

Faculty Day

25 August 2016

Research Overview



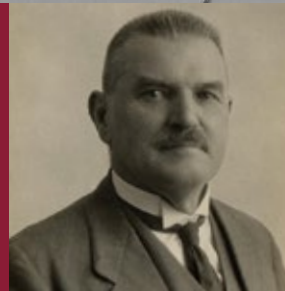
Prof Lucille Blumberg
National Institute for Communicable Diseases,
National Health Laboratory Service, SA



UNIVERSITEIT VAN PRETORIA
UNIVERSITY OF PRETORIA
YUNIBESITHI YA PRETORIA

Faculty of Veterinary Science

Fakulteit Veeartsenykunde
Lefapha la Diseanse tša Bongakadiruiwa



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Brief history of Faculty Day

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

Since the inception of the Faculty of Veterinary Science at Medunsa in the early 1980s, the staff, and later students, were involved in the activities of the "Academic Day",

which was aimed at highlighting the research activities of the University, as well as exposing young researchers to a conference environment.

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

Sponsorships

The Faculty of Veterinary Science wishes to express its sincere thanks to the following sponsors for their very generous contribution in support of the 2016 Faculty Day.



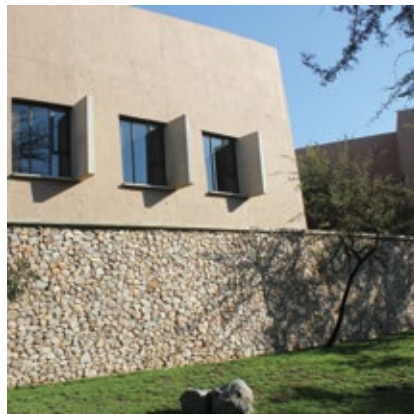
Bayer HealthCare
Animal Health



Faculty Day

Faculty of Veterinary Science
University of Pretoria

25 August 2016



Contents/Programme



08:00 – 08:25 Registration and Coffee in the foyer of the Arnold Theiler Building

Master of Ceremonies: Prof Anita Michel

08:30 – 08:45 Welcoming and Opening Address: Prof Darrell Abernethy, Dean of the Faculty of Veterinary Science

08:45 – 10:00 **First Session Chairperson: Prof Tshepo Matjila**

1. Effects of thiopentone, propofol and alfaxalone on laryngeal motion during oral laryngoscopy in healthy dogs
Smalle T.M.
2. Phytochemicals – an alternative to synthetic chemical acaricides?
Adenubi O.T.
3. Histomorphology, fatty acid composition and molecular characterization of healthy adipose tissue and pansteatitis tissue in the Nile crocodile (*Crocodylus niloticus*)
Azeez O.I.
4. Identification and characterisation of *Ehrlichia ruminantium* vaccine candidate epitopes
Thema N.
5. Sensitivity and specificity of three pregnancy-associated glycoprotein ELISA tests in South African dairy cattle
Motimele B.

10:00 – 10:45 Tea (Arnold Theiler Building Foyer)

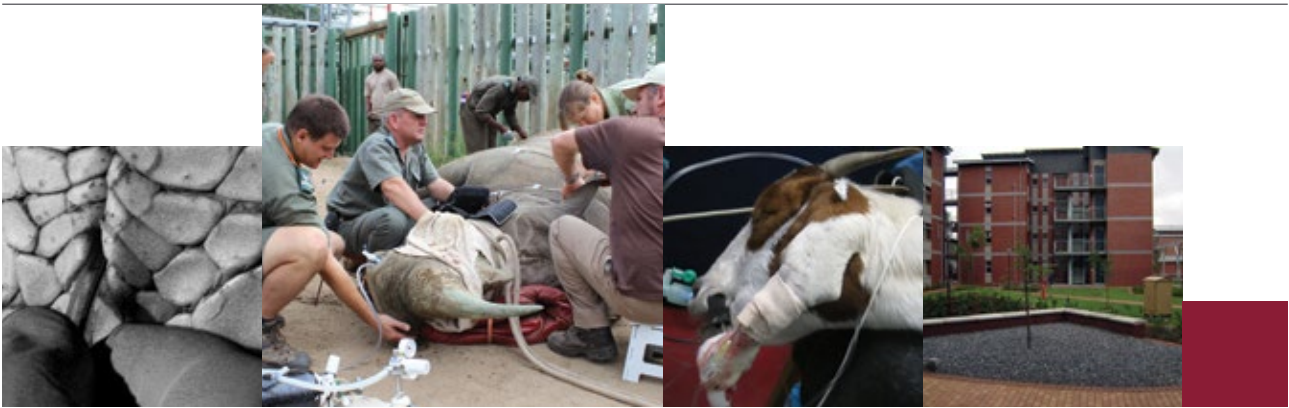
10:45 – 12:00 **Second Session Chairperson: Prof Darrell Abernethy**

