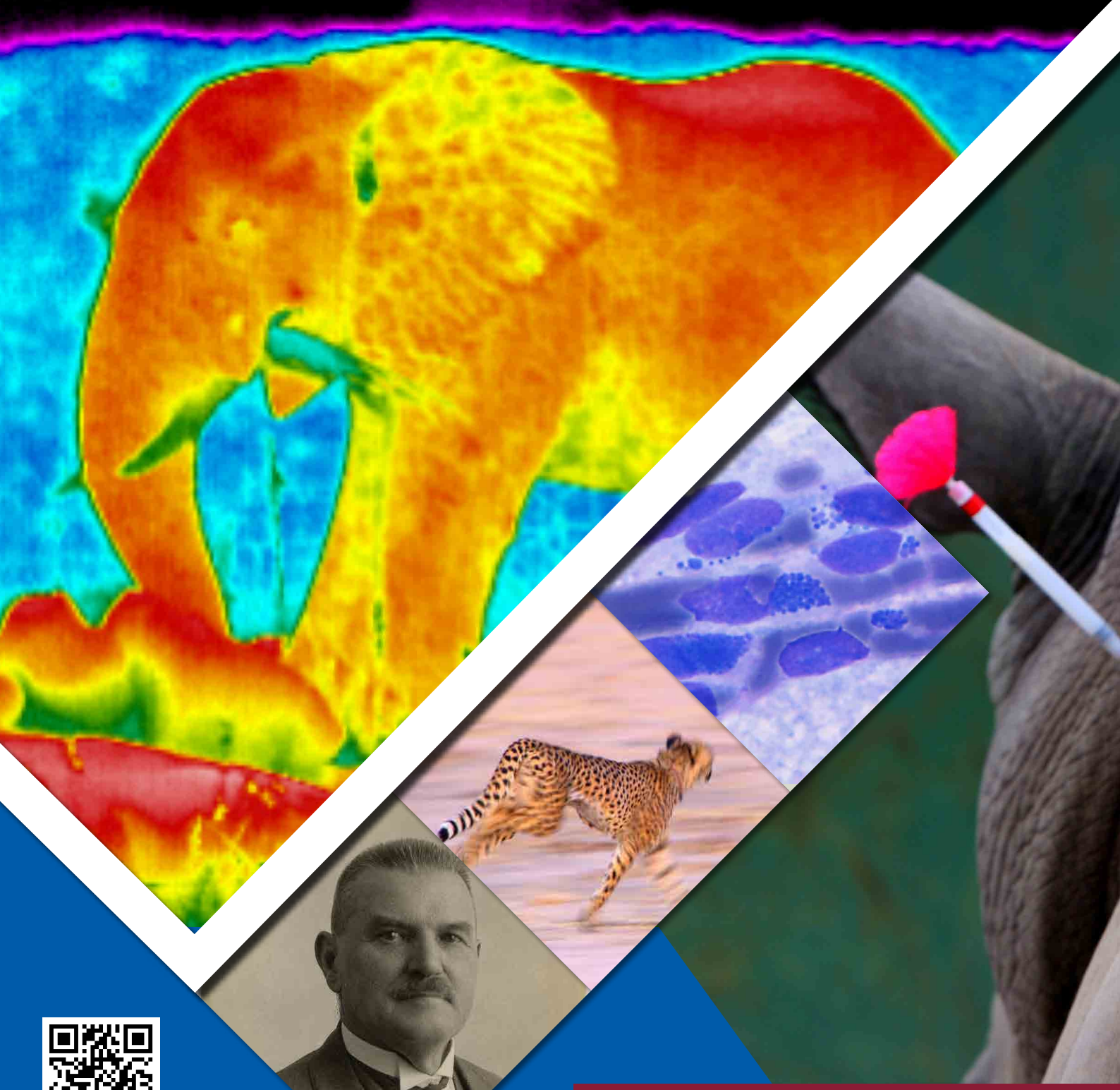


Faculty Day

22 August 2019

Research Overview

UNIVERSITEIT VAN PRETORIA
UNIVERSITY OF PRETORIA
YUNIBESITHI YA PRETORIA

Faculty of
Veterinary Science

Fakulteit Veeartsenykunde
Lefapha la Disaense 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 the Medical University of South Africa (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

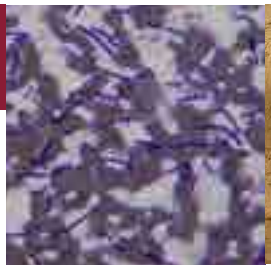
The Faculty of Veterinary Science wishes to express its sincere thanks to the following sponsors for their very generous contribution in support of the 2019 Faculty Day.

Faculty Day

Faculty of Veterinary Science
University of Pretoria

22 August 2019

Contents/programme




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

Master of Ceremonies: Prof Neil Duncan

08:30–08:45 **Welcoming address**

Prof Vinny Naidoo, Dean of the Faculty of Veterinary Science

08:45–10:00 **First Session**

Chairperson: Prof Adrian Tordiffe

1. **The abundance, composition and barcoding of *Tabanidae* within Kruger National Park and their role in the transmission of *Besnoitia besnoiti***
   A Smit, LP Snyman, X Mazibuko, L Neves

2. **Prevalence of radiographic changes in front feet and metacarpophalangeal joints of South African endurance racehorses**
   E Hollenbach, MP Robert, C le Roux, Y Smit

3. **Efficacy of a high potency pentavalent oil-emulsion foot-and-mouth disease vaccine against heterologous challenge with FMDV SAT[1] in goats**
   DD Lazarus, MM Sirdar, J van Heerden, D van der Merwe, PB Mutowembwa, F Peta, L Heath, B Blignaut, PA Opperman, GT Fosgate

4. **Junctional complexes of the blood-testis barrier in the Japanese quail (*Coturnix coturnix japonica*)**
   RA Molele, MC Madekurozwa

5. **Can exogenous sclerostin mitigate the excessive bone formation associated with sclerosteosis?**
   TJ Dreyer, M Shah, C Doyle, G Holdsworth, V Naidoo

10:00–10:45 **Tea (Cafeteria)**

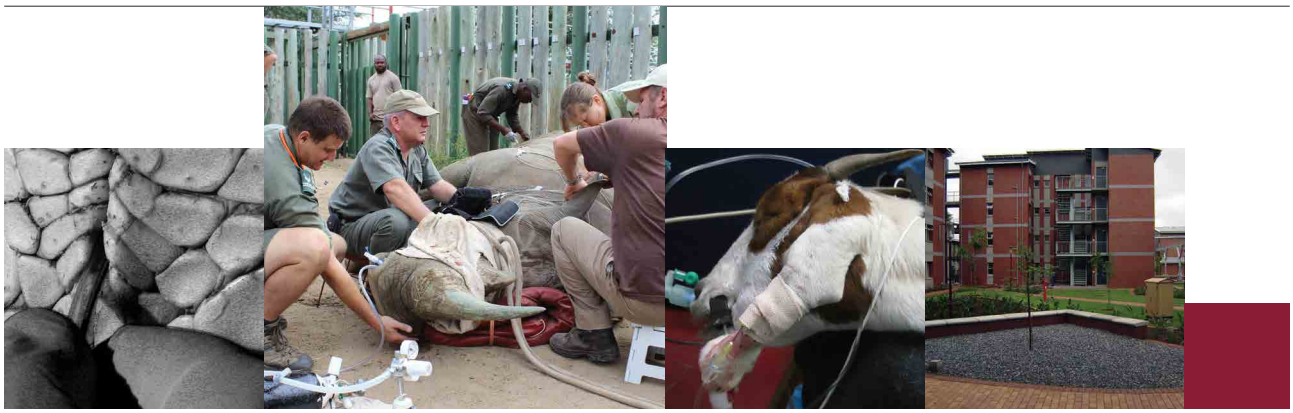
10:45–12:00 **Second Session**

Chairperson: Prof Leith Meyer

Sir Arnorld Theiler Memorial Lecture: Prof Duncan Mitchell
Large mammals facing climate change

12:00–12:30 **Third Session**

Poster session (Cafeteria)



12:30–13:00 **Lunch (Cafeteria)**

13:00–14:15 **Fourth Session**

Chairperson: Dr Nicolize O'Dell

1. **Determining whether colour can be used to assess arterial blood oxygenation in immobilised impala *(Aepyceros melampus)***
   PE Basson, G Zeiler, P Kamerman, LCR Meyer

2. **Gross morphology of the African lion *(Panthera leo)* heart**
   CA Marais, MR Crole

3. **The immunogenicity of two forms of cost-effective, purified, non-living anthrax vaccine candidates compared to the Sterne live spore vaccine with concurrent penicillin G treatment in bovines**
   S Jauro, OC Ndumnego, C Ellis, A Buys, W Beyer, H van Heerden

4 **Retrospective analysis of the epidemiology and clinical presentation of West Nile virus infection in horses in South Africa, 2016–2017**
   F Bertram, M Venter, PN Thompson

5. **Oxidative burst and phagocytic activities of phagocytic cells in canine parvoviral enteritis**
   K du Preez, Y Rautenbach, EH Hooijberg, A Goddard

14:15–15:00 **Interesting research from the Department of Paraclinical Sciences: Prof Lyndy McGaw and Dr Johan Steyl**

15:00–15:15 **Faculty Day Awards**

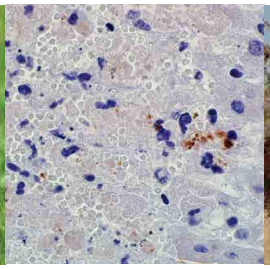
Researcher of the year

Young researcher of the year

Best oral presentation

Best poster

15:15 **Cocktail (Cafeteria)**

POSTER PRESENTATIONS

1. **Physiological and behavioural measure of animal welfare in relation to semicaptive African elephant (*Loxodonta africana*) interaction programs**
   CE Grotto, EV Berkeley, A Ganswindt

2. **Age-related changes in the rete testis and efferent ductules of the Japanese quail (*Coturnix coturnix japonica*)**
   MIA Ibrahim, LI Khumalo, M-C Madekurozwa

3. **Morphology of the Southern Ground-Hornbill (*Bucorvus leadbeateri*) stomach**
   AD Naudé, KN Koeppel, MR Crole

4. **Haematology and biochemistry effects of acepromazine or detomidine standing sedation in horses**
   D Fisher, Y Rautenbach, M Hewetson, G Zeiler

5. **The effects of storage time and temperature on thromboelastographic analysis in dogs and horses**
   A Lemon, A Goddard, EH Hooijberg

6. **Assessing the application of a smartphone modulated ECG device for use in equines**
   G Piketh, T Schliewert, A Williams

7. **Prevalence of bactibilia in apparently healthy dogs**
   E Verwey, A Gal, F Kettner, WJ Botha, P Pazzi

8. **The potential effect of garlium GEM HC as a tick repellent agent in springbok (*Antidorcas marsupialis*)**
   A Fitte, KN Koeppel, J Steyl, L McGaw

9. **Faecal glucocorticoid metabolite concentrations as a measure of stress in black-footed cats (*Felis nigripes)***
   M van Heerden, KN Koeppel, A Ganswindt

10. **Evaluation of the quality of foot-and-mouth disease virus samples in preparation for next-generation sequencing**
    D van der Merwe, J van Heerden, L Heath, B Blignaut, GT Fosgate

11. **Percentage of faecal excretion of meloxicam in the Cape vultures (*Gyps corprotheres*)**
    EO Adawaren, L Mukandiwa, J Chipangura, K Wolter, V Naidoo

12. **The antioxidant and cytotoxic effect of *Cissampelos owariensis* P. Beauv extracts**
    RT Akande, SM Nkadimeng, LJ McGaw

13. **Responses of faecal glucocorticoid metabolites, heart rate variability, body temperature fluctuations and activity patterns to potential stressors in captive cheetahs (*Acinonyx jubatus*)**
    KL Brown, A Ganswindt, G Steenkamp, ASW Tordiffe

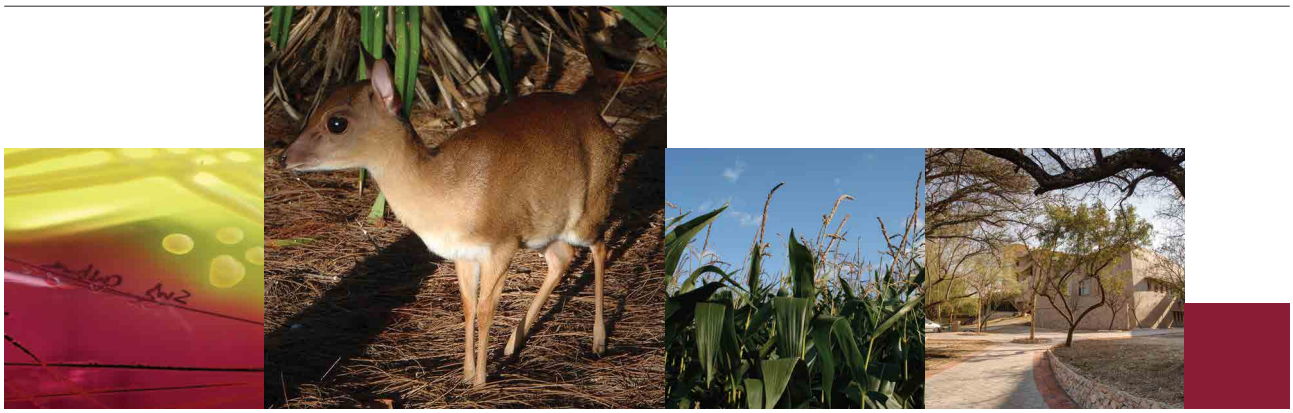
14. **Hematological changes during experimental pathogenic *Theileria* sp. (sable) infection in roan calves (*Hippotragus equinus*)**
    SJ Clift, JCA Steyl, EP Mitchell, JA Lawrence, EH Hooijberg

15. **Evaluation of the antibacterial and antibiofilm potential of three *Combretum* species against selected foodborne pathogens**
    RC Erhabor, JO Erhabor, LJ McGaw

16. **The *in vitro* effect of ionophores on cardiac and skeletal muscle cells**
    D Henn, EA Venter, CJ Botha

17. **Evaluation of *in vitro* neutralization of epoxyscillirosidine by antibodies raised in sheep**
    HI Isa, GCH Ferreira, JE Crafford, CJ Botha

18. **Determination of anti-nutritive factors and toxins in selected fodder trees or shrubs and conventional animal feed during feed scarcity**
    MM Lebeloane, KG Kgosana, LJ McGaw



19. **The microbiome of *Crocodylus niloticus* eggs from commercial southern African crocodile farms and its relationship to foetal mortality and hatching success**
AV Lensink, JG Myburg

20. **The effect of seasonality on the stress and metabolic patterns of an African strepsirrhine**
C Long, ASW Tordiffe, ML Sauther, FP Cuozzo, J Millette, A Ganswindt, J Scheun

21. **Reference intervals for haematology and serum biochemistry in Temminck's ground pangolin (*Smutsia temminckii*)**
K Lourens, E Hooijberg, L Meyer

22. **Activity of three South African plants on phytopathogenic bacteria and fungi of tomatoes and chemical profiling of the extracts**
FN Makhubu, MC Khosa, LJ McGaw

23. ***In vitro* cytotoxicity of isogeigerin acetate, a novel sesquiterpene lactone isolated from *Geigeria aspera* (vermeerbos)**
YZ Mathe, G Fouché, LGJ Ackerman, D Liles, EA Venter, CJ Botha

24. **The effects of water and ethanol extracts of *Panaeolus cyanescens* mushroom on arginase activity in bovine pulmonary aortic endothelial cells**
SM Nkadimeng, CLM Steinmann, JN Eloff

25. **The *in vitro* antibacterial activity and safety of *Morinda lucida* leaf extracts against *Salmonella* serovars relevant in livestock infections**
OS Olawuwo, AO Aro, JO Erhabor, JN Eloff, LJ McGaw

26. **Antimicrobial resistance and biofilm formation in coagulase negative staphylococci isolated from cow milk samples submitted to the Onderstepoort Milk Laboratory**
L Phophi, I Petzer, DN Qekwana

27. **Assessment of infection prevention-control measures and hand hygiene compliance among healthcare workers in the intensive care unit at the Onderstepoort Veterinary Academic Hospital**
DC Sebola, C Boucher, DN Qekwana

28. **The bacterial microbiome of *Rhipicephalus sanguineus* ticks in the Mnisi community, South Africa**
R Ackermann, C Gall, KA Brayton, NE Collins, I van Wyk, J Wentzel, AO Kolo, MC Oosthuizen

29. **Assessment and genotyping of a novel vaccine candidate for *Theileria parva* infections**
LL Borchers, M Tjale, K Sibeko-Matjila

30. **Phylogenetic characterisation of the Palyam serogroup orbiviruses**
K Ebersohn, P Coetzee, LP Snyman, R Swanepoel, EH Venter

31. **Identification and genotyping of predicted host cell phenotype modulators in *Theileria parva***
N Komani, J Liebenberg, KP Sibeko-Matjila

32. **Investigating *Rickettsia africae* infection in *Amblyomma hebraeum* ticks in Mnisi, Bushbuckridge Municipality, South Africa.**
E Mazhetese, Z Lukanji, L Neves, D Morar-Leather

33. **Molecular detection of tick-borne haemoparasites in cattle and buffalo samples from Manicaland province, Zimbabwe**
AAR Modirwa, KP Sibeko-Matjila, R Bhoora

34. **Development and validation of a real time PCR assay to detect *Ehrlichia canis* in dogs**
NF Nkosi, MC Oosthuizen, M Quan

35. **Lesions and cellular tropism of natural Rift Valley fever virus infection in sheep**
L Odendaal, SJ Clift, GT Fosgate, AS Davis

Message from the Dean

What does an institution do to continuously better itself when it is already successful on so many levels? How does it position itself in a world where technology is advancing exponentially as a major positive disruptor in our lives, and where something as small as a smart phone has become an integral part of our lives?

