golden hamster sequences on BLAST analysis. The ectodomains of both Gn and Gc were successfully amplified and cloned in the shuttle vector. Bait proteins were expressed in yeast cells and proteins were confirmed by probing with sera collected from infected animals. No autoactivation or toxicity was detected in proteins expressing ectodomains of Gn and Gc.

In conclusion, the baits, pGBKT7-Gn and pGBKT7-Gc met the requirements as baits to be used in the Y2H assay. The cDNA library from the RVFV susceptible cell line has been constructed successfully and is therefore suitable for studying RVFV-host interactions. Both baits were successfully used to screen the constructed library for interacting proteins. Twelve unique clones were screened (4 with pGBKT7-Gn and 8 with pGBKT7-Gc). Characterization of the clones is in progress.

Prevalence and characterization of *Salmonella* spp. in beef abattoirs, beef and beef products in Gauteng

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Salmonella spp. cause salmonellosis which is the second leading foodborne disease worldwide. There is a scarcity of information regarding the prevalence of *Salmonella* spp., in beef production in South Africa, primarily due to lack of targeted monitoring of foodborne pathogens. The aim of this study was to determine the prevalence, risk factors and characteristics of *Salmonella* spp., from beef abattoirs and retail outlets in Gauteng province, South Africa.

In a cross-sectional study, 824 various types of samples (meat, swabs, water) were collected from 12 abattoirs (n=423) and 31 retail outlets (n=401) during 2015 and 2016. The isolation and identification of *Salmonella* spp., was performed using classical microbiological techniques and characterization of *Salmonella* spp was done using the Kauffman-White scheme. Antimicrobial susceptibility testing was performed for 17 antibiotics according to the Clinical & Laboratory Standards Institute (CLSI) guidelines.

The overall prevalence of *Salmonella* spp. in all samples was 8% (66/823). In abattoirs it was 9.7% (41/423; 95% CI: 7.0–12.9) and in retail outlets 6.2% (25/401; 95% CI: 4.1–9.1). Amongst the samples collected from abattoirs, effluents had the highest contamination of 35% (7/20; 95% CI: 15–59), followed by post evisceration samples with 17% (10/59; 95% CI: 8–29), swabs of wall and floor with 13% (3/23: 95% CI: 3–34), pre-evisceration samples 10% (6/59; 95% CI: 4–21), perineal swabs 7% (4/57; 95% CI: 2–17), rinsates 7% (4/55; 95% CI: 2–18), faeces with 5% (3/59; 95% CI: 1–14), post wash swabs with 5% (3/56; 95% CI: 0.1–15). Prevalence differed significantly between sample types (p=0.010). Samples

from the retail outlets showed the highest prevalence of contamination in minced meat 9% (8/93; 95% Cl: 4–16), followed by cold meat 8% (4/52; 95% Cl: 2–19), boerewors 6% (6/93; 95% Cl: 2–14), brisket 6% (4/71; 95% Cl: 2–14) and biltong 4% (4/92; 95% Cl:1–11); however, these did not differ significantly (p=0.719).

A total of 17 *Salmonella* serotypes was obtained in the study. Serovars found in abattoirs were S. Typhimurium (6), S. Schwarzengrund (5), S. Lagos (3), S. Molade (3), S. Muenster (3), S. Vejle (3), S. Anatum (2), S. Bardo (2), S. Cleveland (2), S. Cremieu (2), S. Amersfoort (1), S. Be (1), S. Irumu (1), S. Kottbus (1), S. Oritameum (1), S. Tibati (1) and S. subspecies (4). Serovars found in the retail survey were S. Schwarzengrund (9), S. Kentucky (5), S. Vejle (5), S. Anatum (4) and S. Typhimurium (2). All the isolates were resistant to penicillin, erythromycin, vancomycin and all were susceptible to ciprofloxacin, ceftriaxone, amikacin, gentamycin. Of the 66 positives, 14% were resistant to cefoxitin, 17% to kanamycin, 42% to cephalothin, 9% to STX, 38% to AMC, 32% to chloramphenicol, 3% to nalidixic acid, 35% to oxacillin, 32% to streptomycin and 59% to tetracycline.

The results of this study showed a high prevalence of *Salmonella* in both abattoirs and retail outlets, with no significant difference between abattoirs and retail outlets in terms of prevalence. Some serovars obtained from abattoir survey were also obtained from the retail survey. These results provide a measure of the potential risk posed by beef to human health if products are not adequately prepared prior to consumption. The resistance of some serotypes to antibiotics is also a serious public health concern.

Prevalence of Q-fever in South Africa: A review of diagnostic laboratory data at the Agricultural Research Council – Onderstepoort Veterinary Research from 2007-2009

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Q-fever is one of the most underestimated zoonoses in South Africa (SA) despite causing significant losses in livestock and game through reproductive disorders such as late abortions, low birth weight or infertility. Humans contract the disease from direct contact with milk, faeces, semen as well as inhalation of aerosolized particles from infected animal placenta causing mild influenza illness. The disease is caused by infection by the intracellular bacterium, *Coxiella burnetii* (*C. burnetii*). The last published report on Q-fever prevalence in animals in SA was in 1987 and there is a need for recent data on Q-fever as well as strains currently circulating in the country. In this study, we reviewed available Q-fever diagnostic laboratory data (DLD) at the Agricultural Research Council-Onderstepoort Veterinary Research Campus (ARC-OVR) from 2007 to 2009 with the aim to establish its prevalence in the country.

Diagnostic samples were obtained from serum samples submitted for Q-fever testing as part of disease surveillance by state veterinarians. A total 740 serum samples comprising 369 bovine, 226 ovine, 76 caprine and 69 from game animals were tested for Q-fever using complement fixation test (CFT).

Overall, the seroprevalence of Q-fever was 0,95% (7/740) which comprised 2 bovine, 0, 27% (2/740) from KZN, 3 ovine 0, 41 % (2/740) from WCP, 1 ovine 0,14 % (1/740) from NCP and 1 ovine 0, 14 % (1/740) from the ECP.

The low number of samples submitted for Q-fever testing between 2007 and 2009 together with the fact that the last published report on Q-fever in SA was more than 30 years ago shows that Q-fever is still one of the most ignored zoonoses despite causing significant losses in livestock and game. Thus frequent testing of livestock and game for Q-fever may provide an important source of recent data for implementation of surveillance and control strategies of the zoonosis.