Sir Arnold Theiler Memorial Lecture: Prof Lucille Blumberg

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

Poster session

12:30 – 13:00 Finger lunch



13:00 – 14:15

Fourth Session Chairperson: Prof Geoffrey Fosgate

1. Pathogenesis of kidney disease in captive cheetahs (*Acinonyx jubatus*): preliminary findings
Lane E.P.
2. Single-incision laparoscopic sterilization of the cheetah (*Acinonyx jubatus*)
Hartman M.J.
3. Osteology of the carpal region of the southern white rhinoceros (*Ceratotherium simum simum*)
Meusel E.G.
4. Host immune response profiles of calves following vaccination with live BCG and inactivated *Mycobacterium bovis* vaccine candidates
Van der Heijden E.M.D.L.
5. The effects of injectable trace mineral supplementation on semen quality in bulls
Ferreira G.M.

14:45 – 15:05

Developing researcher presentation from the Department of Veterinary Tropical Diseases

15:05 – 15:15

Faculty Day Awards: Prof Andre Ganswindt

Researcher of the Year

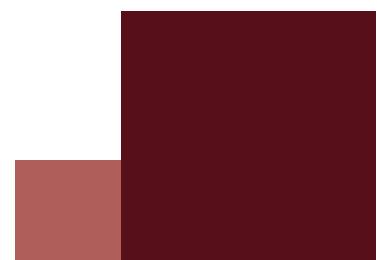
Best oral presentation

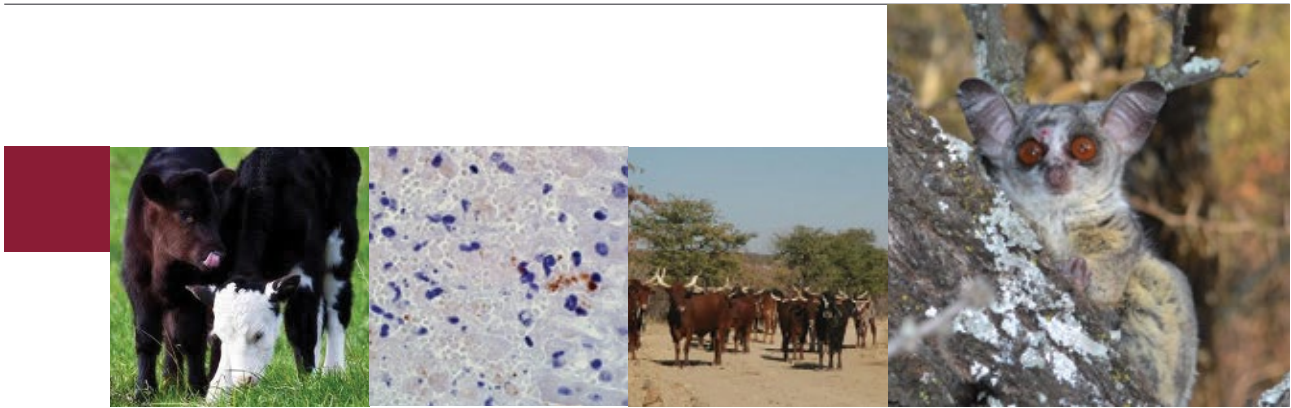
Best poster presentation

2 Book presentations

15:15

Coffee/tea and snacks (Cafeteria)