Prof Vinny Naidoo
Dean: Faculty of Veterinary Science

While embracing this change is inevitable, how we do it is important. For this we need to reassess how we interact with technology and adapt in order to cope with its disruptive effect and subsequently refine what we do to take full advantage of opportunities instead of competing with them. Research by McKinsey suggests that, globally, about half the jobs performed by humans today will be disrupted by automation. A survey of business leaders by the World Economic Forum suggests that 42% of the core job skills required today are set to change substantially by 2022.

This disruptive effect has been defined by current thinkers as the Fourth Industrial Revolution (4IR).

As changes introduced by the 4IR progress, artificial intelligence (AI) will be permeating every aspect of business, industry and our lives, with changes that were once the stuff of science fiction. Try and imagine your daily life in some or other way without the AI algorithms developed and created by Google or without the simplicity of internet banking. Within the veterinary field, AI holds the potential to become a powerful tool in the hands of the clinician and preventative health practitioner.

This potential will be realised on the back of the mining of Big Data and datasets in order to formulate and continuously refine algorithms that are aimed at assisting with or confirming diagnoses. So many predictive opportunities await discovery from our electronic records, accumulated over 20 years at the Onderstepoort Veterinary Academic Hospital (OVAH), of nearly half a million patients. AI could mine this dataset and develop algorithms based on patient signalment and present signs, narrow the diagnostic possibilities and save clinicians hours in diagnostic time.

More importantly, AI could be used to model supplementary data such as environmental conditions, wind patterns and even solar flares to model patterns in disease outbreaks for the better prediction of spread; saving both animal and human lives with the potential of making farming more profitable.

But the greatest benefit to us is that AI has tremendous potential to enhance the ways and means of doing research as more powerful computing gets integrated with research data analytics, creating new ways to solve new problems, with low error rates while handling it more efficiently.

The President of the Future of Life Institute, Max Tegmark, may just have described it best when he said: "Amplifying our human intelligence with artificial intelligence has the potential of helping civilization flourish like never before – as long as we manage to keep the technology beneficial." This is echoed by our own Vice-Chancellor and Principal, Prof Tawana Kupe, with the view that rather than seeing it as something that needs to be managed, the University of Pretoria (UP) sees 4IR as a rare opportunity to steer society in new and better directions.

This includes reimagining and positively transforming higher education. To an extent, this is already being achieved through local and global knowledge exchange that is happening to an unprecedented degree, along with international partnerships that are addressing the world's technological, scientific, economic, survival and societal challenges.

The internationalisation of knowledge and knowledge generated through research initiatives not only serves as a cornerstone to sustainable development and country's economic development, but contributes to a globalised society in which institutions can build on each other's areas of expertise.

Going forward, the Faculty's strategic aim should be to improve the quality and impact of its research, and to embrace technology instead of competing with it. How do we, for example, take advantage of Google analytics to ensure that we have better informed clients so that they don't see an internet search as the preferred preconceived option to a veterinary diagnosis? Can we incorporate smart devices into the monitoring of patients through the use of smart watch technology and drone cameras, and enhance our work in new vaccine and drug development by better incorporating molecular biology tools? All in all, the one thing that is constant in life is change. And change requires continuous adaptation to ensure relevance. I believe that, as a faculty, we are perfectly placed to enter a world of new advancements during the current 4IR and make a positive contribution to the South African economy.

Our annual Faculty Day provides further impetus to the Faculty's pursuit of excellence in support of the University's research-intensive vision. It also provides the opportunity for our researchers to present the results of their studies and share them with their peers. It is a proud tradition and it gives me great pleasure to welcome staff members, students and visitors to the event. A warm welcome also to Prof Duncan Mitchell from the University of the Witwatersrand, who will present this year's Sir Arnold Theiler Memorial Lecture.

Curriculum Vitae: Prof Duncan Mitchell

Prof Duncan Mitchell

Born in Germiston, South Africa, Duncan Mitchell is Emeritus Professor of Physiology at the University of the Witwatersrand, Johannesburg, and Honorary Professorial Research Fellow in its Brain Function Research Group, from which he retired as director in 2006. He is also Adjunct Professor in the School of Human Sciences at the University of Western Australia, Perth.

After completing an honours degree in Experimental Physics at the University of the Witwatersrand, he joined the Research Organisation of the Chamber of Mines of South Africa. While in service of the Chamber of Mines, he completed an MSc and PhD at the University of the Witwatersrand on topics related to the applied physiology of heat stress experienced by deep-level miners. He was guided by legendary thermal physiologist Prof Cyril Wyndham. Thermal physiology became the bedrock of a lifelong research career.

After nine years at the Chamber of Mines, he was offered a research position at the UK Medical Research Council's National Institute for Medical Research, London, England. He left to join Dr Richard Hellon and spend three years in England with his young family. His primary research there investigated the neurophysiological pathways by which body temperature is measured in rats and cats. He also spent one day a week at St Thomas's Hospital, London, exploring the neurochemistry of fever in rabbits and cats with Prof Bill Cranston, a professor of Medicine.

Exploiting the similarity in the neuro-anatomy and neurophysiology of pathways, he and his colleagues at the National Institute for Medical Research extended their research on temperature pathways to study pain pathways in rats. While in England, he was elected to both the UK and the American physiological societies.

In 1975, Duncan Mitchell returned to South Africa to join what is now the School of Physiology at his alma mater. There he continued research on the physiology of fever in rabbits, and on the neurophysiology and neuropharmacology of pain pathways in rats, but he switched his research in thermal physiology first to foetal and neonatal physiology, and then to the ecophysiology of lizards and beetles of the Namib Desert (with desert biologist Prof Mary Seely) and, most recently, to the conservation physiology of large mammals.

He also helped set up a laboratory in the School of Physiology for research in the physiology of human sleep, and supervised the first PhDs completed in this laboratory.

His research on foetal physiology, undertaken with physiologist Prof Helen Laburn, ecologist Prof Graham Kerley and his veterinarian brother (and Onderstepoort graduate), Prof Graham Mitchell, was his first venture into biologging.

The team implanted thermometric radiotelemeters and then thermometric data loggers into foetal sheep and goats; pregnancies proceeded successfully to term and neonates were born instrumented. It was also his research on sheep and goats – on the phenomenon of selective brain cooling – that was his introduction to mammalian wildlife physiology. With his brother, together with German physiologist Prof Claus Jessen, Australian physiologist (then postdoctoral fellow) Prof Shane Maloney and (then PhD student) Prof Andrea Fuller, he used biologging to make the first measurements of selective brain cooling in free-living wild ungulates. Biologging of the physiology of free-living terrestrial large mammals has become a research enterprise in which South Africa now leads the world, and is an enterprise to which Prof Leith Meyer (Director of the University of Pretoria's Centre for Veterinary Wildlife Studies) and other Onderstepoort graduates have made major contributions.

Duncan Mitchell has lectured in 28 countries in the course of his career. He has supervised 30 PhD and 17 masters students, and has published more than 280 papers. He was awarded the Harry Oppenheimer Fellowship, Africa's top research award for an individual researcher, in 2010 and an honorary DSc degree by the University of the Witwatersrand in 2012. He retired from the rating system of the National Research Foundation (NRF) with an A1 rating. With his colleagues and students at the University of the Witwatersrand, University of Pretoria, University of South Africa, University of Western Australia, University of Lethbridge (Canada) and the Gobabeb Research Institute (Namibia), he is actively continuing research in conservation physiology related to climate change.

Research topic summary:

Large mammals facing climate change

The fossil record reveals that it has been the largest mammals that have been the mammals most at risk in previous major global warming events, and they are likely to also be most at risk in the current anthropogenic event, in which global temperature is rising much faster than it has in previous events. The large mammals may succumb to heat disease in what will be increasingly frequent and intense heat waves, or may die of dehydration from the diminishing availability of dietary water (especially in the southern hemisphere), or from the disappearance of their sources of food, or from disease caused by pathogens emerging during global warming. However, what is likely to be more pernicious will be failure of reproduction in the face of warming, drought and food reduction. How cattle are affected by heat stress was a topic of research at Onderstepoort in the days of Sir Arnold Theiler. That ambient warming causes failures of conception, teratogenesis, intra-uterine growth retardation and failure of lactation is now well known to the production animal community, but has yet to make an impact on the wildlife community.

If they are to prosper, large mammals faced with global warming in their current environments will have to move, or be moved, to more benign environments, or will have to adjust genetically or phenotypically to their new circumstances. Although some species of large mammal have the capacity to move thousands of kilometres within a year, anthropogenic land fragmentation will prevent migration being the solution to threats of local warming that it has been in the past, when polar bears migrated to the Canadian mainland, for example. Some charismatic large wild mammals with low population numbers, like the rhinoceros, may be able to be rescued by assisted colonisation. Valuable livestock, like racehorses, could be relocated to higher latitudes or altitudes. However, the scale (and therefore cost) required of the operations is unprecedented, and the demand for financial resources will compete with those that will be required to move humans. While it is a viable option for taxa with rapid reproduction like bacteria (although potentially catastrophic for host species), genetic adaptation is an unlikely stay-put solution for large mammals. With large body size comes increased longevity, slower reproduction and reduced offspring numbers. Large mammal species will not be able to go through sufficient generations for genetic adaptation to result in speciation occurring within the 50- or 100-year horizon of current global warming. However, there is some room for optimism arising from the genetic process of micro-evolution, which is much faster than speciation.

For more than two decades, my research team has been investigating the feasibility of large mammals employing the other stay-put option, namely phenotypic flexibility. Do large mammals have latent physiological talents, autonomic or behavioural, that they do not necessarily require in their current environments, which could be recruited when their environments become warmer and drier? Exploring that question has required the development of a new experimental approach to conservation physiology. It requires the long-term measurement of physiological variables, including behaviour, in identified individual large mammals that are exposed to natural or induced stress, simulating those that will occur with global warming. In the case of wild mammals, at least, studies need to be conducted in free-living mammals in their natural habitats in the absence of human observers, the presence of whom inevitably distorts the mammals' autonomic and behavioural functions.

Such studies have been made possible by the new technology of biologging, which uses onboard instrumentation to measure variables such as location, orientation, movement and temperature in large mammals living free in their natural habitats. We have used seasonal changes in the environment as a proxy for global warming and drying, but have also explored those physiological variables in large antelope in the current extremes of heat and aridity, the Saudi Arabian desert. We have discovered evidence for latent physiological talents, such as switching foraging from day to night, and implementing processes for reducing evaporative water loss, but these are not distributed uniformly across taxa.

Among ungulates, perissodactyls show less flexibility than do artiodactyls. Within the scope of the wild mammalian taxa that we have studied, myrmecophages are the most vulnerable to the consequences of global warming.

Biologging has been employed for studying livestock physiology under ambient thermal stress, for example by ourselves with Angora goats, but not yet nearly to the extent that it should be. The future of livestock under global warming is a hot topic, literally and figuratively, with cattle farming being the main focus. Meteorologists point to the surprisingly large contribution of cattle to greenhouse gases via the eructation of methane and generation of nitrous oxide from mismanaged manure, and to the profligate water requirements of beef production. Cows' milk production is heavily compromised by ambient warming, as is conception.

Economists point to the diminishing capacity to grain-feed cattle in the face of increasing human food requirements. Agronomists point to the compounding effect of a massive decline in cereal crop production, which is anticipated under global warming, including in the "maize belt" of South Africa.

The attractiveness of cattle farming is waning. Yet, there are huge increases in demand for beef, as well as for cows' milk, in developing countries.

Meteorology, conservation and economics argue for a reduction in red meat consumption, at least in developed countries, and for a switch from ruminants to monogastric mammals for meat production.

Climate change biology argues for a switch within ruminants from cattle to goats for milk and meat.

Duncan Mitchell

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"
2018:	Prof Yoshan Moodley	''The story of humanity, told by our oldest commensal: *Helicobacter pylori*''

* 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: 2018–2019

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, it requires increased research outputs through effective postgraduate programmes, and making research 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 UP vision, suggest that the Faculty is well placed to contribute significantly to the University's strategic goals. The Faculty's research publication output increased from 55.3 units in 2006 to 99.75 units in 2018 in 110 Institute of Scientific Information (ISI)-accredited journals, and the higher number of subsidy units earned by the Faculty at one unit per academic staff member. In terms of postgraduate students, the Faculty currently has 156 MSc, 107 PhD, 14 postdoctorate, and 23 postgraduate diploma candidates. The number of postgraduate graduations continues to increase compared to previous years. Challenges to sustain these increases include the clinical nature of academics' work in some departments, the percentage of academics with doctoral degrees or National Research Foundation (NRF) ratings and the percentage supervising postgraduate students. Plans are underway to try to further increase these percentages over the next one to three years and to recruit additional postdoctoral researchers and research fellows. In 2018, the Faculty awarded 21 PhDs and 77 master's degrees at the University's Autumn graduation ceremony, which, combined was the highest number of postgraduate degrees awarded by the Faculty in any given year.

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. An example of this is a postgraduate research study on Rift Valley Fever Virus (RVFV), the findings of which offer insight into the disease. Leading research has also contributed to improving the survival chances of the pangolin, an endangered species. Plasma transfusion from a healthy pangolin to a compromised one was another world first. In a rather unique turn of events, our vets also provided radiation treatment to a lion after it was diagnosed with skin cancer in conjunction with medical doctors. This is a perfect example of how interdisciplinary teams can tackle problems better.

International cooperation and collaboration is an important part of the Faculty's strategic focus in delivering high-quality postgraduate training and research. One such an example is the fourth edition of the collaborative *MSc in Tropical Animal Health*, that officially started earlier this year.

From 29 January to 7 February 2019, a number of staff members of the Institute of Tropical Medicine (ITM), Antwerp, Belgium, joined their colleagues at the Department of Veterinary Tropical Diseases. Twenty-four students, who were carefully selected out of hundreds of applications, got together for the first time. These students, which represented a total of 11 countries, together with a number of academic and administrative staff from ITM and UP, travelled to the Hans Hoheisen Wildlife Research Station, where the students were introduced to the One Health Basic Concepts module, and experienced the challenges of health at the interface of humans, domestic animals, livestock, wildlife and the complex natural environment surrounding the area.

The Faculty is currently training professionals to promote animal health that impacts directly on human health, thereby stimulating economic growth and food security, which is a major Millenium Development Goal. Nonetheless, 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 is explicitly pursued to continue the growth and development of the Faculty. Our vision is to create strong internationally recognised research groups in wildlife health, infectious diseases, primary animal health, food production, One Health and risk assessment, which are already focus areas of the Faculty. 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. These research themes are the following:

Translational medicine

This theme will focus on the treatment of human and animal disease. For the former, it 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 veterinary application, it will either entail the treatment of disease in target animals 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 cross-cutting from the basic sciences, diagnostic medicine to clinical medicine or surgery. In this theme, research will focus on physiology, farming, management, disease management, food safety and disease transmission.

Pathobiology of disease

This research theme is dedicated to the study of diseases in animals, including disease epidemiology. Areas of study include changes in the normal physiology of animals brought about by disease and disease processes. An integral component of this theme includes disease diagnostics from clinical changes observed in the patient, diagnostic imaging, clinical pathological changes, pathological changes, and 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 number of postgraduate students and encouraging teaching staff to submit themselves to the National Research Foundation (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 50% permanent staff members with a doctorate as their highest qualification. Since 2014, there was also a dramatic upsurge in the combined number of master's and doctoral students, and the Faculty has more than doubled its postgraduate output and number of postdoctoral students. The Faculty once again improved on the number of PhDs awarded, with 2018, once again, being higher than the previous years.

Photo: Fransie Lottering

The number of NRF-rated researchers in the Faculty's staff complement has shown a steady growth, increasing from 29 in 2017 to 38 by the end of 2018. The Faculty now has 10 B-rated, 22 C-rated and six Y-rated staff members, with Prof Christo Botha, Head of the Department of Paraclinical Sciences, rated at the B1 level. This makes him the highest NRF-rated researcher in the Faculty. Prof Botha also received an award from the University for outstanding academic achievement.