Abbreviations: NCP-Northern Cape, WCP-Western Cape, ECP-Eastern Cape, KZN-Kwazulu Natal

Species composition and the role of horse-flies in pathogen transmission in south-eastern KNP (Diptera: Tabanidae)

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Tabanidae, commonly known as horse-flies, are a large family of important pollinators, generally believed to be among the most basal brachycerans (Diptera: Brachycera). In addition to their importance in the ecosystem, the females are haematophagous and capable vectors of pathogens. Despite their importance, the family has been severely neglected by science. The current study was aimed at determining the species composition of tabanids in the Kruger National Park (KNP), South Africa. Using three different traps, tabanids in four habitats in southeastern KNP were sampled. In total 14 traps were used, namely: Manitoba (6), Ngu (4) and H traps (4). Manitoba traps captured an average of 1.7 flies/trap/day, Ngu 0.7 flies/trap/day and H trap 2.4 flies/trap/day. A total of 247 flies were captured with the H trap as the most effective. Morphological analyses revealed a total of five different genera, namely: *Haematopota, Tabanus, Philoliche, Chrysops* and *Atylotus*. Thirteen species were collected and the dominant tabanid species (*Tabanus minuscularius*) accounted for 55% (136/247) of the total flies sampled. Investigations are still on going to determine the role of tabanids in pathogen transmission in southern-eastern KNP.

Comparison of decontamination methods for the primary isolation of *Mycobacterium* spp. from milk

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Bovine tuberculosis (BTB) is a chronic respiratory disease of cattle caused by *Mycobacterium bovis* which also affects other domestic animals, wildlife animals and humans (zoonotic TB). Raw milk from BTB infected animals can serve as a vehicle for the transmission of *M. bovis* to humans and the disease is therefore a potential threat to human health and livelihoods, especially in developing countries. The isolation of mycobacteria presents a number of difficulties such as the organism's slow growth rate compared to other microorganisms and the low concentration of the mycobacteria likely to be present in milk of poor microbiological quality. Optimisation of the decontamination method for isolating mycobacteria from milk collected from communal cattle is of importance in the context of higher bacterial contamination often found in milk.

The aim of the study was to compare the efficacy of five decontamination methods applied to milk for the primary isolation of *Mycobacterium* spp. using the example of *M. fortuitum*. This *Mycobacterium* species was selected due to its rapid growth (2–3 days) comparative to *M. bovis* on culture, hence allowing the results to be obtained in a short period of time. Pasteurised milk was spiked with *M. fortuitum, Escherichia coli* and *Staphylococcus aureus* with 10° cells/mL of each organism and serial dilutions were prepared. *E. coli* and *S. aureus* were included to take

the role of contaminants in the experimental samples as these organisms have previously been detected in milk from communal cattle. The spiked milk prior to culture on LJ pyruvate was treated with one of the following methods: 1) 10 mL of 5% Oxalic acid (OA) for 15 min; 2) 7% NaCl and 4% NaOH (HS-SH) (5 mL each) for 15–20 min followed by addition of 5 mL phosphate buffer pH 6.8; 3) 10 mL of 1% CPC for 5 hours; 4) 10 mL of 2% HCl for 10 min followed by addition of 10 mL distilled sterile water for neutralisation; 5) 10 mL of 4% NaOH for 10 min followed by addition of 10 mL distilled sterile water for neutralisation.

This study showed that 5% OA and 2% HCl had a limit of detection at 10^2 cells/mL and 10^1 cells/mL respectively. HS-SH and 4% NaOH were harsh to the mycobacteria which resulted in a limit of detection at 10^5 cells/mL and 10^4 cells/mL respectively. However, 1% CPC was not able to suppress or eliminate the contamination sufficiently. Overall, *M. fortuitum* survived and grew confluent on cultures incubated at 37°C for 2–3 months when milk samples were decontaminated with 2% HCl and 5% OA and inoculated on LJ pyruvate, resulting in a suitable method of isolating the mycobacteria. The period at which the slopes were incubated without contamination indicates that all slow growing mycobacteria will grow confluent when milk samples are treated with 2% HCl and 5% OA.

Prevalence and characterisation of *Salmonella* species from chickens sold in informal poultry markets in Gauteng, South Africa

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Salmonellae are linked to many food-borne cases and epidemics in humans and animals. This cross-sectional study was conducted to determine the prevalence and characteristics of *Salmonella* spp. in chickens sold in informal markets in Gauteng, South Africa.

A total of 151 samples of chicken carcasses and carcass drip were collected from 47 outlets in the informal market and standard methods were used to detect and characterize isolates of *Salmonella* spp. The prevalence of *Salmonella* spp. in carcass drip, cloacal swabs and carcass swabs was 43.0%, 27.8% and 27.2% respectively.

Prevalence of *Salmonella* was high in Alexandra (100.0%), GaRanguwa (80.0%) and Soweto (72.0%) but low in Tembisa (12.5%). Of 268 *Salmonella* isolates typed by multiplex PCR, 157 were typable with the detection of nine serotypes. The predominant serotypes were *S*. Bovismorbificans (31.8%), *S*. Enteriditis (12.7%) and *S*. Dublin (8.9%). Of 16 antimicrobial agents tested, resistance was exhibited to 15 (93.8%) by *Salmonella* isolates from carcass drip and cloacal swabs while to only 6 (37.5%) by isolates from carcass swabs.

The frequency of resistance was generally high to erythromycin (100.0%), spectinomycin (93.0%) and streptomycin (82.8%) but low to chloramphenicol (0.7%), ciprofloxacin (0.7%) and norfloxacin (0.7%). Multidrug resistance was exhibited by 7(2.6%) isolates and the predominant patterns were CEFT-KA (2.6%), AMOX-NA-CN (1.9%) and CIP-NOR-C (0.7%).

All 12 virulence genes assayed for by multiplex PCR were detected in the 268 isolates of *Salmonella* at high frequency for *InvA* (100.0%), *ShdA* (91.0%) and *MgtB* (87.7%) but low to *SpvC* (2.2%), *SefC* (1.5%) and *PefC* (0.4%). Data obtained in the study may assist with the strengthening of intervention strategies for *Salmonella* control in South Africa.