POSTER PRESENTATIONS

1. Etorphine-ketamine-medetomidine total intravenous anaesthesia in wild impala (*Aepyceros melampus*) of 120 minute duration
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7. Important trace element concentrations in ovine liver as determined by energy dispersive handheld X-ray fluorescence spectrometry
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17. Spatial distribution of bovine Trypanosomiasis in Malawi
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Message from the Dean

The University of Pretoria is known as a research-intensive university, recognized for the high quality of its graduates, publications and research. The Faculty of Veterinary Science explicitly endorses this strategy and Faculty Day is a showcase of output from selected post-graduate students. The faculty is on a journey to grow its research, which will only be effective if it remains relevant to the needs of South Africa, further develop its links within Africa, and focuses on expanding its international network.

The development of strong, productive research groups that will support UP's 2025 Vision will be an important element on this journey, which can serve as the basis for Faculty growth and development. In this regard, emphasis will be placed on the Faculty's existing strengths and developing groups that will contribute to both the international ranking of the Faculty and the University's ranking and vision. In identifying these groups, the focus will be on the potential to generate high-impact publications and attract more postgraduate students nationally and internationally. Among others, potential groups that have already been identified include wildlife, infectious diseases, One Health and antimicrobial medicines. We conduct cutting-edge research on a wide range of animal-related diseases and issues that affect people. This is because of the close relationship between people, animals and the environment and the crucial role that veterinarians play in reducing the risk of diseases passing from animals to people, ensuring food safety and promoting food security.

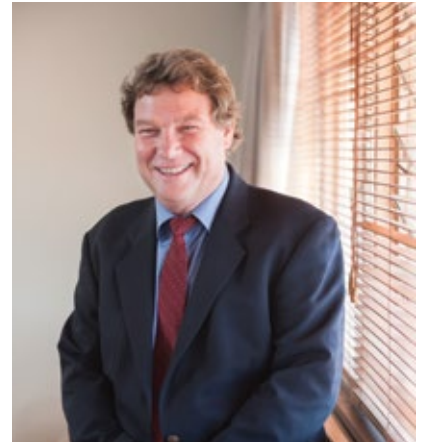
Maintaining this momentum will take teamwork, dedication and a strategic approach to research in our quest to be perceived as a highly productive, world-class veterinary seat of excellence. In further pursuit of this mission we have extended our collaboration by creating various cooperative opportunities over the last two years. Recently the HWSETA committed R15 million to the Faculty, which included 42 postgraduate student bursaries and a range of undergraduate support: 20 undergraduate student bursaries for first year students; work integrated learning for final year students; undergraduate research bursaries; skills laboratory teaching aids; training of 20 learners for manufacturing of teaching aids; 'Adopt a School' pilot project, and articulation with agricultural colleges.

The HWSETA funding and support will make a significant contribution to our training and research programmes and will also assist us in networking with institutions that train veterinary technicians and agricultural students. In this regard the Faculty has wasted no time in setting up partnerships with

agricultural colleges and by the end of 2015, discussions between management at the Faculty and Tsoelo Agricultural and Rural Development Institute (TARDI) in the Eastern Cape were well underway. The support for TARDI by the Faculty of Veterinary Science has been made possible through HWSETA funding and will further supplement HWSETA funding of 50 full bursaries for learners at TARDI to become animal health technicians.