Faculty Day 2018 and research awards

The annual Faculty Day on 23 August 2018 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 Arnold Theiler memorial lecture, entitled "The story of humanity, told by our oldest commensal: *Helicobacter pylori*", was delivered by Prof Yoshan Moodley from the Department of Zoology at the University of Venda. 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 Lyndy McGaw

Nine top researchers in the Faculty

Prof Christo Botha
Prof Geoff Fosgate
Prof Amelia Goddard
Prof Leith Meyer
Prof Anita Michel
Prof Vinny Naidoo
Prof Johan Schoeman
Prof Peter Thompson
Prof Estelle Venter

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Research programme: Oral presentations

The abundance, composition and barcoding of *Tabanidae* within Kruger National Park and their role in the transmission of *Besnoitia besnoiti*

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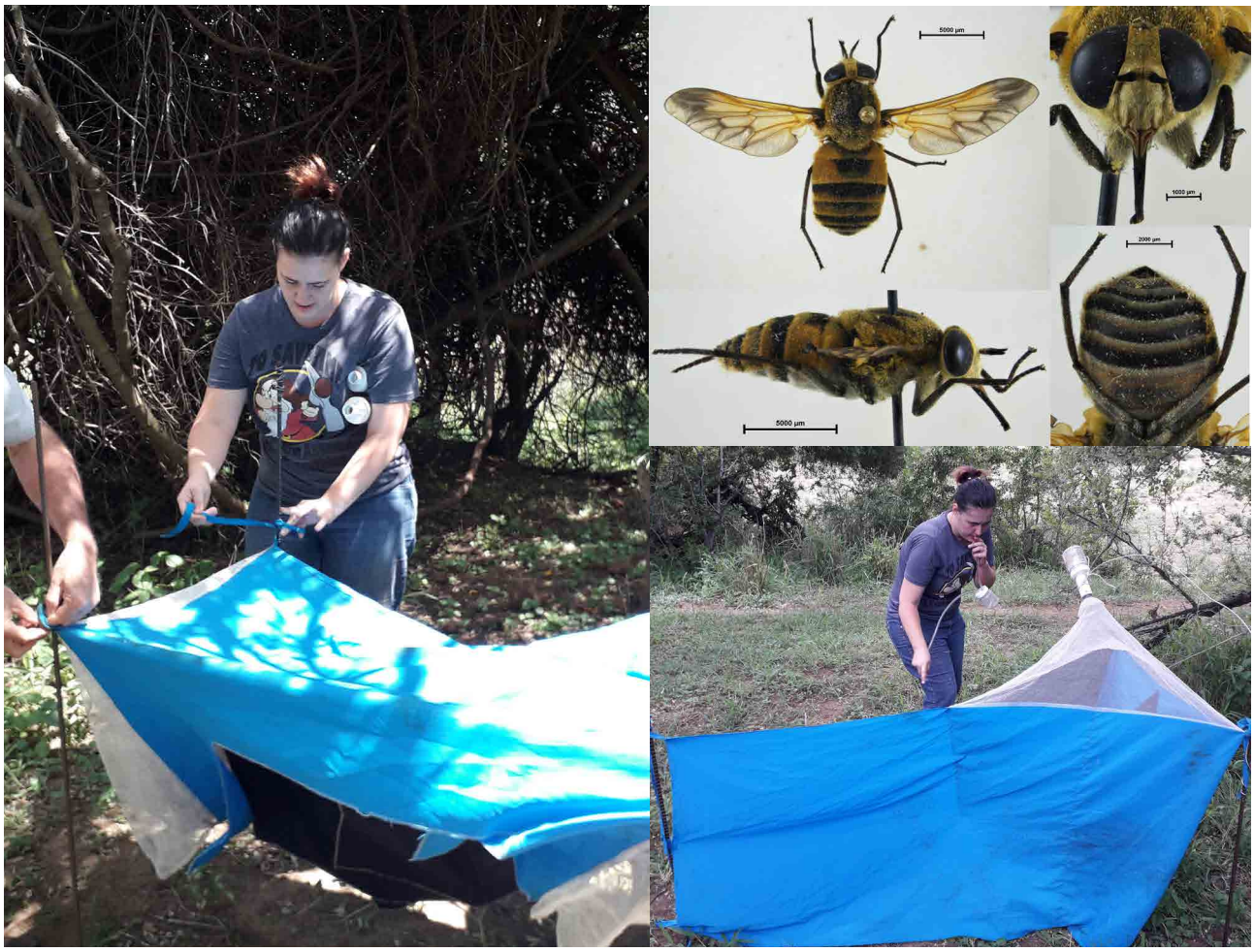
Tabanidae (Diptera) are a diverse hematophagous fly family, known to transmit over 35 livestock pathogens both mechanically and biologically. Little modern taxonomical work has been done on tabanids within South Africa despite their environmental importance. This study aimed at comparing the traditional alpha-taxonomic approach to species delimitation with molecular methods using two gene regions, mitochondrial cytochrome oxidase I (COI) and nuclear alanyl-tRNA-synthetase (AATS). Furthermore, the study aimed to elucidate the role of tabanids in the transmission of *Besnoitia besnoiti*. Tabanids where captured in three locations within Kruger National Park. The flies where morphologically identified then homogenized. A comparative study on DNA extraction methods were conducted, of which the most effective method was selected for DNA extractions. DNA was pooled for the *Besnoitia besnoiti* screening, followed by sequencing and phylogenetical analysis. In total, 854 flies where captured belonging to 14 species under five genera. The phylogenetic analysis indicated sufficient correspondence to that of the morphological identification, however several discrepancies where apparent. The genera *Haematopota, Philoliche* and *Chrysops* were supported across all analyses and clustered into monophyletic groups. Tabaninae, however, formed an unsupported monophyletic group with an unresolved *Tabanus* cluster. It is apparent that the classification of Tabanidae should be placed under scrutiny. A larger sample size, especially with regards to the *Tabanus genus*, will aid in clarifying their relationships. No *B. besnoiti* positives where detected. Furthermore, in-depth research should also be conducted in other regions of South Africa; not only on tabanid ecology and composition but their role as pathogen vectors.



Prevalence of radiographic changes in front feet and metacarpophalangeal joints of South African endurance racehorses.

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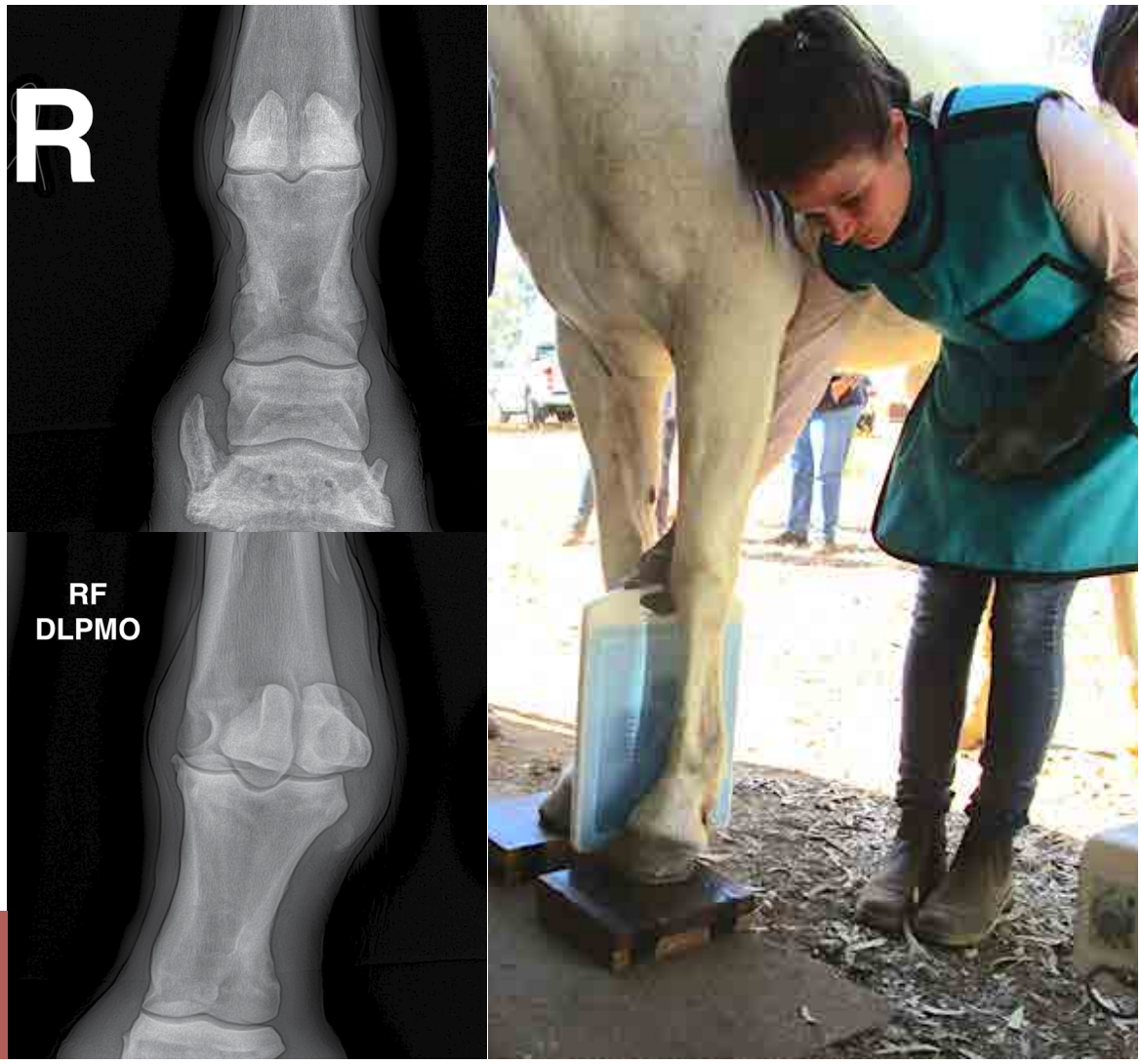
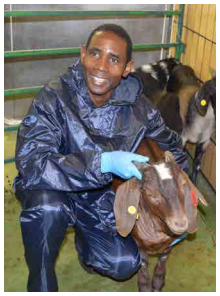
Endurance riding is the fastest growing Fédération Equestre Internationale (FEI) discipline and the second most popular FEI discipline. To the authors knowledge, no studies have been published investigating the prevalence of radiographic changes in the front feet and metacarpophalangeal joints of endurance horses. The experimental study aimed to provide point prevalence and distributive data of radiographic pathology in South African endurance horses. Radiographs were obtained from 160 horses competing during the 2018-2019 racing season. Radiographs included 7 standard views of each distal forelimb. Data analysis revealed a fast majority of horses (95.6%) showed bilateral signs of dorsopalmar hoof imbalance, 77.5% had an abnormal digital axis, with a hyperextended proximal interphalangeal joint being the most common abnormality. Ungulate cartilage ossification was present in 71.9% of at one or both distal phalanges. About one-third (31.9%) of horses displayed signs of metacarpophalangeal osteoarthritis of which 13.8% of these had bilateral changes, however less than 5% of horses showed signs of proximal or distal interphalangeal joint osteoarthritis. Dorsoproximal P1 fragments were observed in 19.1% of horses, while 1.9% had bilateral fragments. Supracondylar lysis of MCIII were evident in 51.2% of horses, which may be indicative of chronic metacarpophalangeal joint distention. One-third (33.1%) of horses showed signs of sesamoid changes such as modelling, osteophytes, lucencies or elongation. Knowledge about the prevalence of specific radiographic changes in South African endurance racehorses would enable equine veterinarians to recognize current pathological changes present and to improve the management of horses affected.





Efficacy of a high potency pentavalent oil-emulsion FMD vaccine against heterologous challenge with FMDV SAT1 in goats

DD Lazarus[1, 3], MM Sirdar[1, 2], J van Heerden[2], D van der Merwe[1, 2], PB Mutowembwa[2], F Peta[2], L Heath[2], B Blignaut[1, 2], PA Opperman[1, 2], GT Fosgate[1]

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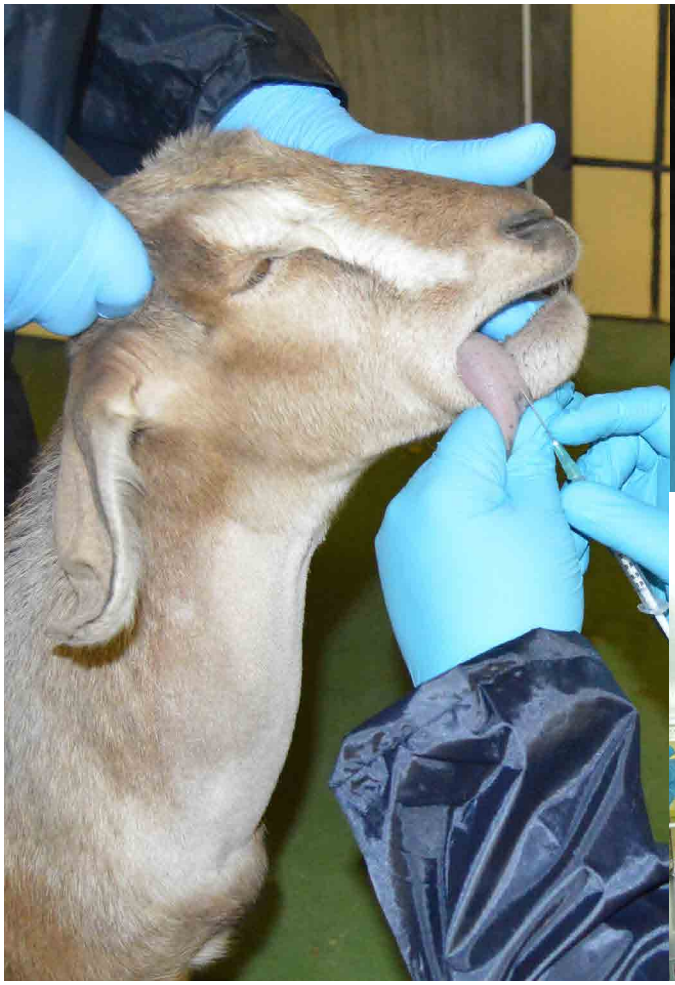
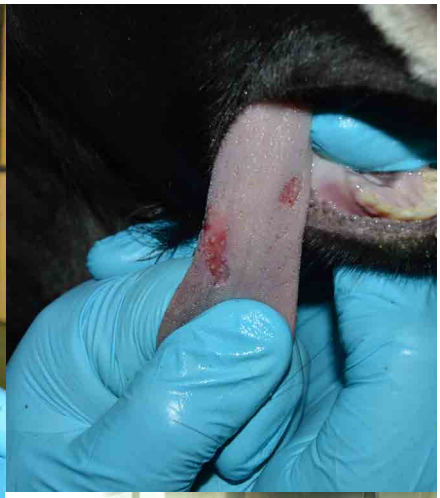
Foot-and-mouth disease (FMD) affects cattle, sheep, goats and pigs but prophylactic vaccination programmes are routinely implemented in cattle only. In South Africa, FMD virus (FMDV) serotypes southern African territories (SAT)1, SAT2 & SAT3 are endemic in African buffalo (*Syncerus caffer*). The objective of this study was to evaluate the efficacy of a pentavalent FMD vaccine in goats against heterologous challenge with a host-adapted pool of SAT1 FMDV. Forty FMD sero-negative indigenous South African goats (6-12 months of age) of mixed sexes were randomly allocated to one of five treatment groups (G): G1 (full cattle dose), G2 (1/3rd cattle dose), G3 (1/6th cattle dose), G4 (1/12th cattle dose) and G5 (placebo control). Goats were vaccinated with an inactivated pentavalent FMD vaccine containing serotypes SAT1, SAT2 & SAT3 on day 0 and revaccinated at day 20 post initial vaccination. Thirty-four goats were challenged by tongue inoculation at day 41 post-vaccination using 104.57 tissue culture infectious dose (TCID)50 FMDV SAT1 pool. Animals were examined daily and clinical signs were scored with secondary lesions indicative of generalized FMD. Rectal temperature was measured daily with temperatures ≥40°C defined as fever. Clinical specimens (oral, nasal and rectal swabs) were collected on days 0, 2, 4 and 6 post-challenge. Specimens were tested for viral shedding by reverse-transcriptase quantitative real-time PCR (RT-qPCR). None of the goats vaccinated with the full cattle dose developed generalized FMD. All vaccinated groups had normal temperature compared to the unvaccinated controls ($P<0.001$). Based on the RT-qPCR results the goats in G5 shed more virus compared to all groups except for G4 ($P<0.05$), while goats in G1 shed less virus than goats in G4 & G5 ($P<0.05$). These results suggest that the 1/6th cattle dose would be sufficient for vaccination of goats to reduce viral shedding after heterologous challenge with FMDV SAT1.