Prevalence and antimicrobial susceptibility of coagulase positive Staphylococcus species from milk samples submitted to the Onderstepoort Faculty of Veterinary Science

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Coagulase-positive Staphylococcus (CoPS) species have been implicated in clinical mastitis cases in dairy cattle. However, limited information is available on the prevalence of CoPS among dairy cattle in South Africa. The aim of this study was to investigate the prevalence and antimicrobial resistance patterns of CoPS isolated from dairy cow milk samples submitted to the Onderstepoort milk laboratory. A multistage sampling technique was used to randomly select milk samples from 2862 dairy cows. Suspected CoPS species were identified based on colony morphology and biochemical tests. The Analytical Profile Index (API) kit was used to cofirm all CoPS isolates. Antimicrobial susceptibility testing was done using the Kirby–Bauer method. Twenty-three percent (23.2%; 665/2862) of samples tested positive for Staphylococcus species. Of these, 21.1% (605/2862) were coagulase negative Staphylococcus species (CoNS) and 2.0% (58/2862) CoPS. Among CoPS, S. aureus (86.2%, 50/58) was the most common followed by S. hyicus (13.8%; 8/58). All CoPS (100%) isolates

were resistant to at least one category of antimicrobials (AMR), while 91.4% were resistant to three or more antimicrobial categories (MDR). The majority of CoPS isolates were resistant to streptomycin (93.1%), erythromycin (63.8%) and ampicillin (63.8%). Ninety-two percent (92.0%) of S. aureus were MDR with majority isolates exhibiting resistance to streptomycin (92.0%), erythromycin (62.0%) and ampicillin (62.0%). Almost half (46.0%) of S. aureus isolates were methicillin resistant but only 8.0% (4 isolates) were resistant to cefoxitin. Seven out of eight Staphylococcus hyicus isolates were MDR. Majority of S. hyicus isolates were resistant to streptomycin (100%), erythromycin (75.0%) and ampicillin (75.0%). Staphylococcus aureus is the predominant CoPS species associated with infectious mastitis among dairy herds in this study. Both CoPS (S. aureus and S. hyicus) exhibited high prevalence of resistance to streptomycin, erythromycin and ampicillin. In addition, MDR and methicillin resistance was very common among S. aureus isolates.

Evaluation of the effectiveness of pulse oximetry at different attachment sites to detect hypoxaemia in immobilized impala (*Aepyceros melampus*)

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Pulse oximetry is a popular, cost-effective and simple way to continuously monitor oxygenation of haemoglobin (SpO₂) in humans and animals. Even though pulse oximetry has been used extensively in wildlife, its efficacy and accuracy has not been validated. We aimed to establish, in immobilized impala, whether pulse oximetry is a reliable method to measure oxygenation of haemoglobin. We also aimed to determine which attachment site gives the most accurate measures. Impala were immobilized with etorphine and arterial blood samples were collected from the auricular artery at 5 minutes intervals during immobilization. An EPOC blood gas analyser was used to measure PaO₂ and calculate cSaO₂ (calculated arterial haemoglobin oxygen saturation) and an AVOXimeter co-oximeter was used to measure SaO₂ (gold standard measure of arterial haemoglobin oxygen saturation) from blood. Pulse oximeter probes were attached at four sites; namely the ear, cheek, rectum and under the tail. Thereafter, pulse oximeter

readings (SpO₂ and pulse quality) were recorded at each site and compared with SaO, and cSaO, using Bland-Altman and Root mean squares (Arms) methods to determine the level of agreement. Overall, the pulse quality measured was generally good at each attachment site. Pulse oximetry measured under the tail was accurate and precise but only when SaO₂ values were above 90% (bias= 0.46, precision=3.47, Arms=3.38). For the ear probe, the overall bias was low with a high precision indicating that pulse oximetry was accurate (bias=-3.02) but imprecise (Precision=10.75). The cheek and rectal probes failed to give accurate or precise readings (cheek: bias=10.86 precision=8.56 Arms=13.72 rectum: bias=4.92 precision=6.9 Arms=8.47). In order to get accurate and precise pulse oximetry readings in immobilized impala, probes must be placed under the tail and SaO₂ must be above 90%. Since SaO₂ values are usually low in immobilized impala, pulse oximeter should be used with caution.

Diversity of the sporozoite antigen gene p67 in *Theileria parva* field isolates from cattle and buffalo in southern and eastern Africa

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Cattle theileriosis is a tick-borne disease caused by a protozoan parasite Theileria parva, which has its natural reservoir in the African buffalo. East Coast fever (ECF) caused by the cattle-derived *T. parva* is a major problem in eastern Africa and some parts of southern Africa. In South Africa, ECF was eradicated in the 1950s; however, Corridor disease (CD) caused by the buffalo-derived *T. parva* remains a concern. The sporozoite gene encoding T. parva antigenic protein, p67, has been targeted for the development of a recombinant DNA vaccine for control of T. parva infections in cattle. Since immunity to T. parva infections depends on the diversity of parasite antigens, it is crucial to investigate the diversity of the p67 gene. Thus, in this study, we assessed the diversity of the p67 gene in *T. parva* isolates infecting buffalo and cattle in South Africa, Mozambique, Kenya, Tanzania and Uganda. A 900bp fragment of this gene was amplified from DNA extracted from blood and the amplicons were subjected to

DNA sequencing following cloning. p67 sequence analysis revealed up to four alleles from buffalo-derived isolates. On the contrary, only a single allele (allele 1) was identified from the cattle-derived isolates from Kenya, Uganda and Tanzania. Notable from South African cattle, CD isolates obtained from clinical cases had four p67 alleles while carriers had one allele (allele 4). Furthermore, an analysis of the two p67 immunodominant B cell epitopes in buffaloderived isolates, including CD isolates from clinical cases in South Africa, revealed nine types of SNPs on epitope 1 (¹⁶⁹TKEEVPPADLSDQVP¹⁸³) and six on epitope 2 (²⁰⁹LQPGKTS²¹⁶). However, both epitopes were conserved in the cattle-derived (ECF) isolates. These findings demonstrate that, to achieve broad protection against both buffalo- and cattle-derived T. parva, the diversity observed on p67 B cell epitopes in buffalo-derived isolates should be considered in the development of a subunit vaccine.