We will always be faced with new challenges in ensuring that we are locally relevant to the challenges of animal health, poverty and food security in southern Africa, while at the same time making an impact internationally with cutting-edge research and high-level collaborations and networks. Examples of these collaborations can be found in the work of, among others, our Exotic Leather Research Centre which serves as technical collaborator for the Department of Trade and Industry (dti)-approved sub-National Exotic Leather Cluster, and is responsible for research, postgraduate training and services to improve the global competitiveness of the South African exotic leather and leather goods industry. Hosting the recent 24th working meeting of the IUCN Crocodile Specialist Group together with the ELRC's partners was seen as a huge success and judged as one of the best CSG working meetings held thus far.

A second example is the unique Rhino DNA Indexing System or RhODIS and eRhodis app initiatives developed by the Faculty's Veterinary Genetics Laboratory (VGL). These initiatives are gaining significant traction internationally thanks to advocacy work of the VGL led by Dr Cindy Harper, a partnership with the World Wildlife Fund (WWF), and the success of the system itself. It has been formally recognised by The Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) and the International Union for Conservation of Nature (IUCN) African Rhino Specialists Group for its pioneering role in tracking poached wildlife and wildlife products. Nine African countries are already part of RhODIS. In addition, the RhODIS team has provided training to more than 400 law enforcement and conservation workers in South



Prof Darrell Abernethy,
Dean: Faculty of Veterinary Science

Africa, Namibia and Swaziland. South Africans trained on the system include SAPS investigators, prosecutors, Green Scorpions, veterinarians and wildlife officials and the system has led to poachers being convicted and fewer rhinos poached in Southern Africa. Recently the VGL was also instrumental in the announcement of the international expansion of rhino horn DNA testing after they hosted an international Rhino DNA Scientific workshop.

Five laboratories of the Faculty were accredited by SANAS over the last two years, the last being the Milk Laboratory which received ISO17025 accreditation in 2016. It means that results obtained by these laboratories are now recognised nationally and internationally.

We are proud of what we have achieved over the last few years, while recognizing that we have still this journey to travel. We are making progress, despite the challenges facing the university sector in South Africa. This year the proportion of permanent staff members with doctorates increased from 44% in 2014 to 53% in 2016 and a record number of 13 PhD students graduated in 2015 with a further 2 to follow at the September 2016 graduation.

Faculty Day provides an opportunity for our researchers to present the results of their studies and share them with their peers. This has become a proud tradition, stretching over a period of more than 30 years. My wish is that Faculty Day 2016 will further provide impetus to our Faculty's pursuit for excellence and distinction in support of the University's research-intensive vision.

This year's Sir Arnold Theiler Memorial Lecture is delivered by Prof Lucille Blumberg, Deputy Director of the National Institute for Communicable Diseases (NICD) of the National Health Laboratory Service, and head of the NICD's Public Health Surveillance and Response Division.

Since Prof Blumberg's tenure started at the NICD in 2002 her path crossed that of a number of veterinary virologists, which stimulated a special interest in zoonotic diseases. Following the SARS outbreak in 2003, an Outbreak Unit was established at the Institute with Prof Blumberg as its founding head. Since many of the disease outbreaks and emerging pathogens have been zoonotic, it was a natural progression to work across the many disciplines in the 'One Health' field. As an active practitioner of One Health, she has worked closely with animal practitioners in outbreak and case prevention and management of a wide variety of zoonotic diseases and in the investigation of cases

of acute febrile illness for these diseases. Her lecture entitled 'One health: a decade of shared experiences and benefits', among others, will focus on how the emergence of 'One Health' as a discipline has helped to improve communications between the human and animal health allied professions, establish a combined outbreak response mechanism, and effect improved public health responses and prevention endeavours.

Specifically relevant to the veterinary profession, she will also elaborate on combined projects that have been initiated to look at the prevalence of zoonoses in potentially highly-exposed humans at the animal/human health interface.

One Health is an essential research focus area in the Faculty's strategic plan and we extend a warm welcome to Prof Blumberg as we look forward to her presentation. Similarly we also welcome all visitors, staff members and students attending this year's event. Congratulations to the Faculty's Research Award winners for 2016 and a special word of gratitude to the Faculty Day Organising Committee for making this event possible.