Junctional complexes of the blood-testis barrier in the Japanese quail (*Coturnix coturnix japonica*)

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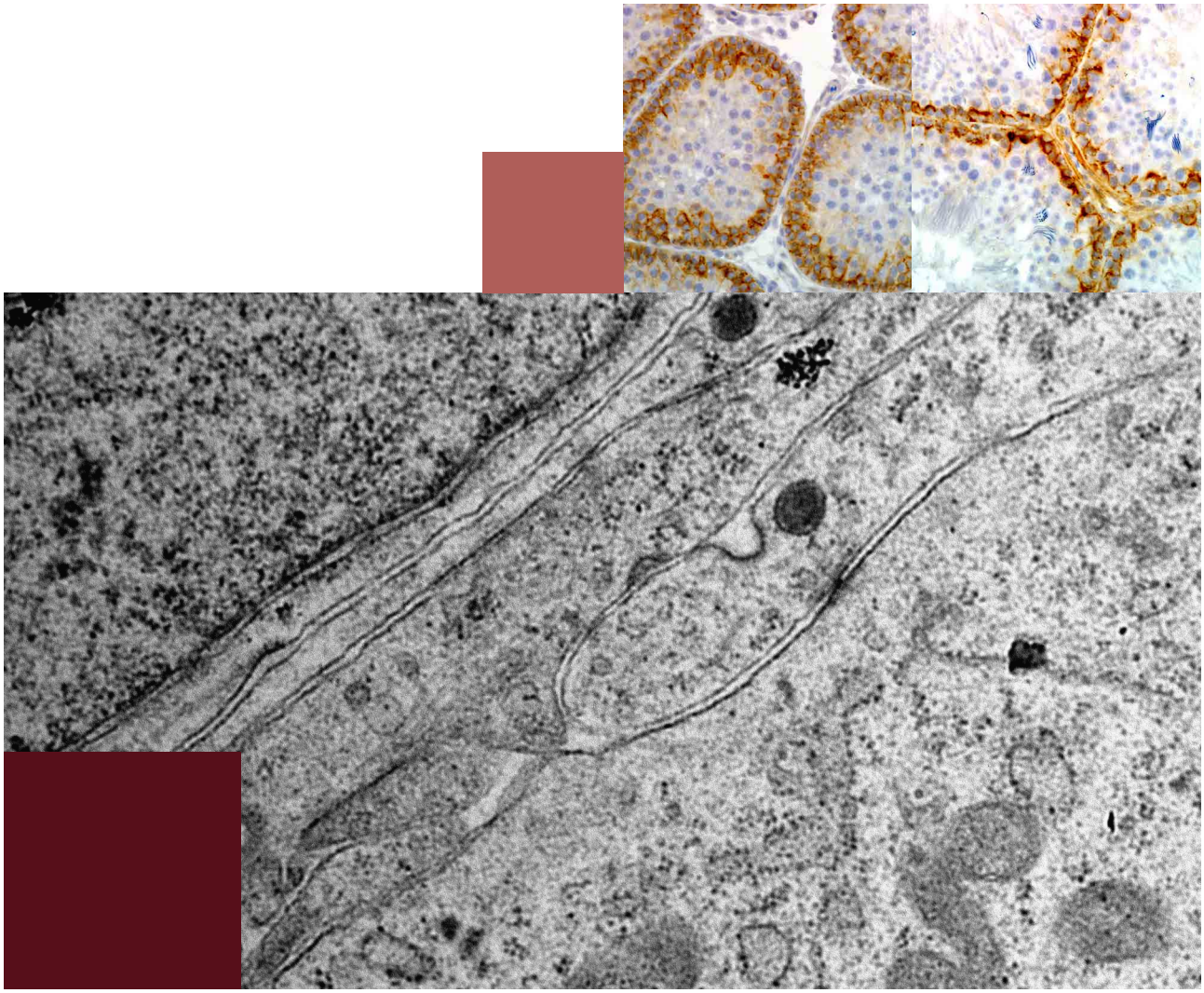
The localization and distribution of proteins forming the junctional complexes of the blood-testis barrier in mammals and reptiles have been well-documented. However, relatively little is known about the proteins forming the blood-testis barrier of birds. The aim of the current study was to investigate developmental changes in the gap junctions, tight junctions, and adherens junctions forming the blood-testis barrier in the Japanese quail. Pre-pubertal (4 weeks old), pubertal (6 weeks old), adult (12 weeks old), and aged (52 weeks old) quails were sacrificed by decapitation. All experimental procedures were approved by the University of Pretoria Animal Ethics Committee (Approval number V034-18). Testicular tissue samples were collected and processed routinely for immunohistochemistry, western blot and transmission electron microscopy. The immunohistochemical and western blot studies utilized primary antibodies against connexin 43 (CX43), zonula occludens 1 (ZO-1), claudin 3, claudin 11, occludin, N-cadherin, E-cadherin and β-catenin. The results of the study showed that the tight junction proteins, ZO-1, occludin, claudin 3 and claudin 11, were localized in the plasma membranes of adjacent Sertoli cells, as well as between Sertoli cells and spermatogonia in all age groups studied. The adherens junction proteins, N-cadherin, E-cadherin and β-catenin, had a similar distribution pattern. The gap junction protein CX43 was localized only between Leydig cells in the testicular interstitium. However, TEM revealed the presence of gap junctions between cells of the seminiferous epithelium as early as the pre-pubertal stage. Furthermore, TEM confirmed the presence of well-developed tight and adherens junctions in the seminiferous epithelia of all age groups. The findings of the study indicate that the junctional complexes forming the blood-testis barrier in the Japanese quail are well established prior to puberty.



Can exogenous sclerostin mitigate the excessive bone formation associated with sclerosteosis?

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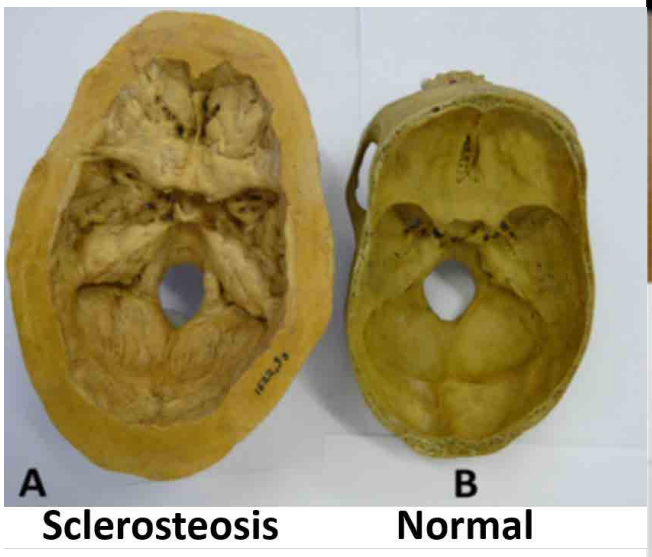
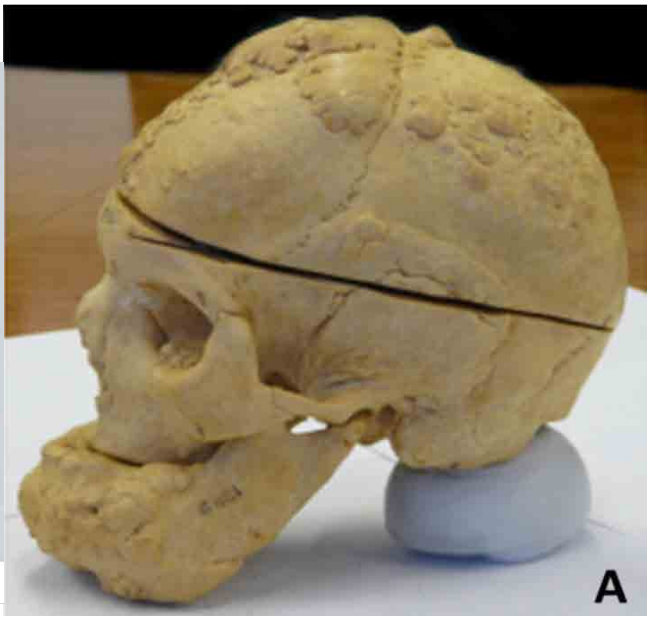
Sclerosteosis is a severe autosomal recessive sclerosing skeletal dysplasia that is characterised by excessive bone overgrowth. It is caused by mutations in the *SOST* gene which lead to loss of expression of the protein sclerostin (Scl). The condition has provided great insights into the importance of sclerostin as a negative regulator of bone formation through its actions as an inhibitor of the canonical Wnt signalling pathway. Approximately 100 cases of this rare disease have been recorded since 1896, of which 66 have been found in the South African Afrikaner population. Bone overgrowth results in facial palsy, hearing loss and potentially lethal elevation of intracranial pressure. The clinical management of sclerosteosis is currently limited to surgical intervention and is challenging due to the thickness and increased density of the bone. Additionally, bone regrowth may cause recurrence of symptoms. New treatment options for sclerosteosis would bring significant benefit to patients. The aim of this study is therefore to explore exogenous recombinant Scl as a potential therapeutic approach in sclerosteosis. Recombinant wild type Scl and various fusions thereof, designed to increase serum half-life, were produced by mammalian expression and were purified using standard chromatography approaches. High affinity (nM) binding of recombinant Scl proteins to the Wnt co-receptor, LRP6, was demonstrated *in vitro*, and addition of purified recombinant Scl inhibited mineralisation of preosteoblast cells cultured in osteogenic conditions. The pharmacokinetic properties of the proteins were explored *in vivo* and recombinant fusion proteins showed improved serum half-life over recombinant wild type Scl. The skeletal consequence of administration of these proteins in a mouse model of sclerosteosis (SOST knock out mice) is currently being examined by microCT. This approach will provide insights into the potential of exogenous recombinant Scl as a treatment strategy for sclerosteosis.





Determining whether colour can be used to assess arterial blood oxygenation in immobilised impala (*Aepyceros melampus*)

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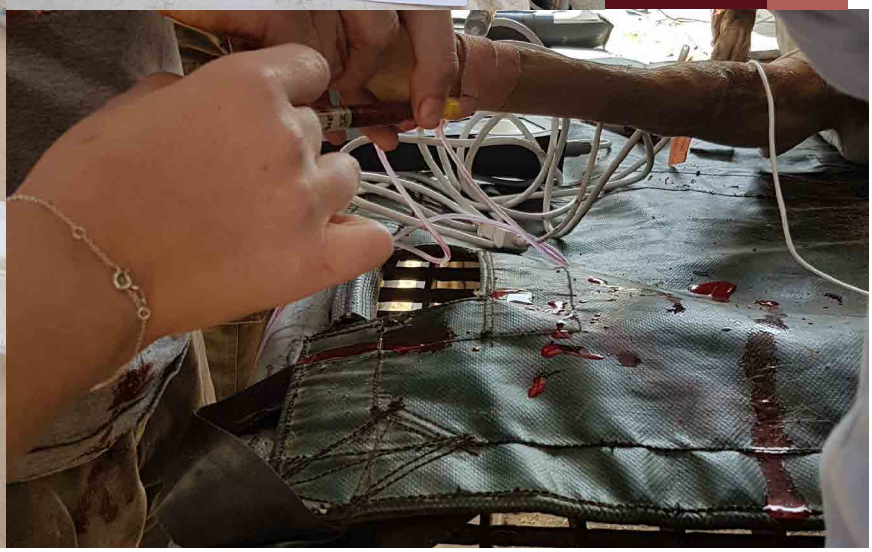
Hypoxaemia often occurs during wildlife immobilisation and poses a risk of morbidity or mortality. A number of methods have been used to assess blood oxygenation in immobilised impala. Pulse-oximetry has been shown to be unreliable, co-oximetry and blood gas analysis is the gold standard but is limited by practicality and cost. With the advent of digital cameras and spectrocolorimeters the assessment of blood colour could be of value for determining blood oxygenation. This study set out to determine whether there is good association between arterial blood colour, as assessed by CIE L*a*b* colour components, and blood oxygenation, as determined by functional oxy-haemoglobin saturation (SaO_2). To obtain arterial blood samples with different blood oxygen levels 11 impala were immobilised with either etorphine or thiafentanyl. Arterial blood samples were collected from the auricular artery at 5 minutes intervals and immediately analysed by means of co-oximetry to measure SaO_2, and spectrocolorimetery, to measure the CIE L*a*b* colour components. The colour components associated better with blood oxygenation (SaO_2) using a quadratic rather than a linear model ($p< 0.001$). The association was strong for each of the colour components (CIE L*a*b*) with pseudo R-squared values for L* = 0.94, a* = 0.93 and b* = 0.92. Therefore functional saturation (SaO_2) is a reliable predictor of all three CIE L*a*b* components of arterial blood colour, and hence blood colour can be used to reliably estimate arterial blood oxygenation of impala. These findings could pave the way for developing colour charts and devices that can be used in the field to cheaply determine blood oxygenation, and detect hypoxaemia, in immobilised or anaesthetised animals.



Gross morphology of the African lion (*Panthera leo*) heart

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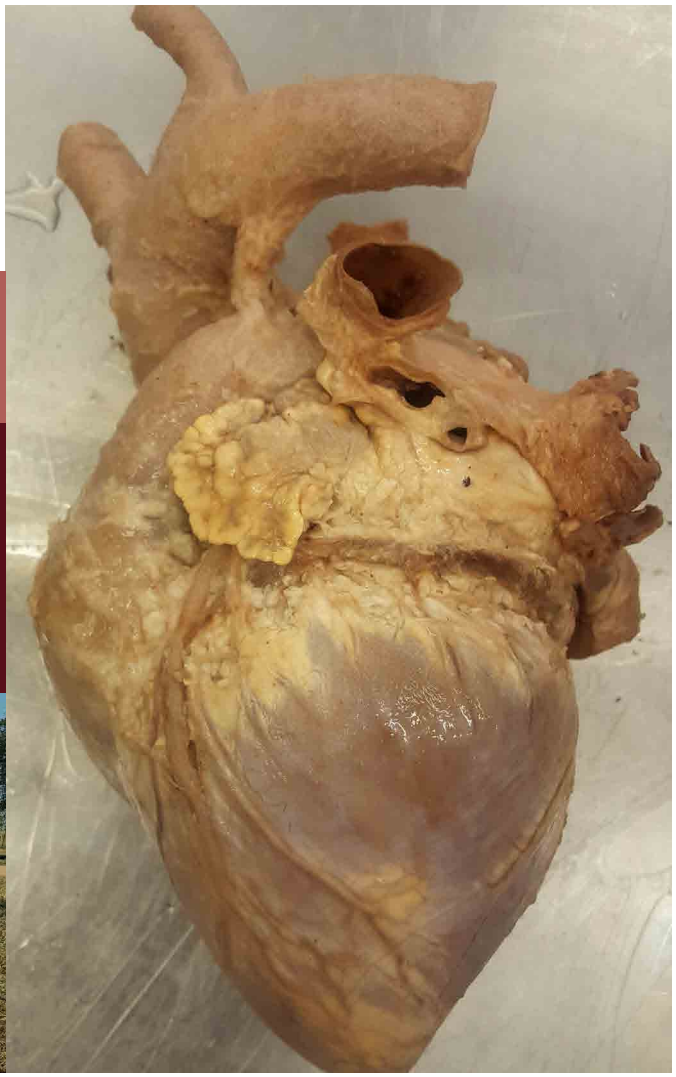
The anatomy of the African lion heart is not well-documented, and assumptions are made that the anatomy is comparable to the domestic cat. Obvious differences may make such assumptions invalid. The increasing demand for veterinary intervention in the African lion warrants sound anatomical knowledge of the heart. Five ±3-year-old captive bred lions were dissected. The thoracic limbs and thoracic muscles were removed, and thoracic topography noted. The heart was removed from the thorax and dissected to expose the external and internal structures. The heart of the African lion is situated caudal to the thoracic limbs between ribs 4-6. It is covered by the left cranial lung lobe, and cranial and middle lobe of the right lung, respectively, with a prominent cardiac incisure present on the right. The sternopericardial and pericardiodiaphragmatic ligaments hold the heart firmly in position. The right coronary artery is dominant, and the right atrium and auricle possess a vast network of *Mm. pectinati.* The massive thoracic limbs, adapted to bring down prey, appear to restrict the cranial thoracic cavity, and as a trade-off, the thoracic viscera are situated more caudally. During intense activity, the heart and lungs compete against each other for space, thus limiting physical activity to short, intense periods. Capacity for sudden increase of cardiac output is facilitated by the extensive pectinate muscles of the right atrium. The sight for intracardiac injections is recommended on the right, ventrally in intercostal space 5. The two pericardial ligaments may help to stabilise the heart during intense activity.



The immunogenicity of two forms of cost-effective purified non-living anthrax vaccine candidate compared to Sterne live spore vaccine with concurrent penicillin G treatment in bovine