Antimicrobial use practices and resistance in indicator bacteria in communal cattle in the Mnisi community, Mpumalanga

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Surveillance of both resistance profiles and antimicrobial use is critical in the containment of resistance to mitigate against a post-antibiotic era, given the rapid resistance emergence and spread and yet limited production of novel antimicrobial agents. Despite the recognition of the importance of surveillance, there is a paucity of published research on antimicrobial use practices and resistance patterns in communal farming systems in South Africa.

The aim of this study was to determine the resistance of the indicator bacteria, *Escherichia coli* and enterococci, isolated from communal cattle and to determine the knowledge levels on and use practices of antimicrobial agents by farmers in Mnisi Ward 1 in Mpumalanga province of South Africa.

Rectal swabs were collected from cattle (n=100) from different households at five dip tanks for culture of *E. coli* and enterococci on MacConkey and blood agar and identification using primary biochemical tests, API 10S, streptococcal grouping kit and differential substrate utilisation. Susceptibility of the isolates to antimicrobial agents belonging to different classes was determined using a broth micro-dilution method. Seventy farmers were also interviewed on antibiotics they have used and where they source the drugs, observation of withdrawal periods, disposal of expired antibiotics and knowledge on resistance. In total, 150 (79 *E. coli* and 71 *Enterococcus* species) bacterial isolates were obtained. The enterococci species isolated were *E. faecium* (33), *E. faecalis* (3), *E. durans* (3), *E. avium*(3) and non-specified *Enterococcus* species (29). *E.coli* isolates exhibited resistance to colistin (16.5%), amoxicillin (8.9%), chlortetracycline (8.9%) and enrofloxacin (2.5%), and complete susceptibility to gentamicin. Enterococci isolates exhibited resistance to amoxicillin (2.8%), enrofloxacin (61%) and erythromycin (38%), and complete susceptibility to chlortetracycline and vancomycin.

The farmers indicated that they source their drugs from the local animal clinic and from an agricultural retailer in the nearby town. Among the various listed antibiotics, farmers (87%) indicated having used tetracyclines yet worryingly nearly all of them (98.5%) neither know what an antimicrobial drug is nor understand the concept of resistance.

Resistance in the indicator bacteria was generally low. However the detection of colistin resistance is a cause for concern and warrants further research. A critical outcome of this study is the identification of the need for farmer education to raise awareness on resistance and promote prudent use of antimicrobial agents in the community.

Pathology in dogs that died following natural infection by African horse sickness virus

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African horse sickness (AHS) is a highly fatal arthropodtransmitted viral disease affecting mainly equids. It is caused by African horse sickness virus (AHSV), an Orbivirus of the family Reoviridae. Dogs are known to contract fatal AHS by ingestion to AHS-infected horse meat. At the Section of Pathology, Department of Paraclinical Sciences, Faculty of Veterinary Science, University of Pretoria a number of canine mortalities ascribed to natural infection of AHS virus have been diagnosed since 2006. African horse sickness as the cause of death in approximately 32 dogs was confirmed at this institution by a combination of diagnostic techniques including macropathology, histopathology, immunohistochemistry and PCR. Limited published information regarding the pathological findings in AHSV-infected dogs however exists. The epidemiology of the canine manifestation of AHS and role of dogs in the transmission of the virus is unclear and may possibly be underestimated because dogs are not recognized as a host that may become infected by means of arthropod-transmission as in the horse. The focus of this retrospective study is to provide a full macroscopic, histologic and ultrastructural description of the pathological changes observed in dogs naturally infected by AHS virus as well as to discuss key epidemiological factors of AHSV in dogs.

Pathology records of dogs diagnosed with AHS at the Section of Pathology, Department of Paraclinical Sciences,

Faculty of Veterinary Science, University of Pretoria, since first being diagnosed in 2006 have been reviewed retrospectively. Macroscopical findings were recorded and formalin fixed tissues were prepared for routine light microscopic examination as well as immunohistochemical labelling for AHSV. Macroscopically the most significant lesions were observed in the lungs and were typical of severe acute interstitial pneumonia associated with mild serofibrinous pleuritis and moderate mediastinal oedema. Histopathologically the pulmonary findings included severe protein rich alveolar and septal oedema with randomly scattered areas of haemorrhage. Marked acute inflammatory changes were present, characterised by mononuclear leukostasis and alveolar exudation, with hyperactivation of the alveolar capillary endothelial cells, alveolar macrophage activation, and severe diffuse hyperaemia. Immunohistochemical labelling revealed AHSV-specific positive labelling of the microvascular endothelial cells as well as scattered monocytes and macrophages. The preliminary ultrastructural changes observed were supportive of vascular injury similar to those observed in horses infected by AHSV. The pathological findings observed in all cases were indicative of acute pulmonary inflammation of haematogenous origin, resulting in significant and fatal vascular injury. Histopathology confirmed the presence of significant endothelial injury resulting in severe protein rich pulmonary oedema in association with interstitial pneumonia.

Pathology and tissue tropism of natural Rift Valley fever virus infection in sheep

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The pathology of Rift Valley fever (RVF) was first characterized following the death of approximately 4,700 lambs and ewes on a farm in Kenya in 1931. There are inconsistencies in the description of the pathology in sheep, and limited immunohistochemical studies of the tissue and cell tropism of natural RVF virus (RVFV) infection are available.

All specimens originated from the carcasses of naturally infected sheep necropsied during the 2010 RVF outbreak in South Africa. A total of 124 cases were available for study of which 99 cases were classified positive for RVF with one or more positive test results for histopathology, rRT-PCR and/or IHC. Tissues were examined by histopathology (haematoxylin and eosin stains) and immunohistochemistry (polyclonal hyperimmune mouse sera and detection with a basic avidinbiotin complex system). Histomorphological features in all available organs were systematically recorded and reviewed within the context of lesions associated with RVFV infection and the results statistically analysed.