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2 Department of Veterinary Microbiology, University of Maiduguri, Maiduguri, Nigeria
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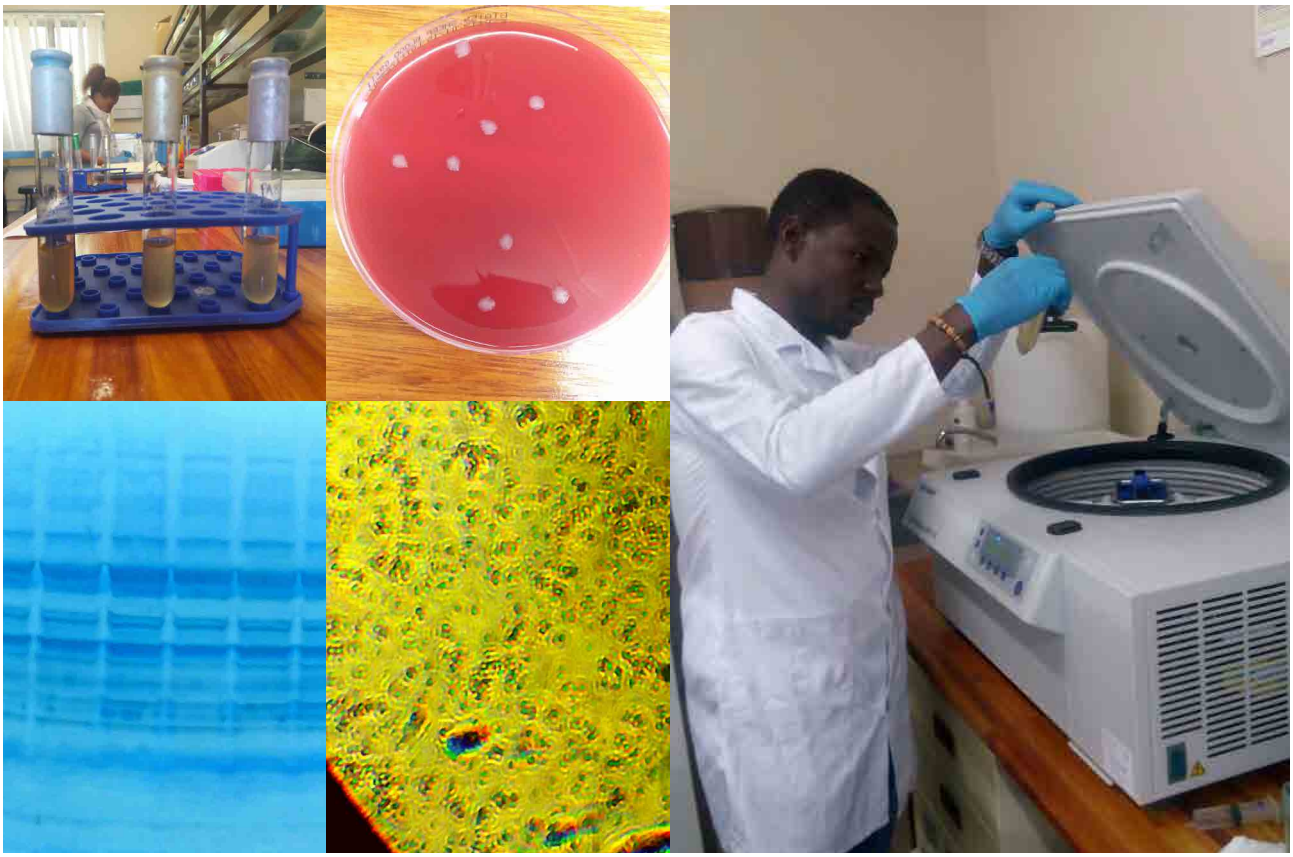
Sterne live spore vaccine (SLSV) is very effective and the OIE recommended anthrax vaccine in veterinary practice in most countries. However, it is unsuitable to use with antibiotic treatment during anthrax outbreaks. Therefore, this study focused on evaluating the immunogenicity of non-living vaccine candidates consisting of purified or crude recombinant protective antigen (rPA) and formalin-inactivated spores (FIS). The non-living vaccine was formulated using purified rPA (PrPA) or crude rPA (CrPA) with FIS and emulsigen-D/alhydrogen adjuvant. Bovine were vaccinated along with Penicillin-G treatment. The vaccinated animals' groups consisted of PrPA+FIS+adjuvant, CrPA+FIS+ adjuvant and SLSV with penicillin-G treatment as well as SLSV without penicillin-G and were vaccinated twice (week 0 and week 3). ELISA (IgM, IgG1, IgG2, and IgG) against rPA and FIS, toxin neutralization assay (TNA) and opsonophagocytic assay were employed for the humoral immunogenicity analysis using blood samples collected before each vaccination and at week 5. The ELISA IgG titre for PrPA and CrPA with penicillin-G had shown a significant increase ($P < 0.0413$ and $P < 0.0341$), whereas the SLSV with penicillin-G group titres were low with a poor immune response compared with the SLSV without penicillin-G treatment group ($P < 0.0001$). Anti-FIS IgG titre followed the same pattern of increase in the titre as seen with PrPA and CrPA with penicillin-G ($P < 0.0173$ and 0.0010) whereas the SLSV with penicillin-G vaccinate group showed significant antibody development without significant difference ($P > 0.3648$) compared to SLSV without penicillin-G. The toxin neutralization was high in PrPA+FIS and CrPA+FIS with penicillin-G. The immunoglobulin isotypes (IgM, IgG1, IgG2) especially IgM and IgG1 demonstrated sudden elevation 2 weeks following vaccination. This revealed the possible ability of PrPA+FIS and CrPA+FIS to stimulate innate immune response in the vaccinated heifers. The antibodies generated against PrPA+FIS and CrPA+FIS enhanced spore phagocytosis significantly by macrophages. Therefore, the purified rPA and more cost-effective CrPA non-living vaccines with penicillin-G treatment were able to stimulate high immune titre and will be tested for protection using passive protection mouse model.



Retrospective analysis of the epidemiology and clinical presentation of West Nile virus infection in horses in South Africa, 2016–2017

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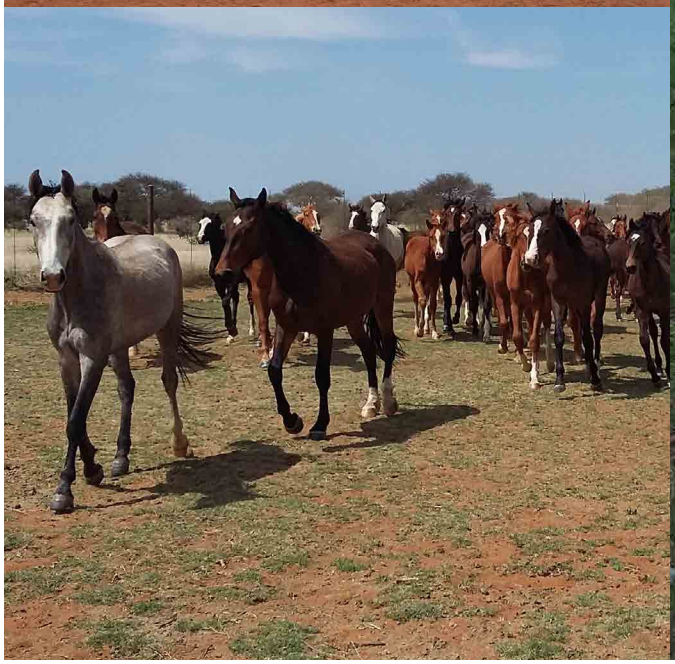
West Nile virus (WNV) has gained international attention in recent years as a globally emerging disease, particularly after large epidemics occurred in North America in the past 20 years. Although endemic in South Africa, it has only been recognised as a significant cause of neurological disease in either humans or horses since 2008. This retrospective study provides an epidemiological and clinical description of WNV disease in horses in South Africa during 2016 – 2017, when 54 cases, most of which occurred during 2017, were diagnosed by passive surveillance at the Centre for Viral Zoonoses (CVZ), University of Pretoria. Cases were followed up and statistically compared to a randomly selected set of 120 WNV negative controls from the CVZ database of the same time period, with similar case descriptions. Clinical presentation of WNV cases was found to be remarkably similar to international trends, with 89% neuro-invasive disease and 39% case fatality rate, mostly displaying typical, significant neurological signs. Approximately half of the cases were pyrexic. Cases which had only neurological signs were more likely to die while cases with pyrexia, with or without neurological signs, were more likely to recover. Cases occurred mostly in highly purebred, WNV-unvaccinated horses less than 5 years old, during the late summer and autumn months after heavy rain in the temperate to warm eastern parts of South Africa. In the multivariable logistic regression analysis, the odds of WNV infection was associated with season (higher during March-April vs. all other times), altitude (higher at 1293-1466 m vs. other categories), breed (lowest in mixed and local breeds), younger age and failure to vaccinate against WNV. Based on these findings, risk-based recommendations may be made to horse owners; in particular, vaccination against WNV, which is currently the most effective prophylactic measure available to reduce disease, severity of clinical signs and mortality.



Oxidative burst and phagocytic activities of phagocytic cells in canine parvoviral enteritis

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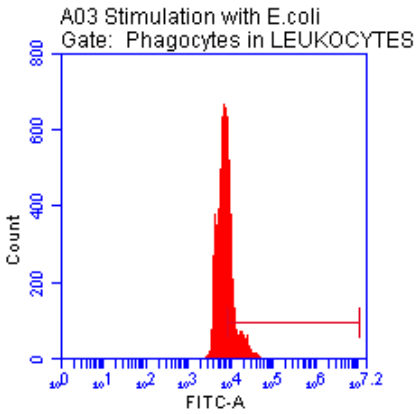
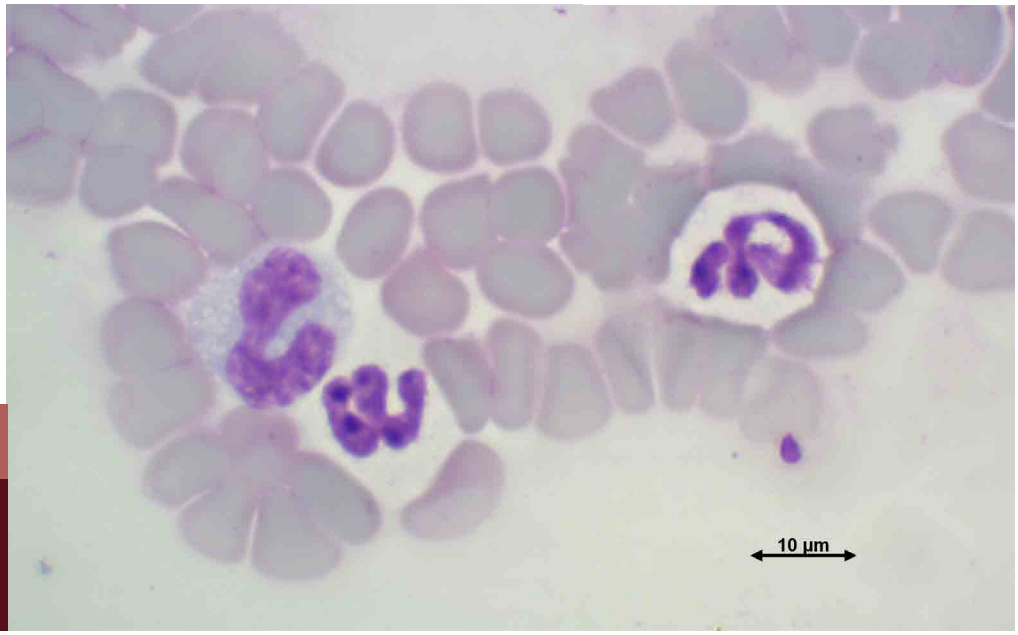
Canine parvoviral enteritis (CPE) is a severe, potentially fatal systemic disease that is both inflammatory and immunosuppressive. CPE can cause leukopenia but the functional capacity of circulating phagocytes (neutrophils and monocytes) has not been fully investigated. The objective of this study was to investigate the functional capacity of phagocytes in CPE by measuring oxidative burst and phagocytic capabilities. Oxidative burst was measured, using flow cytometry, as the percentage of phagocytes producing reactive oxygen species (ROS) and enzymatic activity per cell when stimulated by opsonised E. coli or phorbol 12-myristate 13-acetate (PMA). Similarly, phagocytosis was measured as the number of recruited phagocytes and average number of fluorescein-labelled, opsonised E. coli per cell. Complete blood counts and serum C-reactive protein (CRP) concentrations were determined. Twenty-seven CPE-affected and 8 healthy puppies were included. No significant differences for oxidative burst or phagocytic activities were found between the CPE-affected and healthy control groups. For the neutropenic (<3 x 109/L) CPE-affected puppies the percentage of ROS-producing phagocytes (E. coli: $P<0.05$; PMA: $P<0.01$) and percentage of recruited phagocytes ($P<0.05$) were significantly lower compared to the controls. CRP concentrations were negatively correlated with the percentage of ROS-producing phagocytes (rs -0.577; P<0.01) and percentage of recruited phagocytes (rs -0.426; P<0.05), and positively correlated with phagocytosed bacterial numbers (rs -0.471; $P=0.01$). Based on these results, our conclusions were that neutropenic CPE-affected puppies show evidence of reduced oxidative burst and phagocytic capability compared to healthy control puppies. Additionally, increasing systemic inflammation is associated with decreased oxidative burst capacity and phagocyte recruitment and increased phagocytic ability.





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Research programme: Poster presentations

Physiological and behavioural measure of animal welfare in relation to semi-captive African elephant (*Loxodonta africana*) interaction programs

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Elephant interaction programs, specifically ones that provide elephant back riding, have come under great public scrutiny within the past decade, as claims have been made that captive elephants are "forced" to participate in these programs and experience "unnatural levels of stress" due to these interactions. Thus far, however, no comprehensive information exists to prove whether these claims are just. We examined the potential impact of human interactions and especially ride-based activities on behavioural and physiological stress-related indicators in African elephants. The study focused on the 15 trained semi-captive elephants housed at a private Game Reserve, South Africa, as well as the free-ranging elephant group(s) roaming under the same ecological conditions in the reserve. Frequent faecal sample and behaviour collection took place over 9 months from both groups and collected faecal material was extracted and analyzed at the Endocrine Research Laboratory, University of Pretoria, using an enzyme immunoassay detecting faecal glucocorticoid metabolites (fGCMs) with a 5β-3α-ol-11-one structure. Elephants who participated in elephant-back-safari (EBS) activities showed significant decreases in fGCM concentrations when EBS were discontinued. Similarly, fGCM concentrations of the trained semi-captive individuals who did not participate in EBS also showed decreased steroid concentrations over the same time. Overall, fGCM concentrations of the trained semi-captive herd and the free-ranging herd did not differ significantly. EBS participating elephants and non-EBS participating elephants demonstrated very similar behavioural patterns throughout the entire monitoring period, and followed similar patterns observed in other populations of free-ranging as well as captive elephants. The collected data on physiological stress levels of trained semi-captive elephants during and post participation in EBS will help to better understand the effect of direct anthropogenic interactions on behavioural and endocrinological stress-related markers of elephants. This approach will aid in efforts to optimize welfare and safety management for semi captive elephant populations.

Age-related changes in the rete testis and efferent ductules of the Japanese quail (*Coturnix coturnix japonica*)

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The rete testis plays an important role in the transportation of spermatozoa from the seminiferous tubules to the efferent ductules. The efferent ductules are a major site for fluid reabsorption. There is currently no published information on age-related changes in the morphology of the rete testis and efferent ductules in birds. Therefore, the aim of this study was to investigate age-related changes in the ultrastructure of the rete testis and efferent ductules, as well as alterations in the distribution of cytoskeletal and basement membrane proteins in these two regions. The study was conducted on pre-pubertal (4 weeks old), pubertal (6 weeks old), adult (12 weeks old) and aged (52 weeks old) Japanese quails. The birds were killed by decapitation. This study was approved by the University of Pretoria's Animal Ethics Committee (approval number V034-18). Tissue samples were collected and processed for immunohistochemistry and transmission electron microscopy (TEM). The following cytoskeletal and basement membrane primary antibodies were used: smooth muscle actin, vimentin, desmin, tubulin, cytokeratins (5, 7, 8, 18, 19), laminin, collagen type IV and fibronectin. TEM revealed that the cells of the rete testis and efferent ductules in pre-pubertal quails contained relatively few organelles in comparison to pubertal and adult birds. Furthermore, the rete testis and efferent ductule cells in the aged quails contained numerous vacuoles and spermatozoal debris. Smooth muscle actin was immunolocalized in the rete testis and efferent ductules epithelia of pre-pubertal quails. The intensities of laminin, collagen type IV and cytokeratins (5 and 8) increased with age, while the contrary was observed for desmin, vimentin, tubulin and cytokeratins 19. Cytokeratins 7 and 18 were absent in all cells and tissue types studied. The present study has shown that the rete testes and efferent ductules in the pre-pubertal birds differ from the other age groups, structurally and immunohistochemically.

Morphology of the Southern Ground-Hornbill (*Bucorvus leadbeateri*) stomach

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The Southern Ground-Hornbill (SGH) (*Bucorvus leadbeateri*) is the largest bird species that breeds cooperatively and the only entirely carnivorous hornbill. Currently, there are only an estimated 417 breeding groups in South Africa. As the bird is an obligatory carnivore the stomach may display specific adaptations which could be of significance in the husbandry of this endangered species. Two birds (natural death and euthanised for humane reasons) were studied. The stomachs were immersion-fixed in 10% neutral-buffered formalin. One stomach was sectioned and prepared routinely for light microscopy. The morphology was described and digitally recorded. The most notable organ from ventral view was the gizzard situated between the liver lobes. The proventriculus was small and the gizzard large. The gizzard was simple, distensible and thin-walled in the full state. The *L. epithelialis* of the proventriculus and gizzard presented folds lined by simple columnar epithelium. Additionally, koilin, secreted in distinct layers was present in the gizzard. Massive simple branched tubular, and long simple tubular glands were present in the L. propria of the proventriculus and ventriculus, respectively. Gland openings were the most noteable feature in the proventriculus. The *M. mucosa* was extensive in the proventriculus and absent in the gizzard. The *T. muscularis* of the gizzard was more prominent than the proventriculus. The large proventricular glands and the thin-walled gizzard support enzymatic digestion over the grinding of ingesta. The layered secretion of the koilin may assist this layer to be both tough and flexible to allow vast distension. The heavy reliance on enzymatic digestion is important in the husbandry of these birds as stress may lead to a breakdown in the gizzard lining leading to ulcers.

Haematology and biochemistry effects of acepromazine or detomidine standing sedation in horses

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The objective of this study was to determine the effects of acepromazine and detomidine standing sedation on haematological and biochemical parameters in horses. A joint collaborative blinded randomised, prospective study using a population of twelve Nooitgedachter horses and four Thoroughbred horses was carried out. Prior to experimental procedures, clinical examinations were performed and the horses were randomly assigned to receive a single intravenous administration of either acepromazine maleate or detomidine hydrochloride, or no bolus. Initial samples were taken at T=0 and thereafter samples were collected every 15 minutes until T=60 minutes. All horses were examined to ensure full recovery from treatment prior to being returned to their normal housing and routine. Clinical parameters were altered, as expected, following sedation. Sedation had a dramatic and rapid effect on haematocrit, red and white cell parameters and thrombocyte count. Sedation groups also showed alterations in ALP, ALT, AST and LDH levels, but the most dramatic effects were seen with haematology and glucose levels. Each of the sedation protocols reviewed had an effect on the haematology and serum biochemistry in the horses, these changes were detectable within 15 minutes of sedation and in some cases lasted the hour of observation. This study highlights the clinical relevance of sampling of venous blood in equine patients prior to the administration of sedation or reviewing results of subsequent haematology and serum biochemistry in light of the effects the sedation will have had on the horse in order to obtain accurate results and make informed clinical decisions.