Liver necrosis was confirmed as the most distinctive histopathological feature of RVF cases in adult sheep. Necrosis is distributed irregularly throughout the lobule and focal degeneration of hepatocytes is accompanied by infiltration of the lesion with chiefly neutrophils and histiocytes. Sixtyfour percent (45/70) of the cases where liver, spleen and kidney tissues were available had foci of acute renal tubular epithelial injury in addition to necrosis in both the liver and spleen. Splenic necrosis was most apparent in the germinal centres, mantle zones and marginal zones of the white pulp and was characterized by the presence of cell debris and tingible body macrophages. Splenic necrosis was significantly associated with necrosis in the lymph nodes (rho = 0.532, P = 0.023). Severe changes were also observed in the gutassociated lymphoid tissue in the small intestine that mirrored changes identified in the lymph nodes. Other significant histopathological lesions included foci of necrosis in the adrenal glands, gallbladder, small intestine and skin; frequent pulmonary oedema; rare pulmonary haemorrhage; and haemorrhages in the myocardium and testis. RVFV antigen was detected in the liver, kidney, spleen, lymph nodes, lung, adrenals, heart, gastrointestinal tract, tongue, gallbladder, skin, uterus, and testis. The liver was most consistently and unequivocally positive for RVFV antigen followed by the spleen, kidneys, lung and skin. Notable, three cases had no discernible histological lesions or immunohistochemical labeling in the liver, but RVFV antigen was observed in the kidney. RVFV-laden macrophages were detected in the liver, kidneys, spleen, lymph nodes, lungs and the small intestine. Other RVFV antigen positive cells included hepatocytes, adrenocortical epithelial cells, renal tubular epithelial cells, epidermal keratinocytes, microvascular endothelial cells and vascular smooth muscle cells.

It is recommended that veterinarians and veterinary pathologists take multiple samples from the liver, spleen and kidney of sheep suspected to have RVF to include or exclude a diagnosis. Routine sampling of skin from the nose and ears might also be a useful adjunct sample since 55% of the skin samples in this study tested positive for RVFV.

The *in vitro* antibacterial activity of *Ficus exasperata* leaf extracts against *Campylobacter* and *Escherichia coli* relevant in poultry infections

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The most common cause of bacterial diarrhea in humans worldwide is infection with Campylobacter species, particularly Campylobacter jejuni and C. coli. The disease is zoonotic and domestic animals such as poultry, pigs and cattle may act as reservoirs. Colibacillosis is caused by Escherichia coli which is both a food and water borne zoonotic disease from poultry, calves and pigs. The development of resistant strains against the conventional antibiotics has reduced the efficacy of the antibiotics. Therefore, there is urgency to develop drug leads or templates with good activity against these pathogens. The antibacterial activity and safety of acetone, methanol and aqueous leaf extracts of Ficus exasperata on Campylobacter coli and selected clinical isolates of Escherichia coli was investigated in vitro using serial microdilution and cytotoxicity assays. The MIC values of selected plant extracts against the test organisms

generally ranged from 0.07 to 0.34 mg/ml. MIC values of the acetone extract ranged from 0.05 to 0.30 mg/ml while those of the methanolic extract ranged from 0.07 to 0.30 mg/ ml and the aqueous extract ranged from 0.07 to 0.34 mg/ ml. The LC₅₀ values of the acetone, methanol and aqueous extracts against Vero cells were 0.09, 0.99 and 0.26 mg/ ml respectively. The aqueous extract was relatively toxic to the intestinal CaCo-2 cell line. The selectivity index (SI) values of the acetone extract ranged from 1.28 to 1.80, methanolic extract ranged from 3.30 to 14.14 and aqueous extract ranged from 1.73 to 8.60 indicating that the observed antimicrobial activity was not due to toxicity to mammalian cells. These results highlight the potential of Ficus exasperata as an alternative for treatment of campylobacteriosis and colibacillosis in poultry. However, in vivo data is necessary to determine the potential usefulness of this plant species.

Antimycobacterial activity and toxicity screening of two southern Africa alien invasive plants

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Tuberculosis remains a global threat and one of the leading causes of mortality. The emergence of Multidrug-Resistant (MDR) and Extensively-Drug Resistance (XDR) tuberculosis has become a major challenge especially in Africa. In light of this, the use of medicinal plants as a source of treatment is advocated. Invasive *Chromolaena odorata* and *Tithonia rotundifolia* used traditionally for the treatment of coughs, colds and fever and other related infectious diseases were screened against non-pathogenic *Mycobacterium aurum, M. fortuitum, M. smegmatis* and pathogenic *M. bovis.* The serial microdilution assay was used to determine antimycobacterial activity of acetone, dichloromethane

and hot water extracts of the weed plants. Cytotoxicity and genotoxicity tests were carried out using the tetrazoliumbased colorimetric assay and the Ames test respectively. Both species had a range of antimycobacterial activity against strains tested. However, *T. rotundifolia* displayed better activity against all strains tested with minimum inhibitory concentration values ranging between 0.039 and 0.156 mg/ml. Toxicity tests showed that both species were not cytotoxic at the highest concentrations tested and were not genotoxic. Our results suggest that these plants may be used for the development of antimycobacterial drugs to help combat the spread of the disease.



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Biological activities of selected South African plants used traditionally to treat inflammation and helminth infection

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Inflammation is a major process in the defence system against injury and infective microorganisms and is involved in the healing of damaged tissues. Eleven plant species were selected in this study based on their use in traditional medicine against inflammation and intestinal nematode infections in southern Africa. The crude extracts were tested for their antioxidant, anti-inflammatory and anthelmintic activities. The antioxidant activity was determined by using radical scavenging DPPH and electron reducing ABTS assays. The anti-inflammatory activity of crude extracts was evaluated via the 15-lipoxygenase inhibitory assay and the nitric oxide (NO) inhibition assay using lipopolysaccharide (LPS)-activated RAW 264.7 murine macrophages. The anthelmintic activity was determined against the nematode Haemonchus contortus using the egg hatch assay (EHA) and larval development assay (LDA). The ethanol extract of Carpobrotus edulis had the highest antioxidant (ABTS)

activity with $IC_{_{50}}$ of 1.11 $\mu g/mL$ and antioxidant DPPH activity with IC_{50} = 2.97 µg/mL. The methanol extract had good 15-lipoxygenase inhibitory effects with IC₅₀ of 9.84 µg/ mL, which was significantly (P<0.05) higher than that of quercetin (IC $_{\scriptscriptstyle 50}$ of 26.60 $\mu\text{g/mL})$ and the acetone extract of C. edulis had good NO inhibition and cell viability (97.88% and 100.00% respectively). The acetone crude extract from *Ricinus communis* was the most efficient against *Haemonchus contortus* egg hatching inhibition with IC_{50} =18.55 µg/mL and it also inhibited larval development of *H. contortus* with IC_{50} = 10.64 µg/mL. This study revealed that *C. edulis* had good antioxidant and anti-inflammatory activity. However, R. communis had good anthelmintic activity against Haemonchus contortus. This study confirms the traditional use of C. edulis and R. communis in South Africa. These plants are therefore a potential source of compounds that could be used against inflammation and helminthiasis.