The effects of storage time and temperature on thromboelastographic analysis in dogs and horses

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The utility of thromboelastography (TEG) is limited by short sample storage times and the storage temperature mandated for this method. The objective of this study was to evaluate the stability of canine and equine citrated blood samples for TEG analysis, when stored for extended periods of time, at room (RT) and refrigerator temperature (FT). Whole blood samples from ten healthy dogs and ten healthy horses were stored at RT and FT. Kaolin-activated TEG was performed at 30 minutes, 2 hours, 8 hours and 22.5 hours post collection. TEG data were compared to 30 minute results using ANOVA ($p< 0.05$). Clinical significance of changes was evaluated by comparing results to population- (dogs) or subject-based (horses) reference intervals. In dogs, R was shorter at 2 hr RT, 8 hr RT, 22.5 hr RT and 22.5 hr FT; K was increased at 2 hr FT and 22.5 hr FT; MA was decreased at 22.5 hr FT; no differences were found for α. In horses, R was shorter at 2 hr RT, 8 hr RT, 22.5 hr RT and 22.5 hr FT; K was decreased at 2 hr RT, 8 hr RT and 22.5 hr RT; α was decreased at 22.5 hr FT; there were no differences for MA. Two dogs had clinically significant changes from 8 hrs and one horse had decreased MA at 2 hr RT only. Canine samples can be stored for up to 2 hrs at RT or FT and equine samples for up to 8 hrs at FT without affecting TEG results.

Assessing the application of a smartphone modulated ECG device for use in equines

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Improvements in technology have allowed for the development of smaller diagnostic devices that combine ease of use with cost effectiveness and accessibility. The research presented aimed to determine the most reliable method for application of the *ALIVCOR KARDIA* smartphone modulated electrocardiograph (ECG) device in horses for reliable and clinically useful ECG recordings. Thirty-six Nooidgedachter ponies from the University of Pretoria's research herd were sampled. ECG recordings from three body locations were tested (right and left fourth intercostal space and right triceps). Site preparation and device orientation were also examined. The automatically calculated heart rate from the device application was compared to that acquired via auscultation. The fourth intercostal space on the left hemi thorax yielded the most complete, diagnostic quality ECG tracings. The device is best applied in a vertical orientation with reference to the ground and improved results were obtained when the site was dampened with 70 % alcohol. Using this configuration, the Alivcor Kardia device was able to achieve a 91.67 % acquisition of complete decipherable ECG tracings. An independent t-test conducted on heart rate data yielded a t-value of (-9.8) indicating the means of the two data sets are significantly different. The device can be used to obtain ECG recordings if used in the 4th intercostal space in a vertical orientation with 70 % alcohol applied to the hair. The automatically calculated heart rate is not an accurate measure of true heart rate.

Prevalence of bactibilia in apparently healthy dogs

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Bacterial cholecystitis is a debilitating disease in dogs. The presence of bacteria in bile in ill dogs would be significant if bile was considered sterile; however, the prevalence of bactibilia in healthy dogs is unknown. The primary purpose of this study was to determine the prevalence of bactibilia in healthy dogs. Secondary aims were to determine if differences between bactibillic and non-bactibillic healthy dogs occur with regards to serum liver enzymes activities; and liver and gallbladder histopathology. Sixty-five healthy, abandoned dogs euthanased for non-medical reasons were included in this cross-sectional, prospective study. Dogs were deemed healthy based on clinical and necropsy examinations. Whole blood, bile, gallbladder and liver samples were collected aseptically from all dogs within 30 minutes of euthanasia and submitted for bacterial culture, cytological, biochemical (alkaline phosphatase (ALP), alanine aminotransferase (ALT) and gamma-glutamyl transferase (GGT)) and histopathological analyses. Agreement between cytology and culture was assessed with Cohen κ analysis and analysis of variance of serum liver enzymes activities between dogs with bactibilia and without was performed using the Mann-Whitney test. The prevalence of bactibilia was 13.85% (9/65), with 9.23% (6/65) of dogs diagnosed on cytology and 9.23% (6/65) on bile culture. There was poor agreement between bile cytology and culture (0.449, Cohens kappa; p=0.001). No significant differences in median liver enzyme concentrations and hepatobiliary histopathology were found between bactibilic and non-bactibilic dogs. The prevalence of bactibilia in asymptomatic dogs was 13.85%, with no significant elevation in liver enzyme concentrations or hepatobiliary histopathological changes.

The potential effect of garlium GEM HC as a tick repellent agent in springbok (*Antidorcas marsupialis*)

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Garlic is one of the most popular edible plants in the world that has shown to have antimicrobial functions against bacteria, virus, fungi and protozoa. Our in vivo study with 24 springbok (*Antidorcas marsupialis*) aimed to investigate the repellant effects of garlium on different stages of red-legged ticks (*Rhipicephalus evertsi evertsi*). Engorged females of R. e. evertsi were collected from naturally infested wild ruminants. A group of ten selected engorged ticks were incubated to lay eggs in relative humidity and in a sodium chloride warm bath. Once the eggs hatched, larvae were kept to infest ears of juvenile male springbok during the study. Springbok treated group was fed 1 kg of a total mixed ration (TMR) feed with the addition of a 0.36% of the garlic compound per animal. Garlium was fed for 7 days before a total of 250 larvae of *R. e. evertsi* were introduced to ear bags that were attached to each springbok. Ear bags were then removed after a ten-day period and ticks were counted and weighed. Preliminary results show that garlium treated group has a clear repellent effect on red-legged ticks. The control springbok group had a tick load of 47% more compared to the treated group. Inner ear wax secretion was increased in the treatment group, where fewer ticks were found, suggesting that garlic plays a role in increased ear wax production.

Faecal glucocorticoid metabolite concentrations as a measure of stress in black-footed cats (*Felis nigripes*)

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The black-footed cat (*Felis nigripes*) is one of South Africa's smallest and most enigmatic cats. Their numbers both in the wild and in captivity are rapidly declining. As a consequence, they are currently listed as vulnerable in the IUCN Red List and are also listed under Appendix I by the Convention on the International Trade of Endangered Species (CITES).This project aimed to establish and validate an enzyme-immunoassay (EIA) for monitoring faecal glucocorticoid metabolite (fGCM) concentrations in black-footed cats (BFC) and to compare fGCM levels between BFC in different captive facilities to identify possible stressors. Seventeen BFCs housed in six different captive facilities spread over South Africa were used in this study. Out of this group, two randomly selected, adult individuals (one male and one female), were identified for conducting the ACTH challenge test using a synthetic ACTH analogue (Synacthen® depot, Novartis) to examine the suitability of five EIAs for monitoring adrenocortical function in BFC. Subsequently, fGCM concentrations of focal animals were determined and compared using descriptive statistical analysis. Of the five different EIAs tested, a 5α-pregnane-3β,11β,21-triol-20-one EIA, measuring 3β,11β-diol corticol metabolites, performed best overall, for both male and female BFCs. The comparison of fGCM levels from individually monitored animals exposed to various stressors (temporary enclosures due to construction) including disease (amyloidosis confirmed on histopathology) showed mark increases in fGCMs. The reliably established, non-invasive technique can now be used to assess adrenocortical function in BFC, facilitating the development of management techniques for captive settings, and should help to optimize conservation strategies for free-ranging individuals.

Evaluation of the quality of foot-and-mouth disease virus samples in preparation for next-generation sequencing

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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. Molecular epidemiology is an important tool for studying FMD and the aim of this study is to improve the quality of samples to be used for next-generation sequencing (NGS). NGS can be used to gain insight into the emergence of new strains and serotypes, and has been used for epidemiological tracing of FMD viral transmission. FMDV is an RNA virus and NGS requires suitable concentrations and whole strands of double-stranded DNA (dsDNA) for sequencing, thus FMDV isolates have to be prepared for NGS. This preparation involves extracting RNA from isolate samples and depleting host DNA before first- as well as second-strand synthesis to yield a final dsDNA product, which can be purified and sequenced. NGS does not work if the final dsDNA product is not of high concentration and if the sample is fragmented. The desired concentration of FMDV RNA and dsDNA is a cycle count (Ct) value of ≤25 on a real-time PCR (rt PCR), and fragment size should be between 1 000 and 7 000 base pairs (bp) when visualised on an agarose gel. The current method has been designed to investigate the different characteristics of FMDV samples throughout the sample preparation process. This method involves titrating the viral sample to determine viral infectivity, which relates to sample concentration and fragmentation, rtPCR of the sample at different stages of sample preparation to determine concentration, and visualisation on agarose gel to characterise extent of fragmentation. This process can be used as a quality control of samples being prepared for NGS to ensure adequate results for molecular epidemiological evaluations.

Percentage of faecal excretion of meloxicam in the Cape vultures (*Gyps corprotheres*)

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Asian Gyps vulture species are gradually recovering from the devastating effect of diclofenac being present in contaminated carcasses. This drug was responsible for the death of over 10 million vultures in India, Nepal and Pakistan. To prevent the extinction of vultures, meloxicam was introduced after the ban of veterinary diclofenac. Meloxicam's safety in vultures was attributed to its short elimination half-life in contrast with diclofenac. The reason for the rapid elimination of meloxicam is yet to be explained. The aim of this study was to evaluate the role of biotransformation in the elimination of meloxicam. Six Cape griffon vultures (*Gyps coprotheres*) were treated with 2 mg/kg meloxicam intramuscularly for faecal and plasma quantification of meloxicam concentration over time. In the plasma meloxicam was characterised by a half-life, mean residence time, clearance and volume of distribution at steady state of 0.37 ± 0.10 h, 0.90 ± 0.12 h, 0.02 ± 0.00 l/h kg and 0.02 ± 0.00 l/kg respectively (presented as geometric mean). Over the 24 h monitoring period, the total non- metabolised meloxicam in the faeces was 1.35 ± 0.71% of the total concentration in the plasma. Based on the short meloxicam elimination half-life and low cumulative concentration of total faecal meloxicam over a period in excess of 10 half-lives, this study indicates that Cape griffon vultures are efficient metaboliser of meloxicam, which is suggestive of different set of cytochrome enzymes being involved in the metabolism to that for diclofenac in this species. Identification of orthologous human CYP2C9 and CYP3A4 enzyme families in vultures will be an important further step in explaining the differences in the metabolic pathway(s) of meloxicam and diclofenac for the species.

The antioxidant and cytotoxic effect of *Cissampelos owariensis* P. Beauv extracts

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Tuberculosis is a disease of worldwide occurrence that affects about 10 million people globally. Studies show that free radical activity increases, and total antioxidant status is reduced in all tuberculosis cases, irrespective of treatment status. A decrease in antioxidant status is more pronounced in untreated patients. Some plants from the Menispermaceae family have been used traditionally to treat cough, fever and other tuberculosis related symptoms. In this study, the antioxidant effect of *Cissampelos owariensis (C. owariensis)*, a Nigerian plant from the family Menispermaceae, was investigated. Acetone, methanol:water (4:1) and hot water extracts of *C. owariensis* leaves were evaluated for antioxidant activity using a range of *in vitro assays*. Cytotoxicity of the extracts was determined using a tetrazolium based colorimetric assay against Vero monkey kidney and human liver (HepG2) cell lines. The extracts had good antioxidant activity and the hot water leaf extracts were relatively non-cytotoxic to cells. Additional studies are being carried out to determine the hepatoprotective effect of extracts as well as their activity against non-pathogenic and pathogenic *Mycobacterium strains*. These preliminary results provide support for further investigations on the bioactive compounds of *C. owariensis*.

Responses of faecal glucocorticoid metabolites, heart rate variability, body temperature fluctuations and activity patterns to potential stressors in captive cheetahs (*Acinonyx jubatus*)

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A trend exists whereby at-risk species appear to be less resilient to stress, predisposing them to the deleterious effects of allostatic load. For example, the reported vulnerability of cheetah to husbandry-related stress in captivity, resulting in poor reproduction as well as a high prevalence of several unusual diseases and undesirable behaviours. Using the cheetah as a model, we intend to assess different methods of evaluating stress in species which have demonstrated a low resilience to high allostatic load. This includes the evaluation of biologging technology. A novel technique of gathering biological information about an individual remotely and continuously. Using implanted data loggers we intend to report on heart rate, body temperature and locomotor activity in eight captive cheetahs over six months. Results obtained from a pilot study conducted reveal promising insights into the biology of a wildlife species not previously accessible by traditional technologies. Data transmitted from the biologger showed an average body temperature of 37.4°C maintained within a 24-h range of 2.6°C. This is similar to that previously reported for cheetahs. The high level of nocturnal activity observed is also in agreement with recent studies dispelling the proposition that cheetahs are strictly diurnal. Further the data loggers accurately represented the relationship between locomotor activity and heart rate. We believe that in addition to the measurement of faecal glucocorticoid metabolites (FGCM) and behavioral observations – more established methods of monitoring stress – the data collected by the biologgers will allow for long-term concurrent measurements of multiple functions, providing new understanding into how individuals cope with environmental pressures.

Hematological changes during experimental pathogenic Theileria sp. (sable) infection in roan calves (*Hippotragus equinus*)

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Roan antelope (*Hippotragus equinus*) are rare and endangered, and pathogenic *Theileria sp.* (sable) infection causes significant calf mortality. Schizont-induced transformation of mononuclear leukocytes with subsequent cytopenias characterizes pathogenic theileriosis. However, data on hematologic alterations during acute clinical disease are scarce in roan. The aims of this study were to analyze temporal changes in rectal temperature, quantitative and qualitative hematologic parameters and parasitemia in roan calves with experimental pathogenic theileriosis, before and after treatment. Eight roan calves developed acute theileriosis after inoculation with a *Theileria sp.* (sable)-infected tick tissue stabilate. Consecutive measures of rectal temperature, total leukocyte count, PCV, hemoglobin concentration, manual differential leukocyte counts, leukocyte and erythrocyte morphology and percentage parasitemia were recorded. Data were compared with 15 age-matched PCR-negative calves and nine healthy carrier calves with mixed hemoparasite infections. Six experimental calves required two buparvaquone treatments due to persistent pyrexia. Four calves died despite treatment and two recovered without treatment. Time to pyrexia (>39.5°C) and observation of schizonts and piroplasms was approximately two weeks. Schizonts peaked at 18.75 days post infection, 3 days after the initial treatment. Nonviable schizonts were detected 1-2 days after the second treatment. Piroplasms occurred intermittently at low frequencies (< 1%) after infection and treatment, similar to healthy carrier calves. Total leukocytes were unchanged post infection; neutrophils and monocytes decreased whereas lymphocytes increased. These measures did not differ significantly from healthy carrier calves. However, atypical medium-sized lymphocytes with donut-shaped nuclei and binucleated cells increased substantially in the experimental calves and mitoses occurred exclusively in this group. Hemoglobin concentration and PCV increased postinfection in the experimental group. The role of parasitized and unparasitized lymphocytes, rather than piroplasm-infected erythrocytes, requires further investigation.

Evaluation of the antibacterial and antibiofilm potential of three *Combretum* species against selected foodborne pathogens

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Foodborne pathogens are opportunistic pathogens that can cause persistent infections. This results from their ability to adhere to surfaces and form biofilms as well as to resist antibiotics. Following the need to develop new approaches to prevent and manage disease due to biofilm formation, this study aimed to test plant extracts for anti-biofilm activity. *Combretum molle, C. elaeagnoides, and C. oxystachyum* were selected for this study based on their ethnomedical use and known antibacterial activities. The broth microdilution and crystal violet assays were used to assess the antibacterial and anti-biofilm potential of acetone and methanol leaf extracts against nine bacteria implicated in causing foodborne diseases. These included ATCC strains of *Salmonella Typhimurium, Salmonella Enteritidis, Escherichia coli, Staphylococcus aureus and Campylobacter jejuni, as well as clinical isolates of E. coli, Stenotrophomonas maltophilia, Klebsiella pneumoniae and Enterobacter cloacae.* The antioxidant activity, determined using 2, 2-diphenyl-1 –picrylhydrazyl (DPPH) and 2, 2'-azino-bis (3—ethylbenzothiazoline-6-sulfonic acid) (ABTS) assays as well as the cytotoxicity (via the tetrazolium dye cell viability assay) were also determined. The extracts of the plants were all active against the test organisms with minimum inhibitory concentration (MIC) values ranging from <0.02 to 1.67 mg/ml. The acetone extract of *C. molle* had the best MIC and minimum bactericidal concentration values of <0.02 and 0.02 mg/mL against *Enterobacter cloacae* respectively. The extracts of *C. molle* had biofilm inhibitory activity against most of the tested strains except for S. *Enteritidis* and *S. maltophilia.* Extracts of *C. elaeagnoides* and *C. oxystachyum* also had good antibiofilm effects against some of the tested strains. The extracts of *C. molle* had good radical scavenging activity in the DPPH and ABTS assays. The extracts of the three *Combretum* species were non-cytotoxic to Vero cells except for the acetone extract of *C. molle*. This study indicates that the screened *Combretum* species have good antibiofilm potential and can be explored to further determine the bioactive constituents responsible for this action. The findings also provide motivation for using plant components to assist in combating bacterial resistance associated with biofilms in the food industry.