Histology of the female reproductive tract of the cheetah (Acinonyx jubatus)

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Cheetahs are listed as vulnerable by the IUCN (International Union for Conservation of Nature) with their total worldwide population estimated at 6674 individuals. Ongoing habitat destruction, fragmentation, human animal conflict and predation threaten the survival of the wild cheetah population. Zoos and wildlife parks are tasked with maintaining a genetically healthy population as insurance against catastrophic extinctions. In contrast to reproduction in the wild, reproductive success in captivity has been poor, with only a handful of facilities achieving reasonable success. Both husbandry and spermatic factors have been investigated. Recently the focus has shifted to investigating the role of uterine health in reproductive success. Since the identification of pathology in any organ or system is made with reference to the normal, it was decided to investigate and document the normal histology of the female cheetah reproductive tract.

Six captive female cheetah uteri, obtained from post-mortems after elective euthanasia of cheetahs aged 7–10.5 year, and one uterus, from a 3 year old cheetah that died acutely, were sectioned and processed by routine histological methods. Histological sections were stained with haematoxylin and eosin and studied and photographed with an Olympus BX63 light microscope using bright field illumination and fitted with an Olympus DP72 digital camera. Very early signs of uterine pathology were identified in some sections in four of the seven uteri examined. These few sections were disregarded and only representative sections with no pathological changes were included in the atlas.

The histology of the female reproductive tract of the cheetah resembled that of the domestic dog, the cat and

the lioness with some major differences as follows. The reproductive ligaments of the female cheetah were comprised predominantly of smooth muscle, which was very well developed and supported the entire length of the uterus up to the cervix, similar to the African lioness. The uterine tube opened into the uterine lumen from a microscopically welldefined papilla. Branched tubulo-alveolar papillary glands were found in the papilla and were morphologically distinct to the uterine glands. Cyst like structures were found in the region of the uterotubal junction (UTJ) and appeared to be dilations of the papillary glands. The tunica muscularis of the uterine tube was uniformly thick from the infundibulum to the isthmus. The uterus and uterine glands were lined by a low cuboidal to cuboidal epithelium. No ciliated cells were present in the uterus. The cervix had very few tertiary folds, no mucigenous nor goblet cells and contained no elastic fibres.

The dilations of some papillary glands noted in this study are of importance to pathologists examining uterine sections in the UTJ region as they could easily be confused with cystic endometrial hyperplasia a proposed cause of infertility in captive cheetahs. It is therefore advised that sections for histopathology of the uterus of the cheetah be taken at least 5 mm distal to the UTJ to preclude their accidental inclusion.

The histology reported will be useful for future reference and for comparison with other felids. It will aid pathologists examining biopsies from the reproductive tract of female cheetahs. It will also provide a background reference for use in the future development of research tools to help evaluate reproductive tract health in the female cheetah as well as new artificial reproductive techniques.

Prevalence of *Campylobacter* species from chickens sold in informal poultry markets in Gauteng, South Africa

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Campylobacteriosis, a foodborne disease caused by infection with *Campylobacter* species, is associated with contaminated meat (poultry, beef, sheep and pork) products. Compared to other meat products, consumption of chicken in South Africa is very high and this has increased with the consequent increased demand for chicken. This development has led to the growth of the informal poultry market, a market that is unregulated by appropriate government agency. It is generally believed that the microbial quality of chicken products from these outlets is poor and may pose health hazards to consumers of under-cooked chickens from these sources.

The objective of this study was to determine the prevalence and characteristics (phenotypic and molecular) of *Campylobacter* spp. in chickens sold at informal outlets in Gauteng. The study was conducted in six areas (Germiston, Atteridgeville/Phomolong, Garankuwa, Tembisa/Modise, Alexandra, Soweto) from where a total of 151 samples, each of cloacal swabs, carcass drip and carcass swabs, were collected across 47 outlets. At each outlet, a questionnaire was administered to both the owner and the patrons. *Campylobacter* spp. were isolated from the three types of samples using standard bacteriological methods and confirmation of isolates was achieved using conventional polymerase chain reaction (PCR).

The frequency of isolation of Campylobacter spp. from carcass drip, cloacal and carcass swabs was 40.4%, 37.7% and 24.5% respectively. The frequency of Campylobacter spp. isolation was highest in carcass samples that originated from Germiston (66.7%) and the lowest in carcass samples from Tembisa/Modise (0.0%). The chicken processing methods and practices in these two key areas were different. Seventy percent of the outlets in Tembisa/Modise used the knife shaving method to defeather the chickens, followed by evisceration and placing the carcasses either on the counter or in a bucket. In Germiston, 100.0% of the outlets used the handpicking method to defeather the chickens, followed by evisceration. However, the chickens were stored in improperly functioning mini-freezers with faulty temperature controls, which therefore served as incubators, thus increasing the risk of contamination and multiplication of bacteria. The cloaca is the normal habitat for resident Campylobacter, however the highest frequency of Campylobacter species isolation was detected in carcass drip samples. This could be explained, in part, by contamination from rinse water, processing and sale tables, human handling and knives used at these outlets.

In vitro activity of Psychotria zombamontana and its potential use as a poultry feed additive

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Poultry feed is one of the main constraints, after disease, in poultry production for smallholder farmers in Africa, it also acts as the first step of the food safety chain in the "farm to fork" model. With the poultry industry being the largest individual agricultural industry in South Africa, the nature of the micro-organisms present in the feed is of great importance. Problematic micro-organisms may lead to an increased feed conversion ratio of livestock, illness or disease in livestock, or even disease in humans. The primary aim of this study was to evaluate the activity of Psychotria zombamontana (Rubiaceae) against problematic fungi and bacteria that may be associated with poultry feed. The species was selected following promising preliminary antifungal results. Additionally, further biological activities that may be beneficial to the chickens when added to the feed were evaluated, including antioxidant activity. Acetone leaf extracts of P. zombamontana showed promising activity against the microorganisms tested as well as very high

antioxidant activity. An effort was then made to isolate the active compounds through bio-guided fractionation. These compounds were then tested for activity against selected organisms. The extract and compounds were then investigated for cytotoxicity for preliminary evidence of safe ingestion of plant material or compound by the chickens. Preliminary results show that the extract has no cytotoxic activity and therefore may be safe for ingestion but in vivo studies are necessary to confirm this. These findings coupled with the promising antimicrobial activity and high antioxidant activity suggest that P. zombamontana could be a highly beneficial addition to poultry feed. This encourages more studies in vivo to more closely investigate the effects on chickens when added to feed. Additionally, further studies should aim to observe the curative effect of the extract or compound on artificially infected chickens, as well as any possible preservative effect the material may have on the feed.

A survey of selected pathogens of economic concern within the principle cultivated Tilapia (*Oreochromis* spp) producing regions of South Africa for the period 2017-2018

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Global food security and the need for affordable protein in an increasingly populated world, is spurring the development of aquaculture as an industry, particularly in previously untapped areas. Africa, as a continent with vast land and water resources, has enormous capacity to farm fish, yet lacks diagnostic infrastructure, and epidemiologically structured disease surveillance programs. With focus on the developing commercial tilapia industry in South Africa's chief producing provinces, this study seeks to: assess the presence, distribution and impact of selected economically important diseases; evaluate current levels of farm management and husbandry, water quality, nutrition, biosecurity practices, and their cumulative impact on disease prevalence. Data collected is also serving to quantify commercial farm distribution, identify species farmed and map interprovincial and international movement of fish. Commercial farms over 50 000 litre capacity have been selected. A questionnaire is provided to each farm requesting epidemiological data of the farm that includes parameters such as fish

purchases, management practices, disease history and production records. Ten juvenile grow- out stage tilapia are systematically selected per farm. Each is immobilized on ice, visually appraised, species morphologically identified, weighed, measured, and photographed, and euthanazed. A gill scrape, skin scrape, and gill clip is used to screen microscopically for ectoparasites of significance. Each fish is necropsied and samples of organs (liver, spleen, anterior and posterior kidneys, gills, stomach, intestines, gonads, and brain) collected in 10% formalin for histopathological assessment. Anterior kidneys are swabbed and stored refrigerated in sterile Amies transport medium, and cultured only if histopathological evidence reflects likelihood of bacteraemia. Preliminary results, despite showing little evidence of any serious diseases, are emphasizing general poor husbandry practices, inadequate and poor feed quality, and unacceptable water quality, as overwhelming issues, with potential significant impact on fish health and production levels for the industry.

Endemic circulation of Rift Valley fever virus in far northern KwaZulu-Natal

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Rift Valley fever (RVF) is a mosquito-borne zoonotic disease characterized in South Africa by large epidemics amongst ruminant livestock at very long, irregular intervals, mainly in the central interior. However, the presence and patterns of occurrence of the virus in the eastern parts of the country are poorly known. This study aimed to determine whether RVF virus (RVFV) is circulating in cattle, goats or wildlife in far northern KwaZulu-Natal, close to the Mozambique border, and, if so, to estimate seroprevalence and incidence rate of seroconversion.

Cross-sectional studies were performed in wildlife [nyala (*Tragelaphus angasii*) and impala (*Aepyceros melampus*); n=156] and in communally farmed cattle (n=423) and goats (n=104), followed by longitudinal follow-up of seronegative livestock (n=253) nine times over 18 months, representing 116.2 animal-years at risk. Exposure to RVFV was assessed using the serum neutralization test. In seroconverting animals, multiple imputation was used to impute a random date of seroconversion between last negative and first positive tests, in order to provide an unbiased estimate of seroconversion rate over time. Incidence density was estimated and compared

using Poisson models and seroconversion rate was plotted over time using the derivative of the kernel-smoothed Nelson-Aalen cumulative hazard estimator.

Initial overall seroprevalence was 34.0% (95% CI: 29.5–38.8) in cattle, 31.7% (95% CI: 22.9–41.6) in goats and 45.9% (95% CI: 37.9–54.0) in wildlife. Although it tended to increase with age, it was high in all age groups. Overall rate of seroconversion in cattle was 65.4 (95% CI: 50.3–85.1) and in goats 65.9 (95% CI: 42.5–102.3) per 100 animal-years, varying significantly between localities within a 10 km radius. Seroconversions were detected throughout the year, with incidence rate peaking during the high rainfall months of January to March.

The high seroprevalence in all age groups and evidence of yearround viral circulation indicate a hyperendemic situation in the study area. This is the first study to directly estimate infection rate of RVFV in wildlife and livestock in an endemic area and provides the basis for further investigation of mechanisms for virus circulation in endemic areas and survival during interepidemic periods.

Evaluation of two newly developed lateral-flow immunochromatographic assays for the detection of *Mycobacterium bovis* infections in domestic cattle (*Bos taurus*) and African buffaloes (*Syncerus caffer*)

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The diagnosis of bovine tuberculosis (BTB) is complicated by the complex pathogenesis of the disease as well as the sub-optimal test performance of the available diagnostic assays. Serological assays are thought to detect later stage or progressive disease, which could be missed by more conventional testing strategies alone such as the tuberculin skin test. The existing assays however are often laborious, not very cost-effective and show highly variable specificities (Sp) and sensitivities (Se).

The aim of this study was to evaluate the test performance of two newly developed lateral-flow immunochromatographic assays (LIONEX Animal TB Rapid Test and LIODetect TB-ST, developed for cattle and humans, respectively) as compared to that of an existing commercially available antibody test for the detection of *Mycobacterium bovis* infections (IDEXX TB ELISA). Sera from cattle and African buffaloes with a known exposure/infection history that had previously been tested in the IDEXX TB ELISA were divided into the following groups: 1) gold-standard positive (cattle n=10; buffaloes n=16); 2) testpositive, culture negative (buffaloes only; n=16) (considered true positives); 3) experimentally infected with non-tuberculous mycobacteria (cattle only; n=31) (considered true negatives); and 4) gold-standard negative (cattle n=15; buffaloes n=12). All sera were tested using both lateral-flow assays. Briefly, 20 μ L of serum was added to the sample well of both tests. For the Animal TB Rapid test, two drops of diluent buffer were subsequently added and a third drop was added 5 min later. For the LIODetect TB-ST, three drops of diluent buffer were added immediately after addition of the serum. Test results for both assays were read and recorded 20 min after the last drop of diluent buffer was added. The tests were only considered valid if the control line (C) was visible.