The *in vitro* effect of ionophores on cardiac and skeletal muscle cells

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Ionophores are polyether antibiotics with the ability to form dynamically reversible complexes with cations and, as a result, transfer these ions across biological membranes. The carboxylic ionophore class consists of ionophores that facilitate electrically neutral ion exchange and are tolerated relatively well by animals. These compounds are often used in the cattle and poultry industries as feed additives. They are used for the control of coccidiosis and the promotion of growth and feed efficiency. Unfortunately, cases of ionophore toxicity do occur and primarily affects the cardiac and skeletal muscle of livestock. Ionophores alter the ion homeostasis of the cell and cause changes in the intracellular pH, calcium overload, lipid peroxidation and disrupt the plasma membrane. The aim of the study is to determine the effect of different ionophores on the viability and cytoskeleton of cardiac and skeletal muscle cells in vitro. The MTT viability assay will be used to investigate the cytotoxicity of monensin, salinomycin and lasalocid, ionophores that selectively transport sodium, potassium and calcium ions, respectively. The effect of these ionophores on the viability of rat cardiac and skeletal muscle myoblasts and their differentiated myotubes will be determined over 24, 48 and 72 h. In addition, immunocytochemistry techniques will be used to investigate the effect of these ionophores on the cytoskeleton of cells

Evaluation of *in vitro* neutralization of epoxyscillirosidine by antibodies raised in sheep

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Intoxication by *Moraea pallida Bak.* (yellow tulp) in livestock is of great importance in South Africa, ranking top among all plant induced cardiac glycoside toxicosis. The toxic principle, a bufadienolide, is 1α, 2α-epoxyscillirosidine. Treatment of poisoning is challenging and affected livestock often succumbs due to the stress of handling. Manipulating animals to resist poisoning is a potential management strategy. The aim of this study was to explore the potential to develop a vaccine against epoxyscillirosidine by raising antibodies against epoxyscillirosidine in sheep and to assess the neutralization ability of the antibodies *in vitro*. Epoxyscillirosidine was successfully conjugated to keyhole limpet haemocyanin (KLH) and bovine serum albumin (BSA) rendering them immunogenic. The sheep, vaccinated with epoxyscillirosidine-KLH conjugate (n=4) and KLH (n=2) with Montanide, as adjuvant, developed antibodies as determined with an indirect enzyme linked immunosorbent assay (ELISA). Total immunoglobulins from sera of vaccinated and control sheep that were purified and concentrated using ammonium sulphate precipitation were 11 940 and 7 850 µg, respectively. The *in vitro* neutralization assay, using the methyl blue tetrazolium bromide (MTT) cell viability assay, indicated no significant difference ($p>0.05$) between anti-epoxyscillirosidine-KLH and KLH antibodies. In fact, the antibodies seemed to enhance the cytotoxicity of epoxyscillirosidine in H9c2 cells. Thus, it is necessary to develop improved conjugation methods and vaccination regimens to generate antibodies capable of neutralizing the functional group responsible for epoxyscillirosidine toxicity.

Determination of anti-nutritive factors and toxins in selected fodder trees or shrubs and conventional animal feed during feed scarcity

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Fodder trees and shrubs are important source of nutrients for livestock in developing countries during annual feed scarcity. However, the feeding value of non-conventional fodder is restricted by the presence of anti-nutritive factors (ANFs) and toxins. Thus, the objective of this study was to evaluate ANFs and toxins such as saponins, tannins, phenols, flavonoids, cardiac glycosides and oxalates in thirteen selected species of the genera *Acacia, Combretum, Gleditsia, Ceratonia* and *Peltophorum*, which are browsed or fed to livestock by rural smallholder farmers in Onderstepoort, Pretoria North. Standard commercial animal feed was used as a negative control. Aqueous (infusion and decoction) and organic solvent (hexane, chloroform and methanol) extraction methods were employed and the resulting extracts were qualitatively analyzed using standard methods while quantitative analysis was carried out using spectrophotometric techniques. Cytotoxicity (colorimetric) of the extracts to Vero monkey kidney cells was also investigated. The contents of ANFs and toxins were compared with the conventional animal feed for safety assessment. The qualitative results revealed the presence of cardiac glycosides, saponins, phenols, flavonoids, terpenoids, tannins, alkaloids, oxalate, quinones, proteins and amino acids, mostly in the methanol and aqueous extracts. The difference in ANFs and toxins between conventional animal feed and selected fodder was significant ($p<0.05$). However, the estimated levels of ANFs and toxins in selected fodder were too low (<2%) to cause any health and production problem. This was supported by the results of the cytotoxicity assay which showed that most selected species were not cytotoxic. This suggests that the selected browse material is within acceptable safe limits for animal consumption but *in vivo* tests are required to confirm this assertion.

The microbiome of *Crocodylus niloticus* eggs from commercial southern African crocodile farms and its relationship to foetal mortality and hatching success

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Crocodile products are sought after export items from southern African countries. Due to the high demand of these products, effective captive breeding and rearing of crocodiles is of critical importance to maintain a renewable resource rather than exploiting existing populations. The lack of information and scientific reports contribute to unsustainable commercial farming, one of which is low hatching rates (roughly half of that seen in the wild). Infections of the eggs may play a significant role in the low hatching rates seen. Therefore, identification of potential pathogens, sources of the pathogens and epidemiological factors influencing foetal mortality could advance optimized husbandry and incubator management, and thereby increasing farming productivity. Environmental samples (including; water, nesting material and samples from the artificial incubation environment) and samples from eggs (collected internally and externally from healthy eggs, infected eggs and eggs with foetal mortality) was collected. The bacterial and fungal communities present were identified using biomolecule-analysis and DNA-sequencing. Together with this, selected samples were processed for microscopic evaluation to elucidate mechanisms of penetration, infection and possible interactions. Over 100 different bacterial species were identified, including the following genera: Aeromonas, Citrobacter, Escherichia, Proteus, Pseudomonas and Salmonella. A third of these are known to be opportunistic pathogens and several has been implicated in reptile disease and embryonic death in oviparous animals. From the 22 species of fungi identified (including; Aspergillus, Fusarium and Lichtheimia) at least five is well-known causative agents for crocodile disease and a few is problematic in poultry farming as they are able to infect egg contents. Microscopy shown that in samples where penetration of the limiting-membrane (infection of the egg contents) occurred, close association of bacterial and fungal agents is predominantly seen. This might suggest a symbiotic or cooperative interaction between these organisms that possibly alter their pathogenicity or the host defence mechanisms.

The effect of seasonality on the stress and metabolic patterns of an African strepsirrhine

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The dramatic decrease in primate populations globally has been linked to climate change and anthropogenic stressors. As such, it is imperative to study physiological responses to environmental changes to understand primate adaptability and to enhance species conservation strategies. To assess the physiological responses to environmental change in a strepsirrhine primate, we studied the greater thick-tailed bushbaby (*Otolemur crassicaudatus*). We examined the effects of seasonality on annual faecal glucocorticoid (fGCMs) and faecal triiodothyronine (T3) metabolites (fTMs) of free-ranging male and female bushbabies in a high-altitude seasonal habitat whereby rainfall and temperature follow seasonal patterns. Prior to the hormone analysis, we validated a cortisol enzyme immunoassay (EIA) to monitor fGCM concentrations using a handling event and a T3 EIA using a thyroid stimulating hormone challenge. We collected 330 samples for glucocorticoid and T3 hormone analysis from free-ranging *O. crassicaudatus* from the Lajuma Research Station, Soutpansberg Mountains. Season was defined by rainfall and temperature variations; that is, dry-cold, and wet-hot. Additionally, seasonal insect availability was estimated using light traps. For females, the results revealed that season best explained the variation in fGCM concentrations. For males, rainfall showed significant effects on the fGCM concentration variation. Nonetheless, males expressed the highest fGCM levels during the mating period, while female levels peaked during late gestation and lactation, suggesting reproduction may play a role in driving stress levels. The fTM patterns reveal season and reproduction have significant effects on the T3 concentration. These data coincide with seasonal insect population fluctuations indicating nutritional quality may influence the metabolic patterns, supporting outcomes of previous studies. Overall, our findings show that changes in the natural environment can have significant effects on the stress and metabolic patterns in a strepsirrhine species and demonstrates the efficacy of integrating stress and metabolic hormones to better understand climate change ecology.

Reference intervals for haematology and serum biochemistry in Temminck's ground pangolin (*Smutsia temminckii*)

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An alarming number of pangolins are currently illegally traded for their scales and meat. Many pangolins confiscated from the trade are severely clinically compromised. Unfortunately, little is known about the physiology and normal health of pangolin, making it difficult to identify disease processes and treat them. The purpose of this study was to establish reference intervals (RIs) for haematology and plasma clinical chemistry in the Temminck's ground pangolin. Blood samples were collected from 27 healthy free-living or rehabilitated pangolins and reference intervals were generated according to international guidelines. Clinical chemistry analysis was performed using the Abaxis VetScan VS2 and haematology was performed using the Abaxis VetScan HM5 analyser. Plasma clinical chemistry RIs were: albumin 26-41 g/L, amylase 316-1014 U/L, ALP 29-153 U/L, ALT 25-307 U/L, bilirubin 1.5-10.8 □mol/L, calcium 1.8-2.5 mmol/L, creatinine 9.7-46.3 □mol/L, glucose 3.8-10.0 mmol/L, potassium 3.6-5.9 mmol/L, phosphate 1.3-2.6 mmol/L, sodium 132-142 mmol/L, total protein 53-84 g/L and urea 5.6-19.9 mmol/L. Haematology RIs were: WBC 1.8-10.71 x109/L, RBC 3.88-8.31 x1012/L, HGB 73-150 g/L, HCT 26-51%, MCV 55-72 fL, MCH 15.6-21.4 pg, MCHC 242-332 g/L and RDW 14.3-19.1%. RIs for some measurands were wide, probably due to the small sample size. Nevertheless, these are the first RIs generated for the Temminck's ground pangolin and the results presented here will allow veterinarians to better determine the health status of pangolin patients, thus enabling them to formulate optimal treatment plans in the hope of increasing patient survival rates of this endangered species.

Activity of three South African plants on phytopathogenic bacteria and fungi of tomatoes and chemical profiling of the extracts

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Soil-borne pathogens in the fungal and bacterial kingdom cause destruction of large fields of agricultural crops, with great economic loss worldwide. The aim of this study was to evaluate three South African plant extracts for activity against phytopathogenic bacteria, fungi and to profile their chemical constituents. A serial microplate dilution method was used to determine the minimum inhibitory concentration (MIC) of *Leonotis leonurus, Clausena anisata* and *Lantana rugosa* crude extracts and fractions prepared using solvent-solvent fractionation. Antibacterial activity was determined against *Xanthomonas perforans, Xanthomonas vesicatoria, Ralstonia solanacearum, Ralstonia pseudosolanacearum* and *Clavibacter michiganensis subsp. michiganensis (Cmm)* while antifungal activity was evaluated against *Fusarium oxysporum f. sp. lycopersici.* Gas chromatography-mass spectrometry (GC-MS) was used for profiling the phytochemicals from the acetone and dichloromethane/methanol extracts. *L. leonurus* and *L. rugosa* extracts had moderate to weak activity with MIC values ranging between 0.156 to 2.5 mg/mL. *L. rugosa* fractions were more active with MIC values ranging between 0.078 to 0.156 mg/mL against most phytopathogenic bacteria, followed by dichloromethane and ethyl acetate fractions of *L. leonurus*. All extracts and fractions were inactive against Fusarium spp. except the water extract of *L. leonurus* with MIC of 0.156 mg/mL. The selectivity index of *L. rugosa* extracts (determined using cytotoxicity against Vero cells) was less than 1 in all extracts making it unsafe. The GC-MS analysis of *L. leonurus* dichloromethane/methanol extract revealed a high quantity (32%) of 9,12-octadecadienoyl chloride, (Z,Z)-. This was followed by 9-octadecenamide, (Z)- with 20%, and this compound is known to have antibacterial activity. This study supports further investigation of *L. leonurus* for management of pest diseases of tomatoes.

In vitro cytotoxicity of isogeigerin acetate, a novel sesquiterpene lactone isolated from *Geigeria aspera* (vermeerbos)

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Ingestion of *Geigeria* species induces 'vermeersiekte' (vomiting disease) in sheep, an economically important plant poisoning in South Africa. *Geigeria aspera* were collected in the Vrede district, Free State (27° 25' 48" S; 29° 9' 36" E). The plant material was dried, milled and the toxic principles were extracted and isolated following chromatographic procedures. Besides geigerin and ivalin, a novel sesquiterpene lactone was also isolated. The compound, isogeigerin acetate, was characterised by nuclear magnetic resonance (NMR), mass spectrometry and absolute configurations were determined using X-ray crystal diffraction. Cytotoxicity of geigerin, ivalin and isogeigerin acetate were compared by exposing a murine myoblast (C2C12) cell line to varying concentrations of the three sesquiterpene lactones isolated. Cell viability was assessed using the methyl-thiazolyl-tetrazolium (MTT) assay. After 48 h exposure, the median effective concentrations (EC50) were calculated. The EC50's were 0.0029 mM, 3.746 mM and 3.792 mM for ivalin, geigerin and isogeigerin acetate, respectively. A concentration- and time-dependent cytotoxic response was observed following exposure of the cell line to ivalin and geigerin for 24, 48 and 72 h. The results indicate that ivalin is more toxic than geigerin and isogeigerin acetate.

The effects of water and ethanol extracts of *Panaeolus cyanescens* mushroom on arginase activity in bovine pulmonary aortic endothelial cells

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Increased arginase activity has been implicated in various conditions characterised by impaired endothelial dysfunctions including pulmonary hypertension. Arginase competes with nitric oxide synthase (NOS) for intracellular L-arginine substrate and as a result contributes to reductions in endothelial nitric oxide generation, promote reactive oxygen species production and endothelial NOS uncoupling. *Panaeolus cyanescens (P. cyan)* is a potent psilocybin mushroom in the *Panaeolus* genus that grows in different countries including South Africa. Psilocybin and psilocybin mushrooms have been found to possess antidepressant effects with temporary increase in blood pressure (BP) and mechanisms are not known. We hypothesized that *P. cyan* increases BP by mechanisms that involve arginase activity pathways in endothelial cells. The mushrooms were oven dried and extracted with ethanol, cold and hot water. Antioxidant activity was measured with 1,1-Diphenyl-2-picrylhydrazyl (DPPH) and 2,2'-azino-bis(3-ethyl-benzothiazoline-6 sulfonic acid) diammonium salt (ABTS) assays. The *P. cyan* extracts were tested for cytotoxicity using the 3-(4,5-dimethylthiazol-2-yl)-2,5 diphenyltetrazolium bromide(MTT) assay on bovine pulmonary aortic endothelial cells (BPAEC). When grown to confluence, BPAEC cells were induced with stress and then treated with the three extracts (50 and100µg/mL) over 48 hours with and without Nw-nitro-L-arginine methyl ester (L-NAME), a NOS inhibitor 10 and 100µM; S-(2-Boronoethyl)-L-cysteine hydrochloride (BEC), an arginase inhibitor 10 and 100µM and 200µM L-arginine supplement. Apoptosis, nitric oxide production and arginase activity was measured using caspase 3, nitrite and arginase activity assay kits. Results showed that *P. cyan* mushroom extracts have poor antioxidant activity. The results also showed that stressed induced cells had lower cell viability, higher caspase 3 and arginase activities than the normal cells. The results suggested that *P cyan* extracted with hot water is safer and has beneficial arginase-downregulation effects. The results also suggested that the cold water extracts of *P. cyan* has upregulating-arginase activity which may promote BP increase and caution needs to be taken when consumed in conditions such as hypertension.

The *in vitro* antibacterial activity and safety of *Morinda lucida* leaf extracts against Salmonella serovars relevant in livestock infections

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Salmonella infections are of great importance in human and animal health. They cause significant morbidity and mortality worldwide. Infections caused by non-typhoidal *Salmonella* are either non-invasive or invasive systemic infections that require effective antimicrobial therapy. The development of resistant strains has reduced the efficacy of the conventional antibiotics. Therefore, there is an urgent need to develop drug templates with good activity against these pathogens. *Morinda lucida* has been used extensively in African traditional medicine for the treatment of symptoms similar to human typhoid and malaria fever. The aim of this study was to investigate the antibacterial activity and safety *in vitro* of the leaf extracts against eight *Salmonella* serovars. Acetone and aqueous leaf extracts of *M. lucida* were screened for antibacterial activity against several serovars of *Salmonella enterica subsp. enterica* including *S. enterica* serovar Gallinarum (birds), Dublin (birds and ruminants), Choleraesuis (pigs), Braenderup (birds), Idikan (humans and birds), Kottbus (birds), Typhimurium (birds and ruminants) and Enteritidis (birds and humans) using a serial microdilution assay. The cytotoxic and anti-biofilm potential of the acetone and aqueous extracts were also determined against human colon cancer (Caco-2) cells and biofilm formation respectively. The minimum inhibitory concentration (MIC) of the extracts ranged from 0.09 to 1.87 mg/ml. The LC50 values of the acetone and aqueous extracts against the Caco-2 cells were 0.46 and 0.33 mg/ml respectively. The acetone extract had the strongest anti-biofilm activity against S. Enteritidis. The range of selectivity index (SI) values of the acetone and aqueous extracts was 1.00 to 6.57 and 0.23 to 8.28 respectively. The potential usefulness of this plant species as an alternative for treatment of human and animal salmonellosis was supported by these results. However, in vivo data is necessary to further investigate this claim.