The results of the various assays as well as the test performance are listed in the table below:

Species	Assay	True positives ¹	False positives ¹	True negatives ¹	False negatives ¹	Sensitivity (%)	Specificity (%)
Cattle	IDEXX TB ELISA	10	1	45	0	100	97.8
	Animal TB Rapid	5	3	43	5	50	93.5
	LIODetect TB-ST	3	0	46	7	30	100
Buffalo	IDEXX TB ELISA	9	0	12	23	28.1	91.7
	Animal TB Rapid	9	1	11	23	28.1	91.7
	LIODetect TB-ST	1	0	12	31	0.03	100

¹True diagnosis was determined on the basis of history and gold standard assays

Overall, both assays showed a higher test performance in cattle as compared to buffaloes. The Animal TB Rapid showed a higher test performance than the LIODetect TB-ST in both species. The IDEXX TB ELISA showed the highest test performance. The low sensitivity of the Animal TB Rapid test in both species, which is in general accordance with other serological assays for BTB, may render it unsuitable to rule out disease, but due to its ease of application the assay could be useful in the preliminary screening of populations with an unknown BTB status.

Adaptation of SAT2 foot-and-mouth disease viruses in cattle and goats

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Foot-and-mouth disease (FMD) is a highly contagious viral disease that affects cloven-hoofed animals. Infection of susceptible livestock species with FMD virus (FMDV) causes an acute, febrile illness characterised by a rapid onset of clinical signs. The Southern African Territories (SAT) type viruses occur in sub-Saharan Africa and are maintained within African buffalo (Syncerus caffer) populations. Considering the fact that this natural reservoir cannot be exterminated for ethical and ecological reasons, vaccination of at-risk livestock and prevention of contacts between domestic animals and wildlife play an important role in the control of FMD in endemic areas. In southern Africa especially, goats (caprine) and cattle (bovine) are extremely important to rural communities, providing food security and wealth. However, many of these rural communities are found close to or bordering wildlife reserves and national parks and infected wildlife may transmit FMDV to domestic livestock.

There are few data available on the adaptation and comparison of infection of the SAT2 viruses once they have entered hosts. Therefore, a project was carried out wherein bovine and caprine were infected with SAT2 outbreak isolates. Next-generation sequencing (NGS) was applied for SAT2 challenge and recovered viruses, and the variation between the genomes analysed. The FMDV genome regions that underwent non-synonymous changes for both the bovine and caprine samples were the Leader, VP2, VP1, 2B and 2C regions. The results indicated that there was selection of a single Challenge Virus (CV) throughout the course of the experiment, and that this was the same for both species. A second round of challenge was performed, but different viruses were selected that varied for the host species, namely bovine (CV3) and caprine (CV1). The fact that CV3 is found to be dominant in bovine, while CV1 is dominant in caprine, suggested that caprine became infected with FMDV during the early stages of the outbreak from which the CVs were obtained. This is supported by the fact that CV1, which was an isolate obtained during the beginning of the outbreak, was found to be dominant in caprine in this study, indicating that this CV was better adapted to caprine than bovine. FMDV-infected caprine often do not show clinical signs of the disease, and subclinically infected caprine may have transmitted the virus to bovine. CV3, which was collected during the end of the outbreak, was found to be better adapted to bovine, indicating viral adaptation to host-species during the course of the outbreak.

Understanding the host-virus interaction is key to both understanding transmission between animals and the rational design of intervention strategies. These data will be beneficial for improving our understanding of virus adaptation in domesticated FMD host species, which will in turn aid in the fight against FMD and the negative effects associated with FMD outbreaks.

Long bone fractures in impala (*Aepyceros melampus*): A classification system and review of 62 fractures

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The importance of antelope for conservation and their value in zoological and private wildlife collections, has led to a surge in the need for sophisticated veterinary care. The demand for diagnosis and treatment of long bone fractures in certain antelope species, especially impala (*Aepyceros malampus*), have increased accordingly. The foundation of fracture management is a fracture classification system that guides treatment, prognosis, comparative and retrospective studies. Ideally this system should be simple, precise, repeatable and applicable to the species in question (Unger, Montavon & Heim 1990).

The purpose of this study was to introduce a fracture classification system and reports the findings of 62 impala fractures classified with this system. Due to the lack of a long bone fracture classification system available for ungulates and equines, the Unger fracture classification was modified to be applicable to impala. In this retrospective analysis, one hundred and thirty radiographs of 62 impala long bone fractures were studied. Each fracture was classified and assigned a four symbol alpha-numeric code using the modified-Unger classification. The following information was captured from patient records: Patient signalment, skeletal maturity, fractureassociated soft-tissue changes, the presence of fissure lines, the cause of the fracture and all fracture fixation methods. The overall fracture distribution based on location found that tibial fractures (n=17) were the most common followed by metacarpal (n=13) and metatarsal (n=10) fractures. Seventy eight percent of the cases had diaphyseal fractures. The fracture distribution based on type was 47% simple, 28% wedge and 26% multi-fragmentary type fractures. The sex of 36 cases were recorded, of which 58% were male and 42% female. Based on skeletal maturity 68% cases were immature and 32% mature. Fifty three percent of cases had open fractures. Fissure lines were detected in 34% of cases. The cause in the majority of fractures was unknown (n=53/62). Fractures were treated with a range of internal and external fixation methods, or combinations thereof.

To the authors' knowledge, this is the first fracture classification system for long bone fractures in ungulate species containing the metacarpus and metatarsus. Our modified-Unger fracture classification was found to be applicable in classifying 62 fractures in impala. This classification should provide a foundation for further advances in veterinary and comparative ungulate, and particularly antelope, orthopaedics and traumatology.



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