Antimicrobial resistance and biofilm formation in coagulase negative staphylococci isolated from cow milk samples submitted to the Onderstepoort Milk Laboratory

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Increased prevalence of antimicrobial resistance, treatment failure, and financial losses have been associated with coagulase negative staphylococci (CoNS) in dairy cattle with clinical mastitis. The objectives of this study were to investigate the antimicrobial resistance patterns and biofilm formation in CoNS isolated from cow milk samples submitted to the Onderstepoort Milk Laboratory. A total of 142 confirmed CoNS isolated from cow milk samples were used for this study. Isolates were subjected to the tissue culture plate method for biofilm formation testing and antimicrobial susceptibility testing against a panel of 11 antimicrobials using the disk diffusion method. Biofilm formation was identified in 18% of CoNS tested. *Staphylococcus* chromogenes (11%) had the highest proportion of biofilm formation followed by *S. haemolyticus* 4.0% and *S. epidermidis*, *S. hominis, S. xylosus,* and *S. simulans* with 1% respectively. Ninety percent of CoNS isolates were resistant to at least one antimicrobial (AMR) and 51% were multidrug resistant (MDR). Resistance among CoNS was the highest to ampicillin (90%), penicillin (89%) with few (9%) isolates resistant to cefoxitin and vancomycin respectively. The most common resistance patterns among the CoNS was penicillin-ampicillin (16%) and penicillin-ampicillin-erythromycin (10%). Forty-two percent of biofilm positive CoNS were MDR. At the species level, MDR was common among *S. epidermis* (65%), *S. chromogenes* (52%) and *S. haemolyticus* (44%). Biofilm formation was uncommon among the CoNS in this study. However, almost half biofilm producing organisms were MDR. Most CoNS isolates exhibited resistance penicillin and ampicillin.

Assessment of infection prevention-control measures and hand hygiene compliance among healthcare workers in the intensive care unit at the Onderstepoort veterinary academic hospital

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Although studies have been done in humans on infection prevention and control (IPC), there is a paucity of information in veterinary health care facilities in South Africa. Therefore, the aim of this study was to evaluate IPC measures including hand hygiene compliance among healthcare workers in the intensive care unit (ICU) in the Onderstepoort Veterinary Academic Hospital. A cross-sectional study was conducted among healthcare workers (HCW) (doctors, students, and nurses) in the ICU using the infection control assessment tool (ICAT). In addition, direct observations of the five hand hygiene moments were also conducted. A total of 296 observations were recorded consisting of 734 hand hygiene opportunities. The overall compliance level in ICU was 24.3% (178/734). Most HCWs did not sanitise stethoscopes and leashes, before and between patients and majority were not bare 'below the elbows.' Hand hygiene compliance was higher among nurses (44%, CI:34.21- 54.19) compared to students (22 %, CI:19- 26) and doctors (15%, CI: 9- 26). Similarly, compliance was higher after body fluid exposure (41%, CI: 27- 58) compared to after patient contact (32.2%, CI:27-38), before patient contact (18.8%, CI:15- 24), after contact with patient surroundings (16%, CI:10-24) and before an aseptic procedure (15%, CI: 6-34). Among the doctors, low hand hygiene compliance was observed before patient contact (3%, 2/54) and among the nurses was low after body fluid exposure 5.56% (2/36). While student had lower compliance after body fluid exposure (33%, 12/36). The low overall hand hygiene compliance in this study raises a concern of potential transmission of HAIs and zoonotic disease in the ICU. Compliance was lower among doctors compared to students and nurses. In view of this, educational interventions are needed to address low level of compliance in the ICU in this study.

The bacterial microbiome of *Rhipicephalus sanguineus* ticks in the Mnisi community, South Africa

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Rhipicephalus sanguineus is a three host tick that completes all its life stages on domestic dogs. It is, however, capable of parasitizing most vertebrates, including wildlife, cattle, dogs and humans. It is known to transmit various tick-borne diseases. In Mnisi, a rural community in Bushbuckridge, Mpumalanga, South Africa, *R. sanguineus* is one of the most prevalent ticks found on dogs. The community lies at the wildlife-livestock-human interface where humans are at risk of infection with various tick-borne zoonotic diseases. The aim of this study was to investigate the prevalence and diversity of tick-borne bacterial pathogens in *R. sanguineus* that may impact human and animal health. To achieve this, we analysed the microbiome of ticks sampled from community dogs over a 12-month period to detect bacterial pathogens and symbionts. To date, *R. sanguineus* (n = 582), *R. simus* (n = 82), *Amblyomma hebraeum* (n=97), as well as 183 unidentified ticks have been collected from 51 dogs. Ticks were kept in a humidity and temperature controlled chamber for two days to allow them to digest their blood meal. Ten *R. sanguineus* ticks from each dog were surface sterilized, and dissected to remove their midguts and salivary glands and then pooled. Genomic DNA was extracted and PCR amplified using universal 16S rDNA barcoded primers. Sequencing will be done at Washington State University using Pacific Bioscience's circular consensus sequencing strategy. Environmental conditions and other factors that could influence the tick population or tick microbiome were also analysed. Preliminary data shows that the microbiome is dominated by *Coxiella spp*, including *C. burnettii*, the causative agent of Q-fever. The information gained about the bacterial communities that abound in *R. sanguineus* will undoubtedly aid health care practitioners in the area with the diagnosis of important tick-borne diseases in animals and humans.

Assessment and genotyping of a novel vaccine candidate for *Theileria parva* infections

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Theileriosis is a lymphoproliferative tick-borne disease of cattle and other wild ruminants. It is caused by infection with a protozoan, *Theileria parva*, which is vectored by *Rhipicephalus sp*. ticks. This disease is prevalent in cattle throughout most parts of Central, East and southern Africa. There are various control and treatment methods in place for *T. parva* infections; however, they all have drawbacks and limitations. The available vaccine, Muguga cocktail does not confer protection against all field strains, particularly buffalo-derived *T. parva* infections. Attempts to develop a subunit vaccine using different candidate antigens have been promising but these have shown limited efficacy due to antigenic and genetic diversity of *T. parva* strains in the field. Thus, there is a need to search for additional vaccine candidates. Our laboratory identified potential vaccine candidates using a genome-wide in silico approach. Secreted antigens expressed in the pathogenic schizont stage of the parasite were the main targets. Ideally, a vaccine candidate that is genetically conserved in both cattle- and buffalo-derived *T. parva* isolates is preferred to provide broad spectrum immunity against the different types of *T. parva* strains. Thus, the aim of this study was to assess and genotype one of the possible antigens identified as a novel vaccine candidate. The identified candidates were ranked based on their level of expression in the schizont stage of the parasite. RNA sequence data produced from the transcriptome analysis of two *T. parva* stocks representing cattle- and buffalo-derived parasites, using next-generation sequencing, was employed. The candidate with the highest expression levels was selected for genetic diversity analysis, in cattle- and buffalo-derived *T. parva* isolates. Thus, specific primers were designed and optimised for PCR amplification and sequencing of the gene encoding the target 'antigen'. The selected candidate seems to be conserved across both buffalo and cattle samples as well as between geographical areas. These findings indicate that this hypothetical protein could be a good vaccine candidate.

Phylogenetic characterisation of the Palyam serogroup orbiviruses

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The Palyam serogroup, genus *Orbivirus* and family *Reoviridae*, are arthropod-borne viruses that have been isolated in Africa, Australia and Asia. Some of the viruses are associated with abortion and teratogenesis in cattle and other ruminants. Of the 13 serotypes identified, the full genome sequence of only one, Kasba, has been published. The objective of this study was to perform Next Generation Sequencing (NGS) and phylogenetic analysis on 12 Palyam serotypes and field isolates of the African serotypes in our possession. The viruses were propagated, full-length amplification of cDNA (FLAC) was performed and the amplicons were sequenced on an Illumina® Mi-Seq sequencer. Sequence data were analysed using the CLC Genomics Main workbench, version, 8.0.1 and Bayesian analyses was performed in MrBayes version 3. The Palyam serogroup was found to be most closely related to the African horse sickness virus group and showed the most distant evolutionary relationship to equine encephalosis viruses (EEV). Amino acid sequence analysis revealed that the gene encoding VP7 was the most conserved within serotypes and VP2 and VP5 showed the highest degree of variation. A high degree of sequence identity was found for isolates from the same geographical region. The phylogenetic analysis revealed two clades where the African serotypes were all very closely related in one clade and the other clade contained the Australian and Asian serotypes and one African serotype, Petevo. Results obtained from sequence data indicated that the geographical origin of Palyam serogroup viruses played an important role in the development of the different serotypes.

Identification and genotyping of predicted host cell phenotype modulators in *Theileria parva*

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Theileria parva is one of the two *Theileria* species known to cause reversible transformation of their host cells. This protozoan parasite belongs to the *Apicomplexan phylum*, and causes cattle theileriosis in cattle in eastern, central, and southern Africa. The cattle-derived *T. parva* isolate is responsible for the form of theileriosis known as East Coast fever while the buffalo-derived causes Corridor disease. The schizont is the pathogenic stage of the parasite, responsible for the transformation of infected lymphocytes, yet little is known about virulent proteins responsible for host cell transformation in this stage. Thus, the aim of this study is to predict *T. parva* host cell phenotype modulators (HCPMs) using in silico methods, determine their expression and to analyse the conservation of their gene and protein sequences. From in silico analysis using a combination of bioinformatics tools targeting secreted, membrane and cytoplasmic proteins, 145 proteins with predicted functions associated with host cell transformation were identified after excluding proteins with homologs or orthologs or paralogs in non-transforming parasites and the bovine host. The expression of genes encoding the predicted HCPMs was determined using RNA sequence data, previously obtained from the analysis of the transcriptome of the schizont stage of two *T. parva* stocks, representing the cattle-derived and buffalo-derived parasites. The transcriptome analysis revealed expression of 47 genes encoding possible HCPMs, in both *T. parva* stocks, with average RPKM values ranging from just above 10 to > 3000. Therefore, the top three genes with the highest expression in both isolates were selected for genetic diversity studies, comparing gene, and protein sequences from cattle and buffalo-derived parasites. Genetic diversity analysis is ongoing, which will give us an insight on the evolution, diversification, and migration of this parasite in the African continent based on the allelic diversity pattern among strains.

Investigating *Rickettsia africae* infection in *Amblyomma hebraeum* ticks in Mnisi, Bushbuckridge Municipality, South Africa.

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Rickettsia africae is a gram-negative bacterium, which causes African Tick Bite Fever (ATBF) in humans. African TBF is a febrile disease mainly affecting travellers to Southern Africa. This bacterium is known to be transmitted by *Amblyomma hebraeum* and Amblyomma variegatum ticks. In southern Africa the principal vector is *A. hebraeum*. This project was performed in a rural community in Mpumalanga province and aimed at addressing knowledge gaps in *R. africae* infection in *A. hebraeum* ticks. Infection rates in adult ticks, eggs and larvae as well as transovarial transmission efficiency of *R. africae* from the tick to its offspring was determined. To accomplish this, adult *A. hebraeum* ticks were collected from cattle and larvae were collected by dragging at the targeted dip tanks. Engorged female *A. hebraeum* ticks were also collected and they were put in a humidifier to oviposit. DNA was extracted from the engorged ticks and the egg masses as well as from the adult ticks and the larvae. After DNA quantification, a Rickettsia qPCR was performed to screen all samples for the target gene, gltA. Samples positive for *gltA* were subjected to conventional PCR targeting the *ompA* gene, which is specific for the Spotted Fever Group to which R. africae belongs. From the sampled adult ticks, engorged females and egg masses 95.75%, 87.74% and 91.67% were positive for the *gltA* gene respectively. Results from the *ompA* gene screening revealed that 14.28% of adult ticks, 26.56% engorged females and 19.81% egg masses and were positive. The samples positive for amplicons of *ompA* gene were sequenced to determine the rickettsia species present. The sequences were analysed and all the samples were found to be 99.84% identical to *R. africae*. The presence of *R. africae* in this area necessitates public awareness.

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Molecular detection of tick-borne haemoparasites in cattle and buffalo samples from Manicaland province, Zimbabwe

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In sub-Saharan Africa, the most important tick-borne diseases (TBDs) affecting cattle are babesiosis caused by *Babesia bovis* and *B. bigemina*, theileriosis caused by *Theileria parva*, anaplasmosis caused by *Anaplasma marginale* and heartwater caused by *Ehrlichia ruminantium*. These TBDs cause major constraints to livestock production in developing countries such as Zimbabwe. DNA was extracted from 87 (80 cattle and 7 buffalo) whole blood samples and subjected to Reverse-line blot hybridization (RLB) and quantitative real-time polymerase chain reaction (qPCR) analyses. The RLB analysis revealed TBD infections in 58 samples (67%); 48 (55%) of which hybridized to the genus-specific probes only. The species detected by the genus-probes will be confirmed by 16S and 18S rRNA gene sequencing. Nonetheless, TBDs detected by RLB included three *Theileria* (*T. mutans, T. velifera, and Theileria sp. sable*), two *Anaplasma* (*A. marginale, A. centrale*) and two *Babesia* (*B. bigemina, B. bovis*) species. The most commonly occurring infections detected by qPCR assays were A. marginale (n = 27, 70.97 %) and B. bigemina (n = 7, 22.58 %); followed by *A. centrale* (n = 1, 14.28 %). Notably, all the buffalo samples tested negative for *B. bigemina* and *B. bovis*. Moreover, none of the samples (n = 87) tested positive for either *T. parva* or *E. ruminantium* by any of the assays used. Our results did not follow the common trend for the prevalence of TBDs in Zimbabwe. According to recent reports, cattle theileriosis is the major killer of cattle, followed by babesiosis, heartwater, then anaplasmosis. Thus, our data suggest that the trend of occurrence of TBDs may vary between provinces depending on the vector-parasite-host-environment dynamics for each province. Finally, this study confirms that buffalo in this area are carriers of TBDs that pose risk to the cattle population.

Development and validation of a real time PCR assay to detect *Ehrlichia canis* in dogs

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Ehrlichiosis is caused by a pleomorphic gram-negative bacteria and an important zoonotic tick-borne disease, with a potential to be fatal. This bacteria occurs worldwide and species affected by it include human, domestic and wild animals. Although direct detection of the bacterial antigen by ELISA has been used successfully to diagnose the disease, a challenge remains in dogs where co-infection of infectious agents is common due to pathogens being transmitted by the same vectors. Cross-reactivity of the serology assays makes it difficult to make species-specific diagnoses. A more sensitive and reliable molecular technique that can detect and identify pathogens at species level is needed to enhance disease diagnosis. Real-time PCR is a highly sensitive, efficient, rapid, and reproducible method but has limited multiplexing capabilities. In this study we developed a real time PCR assay that employs group specific primers and an *E. canis* TaqMan® MGB probe. The group specific primers were designed in the conserved region of the 16S gene and the forward primers included redundant base pairs to accommodate other species in this genus. The efficiency of the assay was 93%, specific and sensitive. The 95% limit of detection = 33 bacteria/µl of blood (95% confidence interval: 23 - 58). The inter-run standard deviation (SD) ranged between 0.33 - 1.29 and the intra- run SD 0.04- 1.14, consistent repeatability was observed. Diagnostic validation was performed on field samples. The assay proved to be *E. canis* specific when tested against other pathogens, there was no cross reactivity observed. This assay will be a useful tool for the early diagnosis of *E. canis* and this will aid in timely treatment.

Lesions and cellular 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, real-time reverse transcription PCR and/or immunohistochemistry (IHC). Tissues were examined by histopathology (haematoxylin and eosin stains) and IHC (polyclonal hyperimmune mouse ascitic fluid and detection with a basic avidin-biotin 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 macrophages. Sixty-four 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 gut-associated 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 antigen was present in macrophages 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. From results obtained in this study it is recommended that the minimum set of specimens to be submitted for histopathology and IHC to confirm or exclude a diagnosis of RVFV are liver, spleen and kidney. Skin from areas with visible crusts and lung could be useful additional samples. In endemic areas, cases of acute renal tubular injury should be investigated further if other more common causes of renal lesions have already been excluded. RVFV can also cause an acute infection in the testis, which requires further investigation.

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Research Awards 2018

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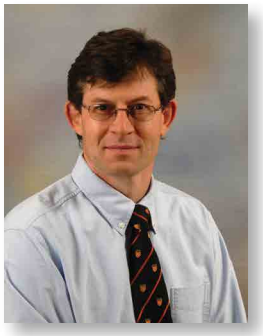

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University of Pretoria Faculty of Veterinary Science - Faculty Day 22 August 2019

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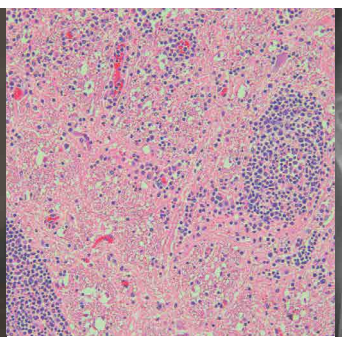
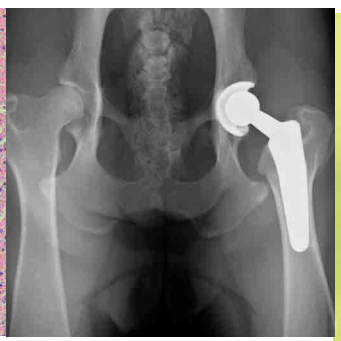
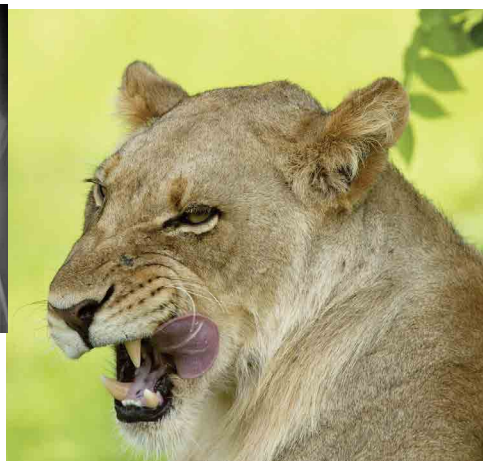

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