May Faculty Day again serve as an inspiration to all of us in pursuit of the Faculty's predominant research goals by sharing new scientific conclusions, new ideas and innovative concepts.

Curriculum Vitae: Lucille Blumberg



Prof Lucille Blumberg

Professor Lucille Blumberg was born in Johannesburg, attended school there and qualified in medicine at the University of the Witwatersrand in 1975. She has many years of experience in paediatrics, critical care, medical microbiology, and infectious diseases and public health. While working in intensive care at Baragwanath Hospital in the 1980s she developed a keen interest in the treatment and prevention of infectious diseases,

after recognizing that many of the patients admitted to ICU had diseases that were preventable by addressing the public health issues underlying them. She went on to obtain her

specialist qualifications in medical microbiology and infectious diseases and developed a special interest in travel-related and tropical diseases, especially malaria and sleeping sickness. She moved to the newly established National Institute for Communicable Diseases (NICD) in 2002 to take up a position as medical consultant in haemorrhagic fevers and rabies. Here her path crossed that of a number of veterinary virologists, which stimulated a special interest in zoonotic diseases. Following the SARS outbreak in 2003, an Outbreak Unit was established at the NICD with Prof Blumberg as its founding head. Since many of the disease outbreaks and emerging pathogens have been zoonotic, it was a natural progression to work across the many disciplines in the 'One Health' field. As an active practitioner of One Health, Prof Blumberg has worked closely with animal practitioners in outbreak and case prevention and management of avian influenza, Rift Valley fever, rabies, Zika virus, and in the investigation of cases of acute febrile illness for zoonotic diseases. She serves on a number of international and local advisory committees in the zoonosis field. Prof Blumberg was awarded the SASVEPM (The Southern African Society for Veterinary Epidemiology and Preventive Medicine) Annual Epidemiology Prize for contributions to the society and its objectives in 2012 and the World Small Animal Veterinary Association (WSAVA) One Health Director of the National Institute for Communicable Diseases of the National Health Laboratory Service, and currently head of the NICD's Public Health Surveillance and Response Division.

Sir Arnold Theiler Memorial Lecture

One Health: a decade of shared experiences and benefits

Professor Lucille Blumberg

MB BCH MMed (Micro) ID (SA) FFTM (RCPS, Glasgow) DTM&H DOH DCH
Division of Public Health Surveillance and Response National Institute for Communicable Diseases, a division of the National Health Laboratory Service (lucilleb@nicd.ac.za)

Zoonotic diseases have been responsible for a significant number of outbreaks of emerging diseases affecting humans in the past 30 years. These have included SARS, MERS-CoV, Ebola virus disease and more recently the Zika virus, with the two latter diseases being declared Public Health Outbreaks of International Concern (PHEIC) because of the risk of international spread and the need for a co-ordinated international response for research and control. In southern Africa, outbreaks of rabies, influenza A (H5N2), Rift Valley fever and the newly-identified

arenavirus, the Lujo virus, have required animal and human health practitioners to work together to effect a response. Zoonotic diseases are under-recognized in humans due to a low diagnostic index of clinical suspicion, the non-specific nature of clinical disease, especially on a high background prevalence of HIV and TB, the absence of active surveillance programmes, and limited availability of diagnostic testing facilities. The widespread use of antibiotics in livestock and the poultry industry has likely contributed to the emergence of the 'Superbugs', highly antibiotic-resistant bacteria in humans, leading to untreatable systemic infections. The emergence of 'One Health' as a discipline has helped to improve communications between the human and animal health allied professions, establish a combined outbreak response mechanism, and effect improved public health responses and prevention endeavors. In addition, combined projects looking at prevalence of zoonoses in potentially highly-exposed humans at the animal/human health interface have been initiated, and have provided useful information on control, prevention and treatment.

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 Huisman	"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	"Fumonisin: 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 Guillet 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.

** 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: 2015 – 2016

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, requires increasing research outputs through effective postgraduate programmes, and making research a primary thrust. The Faculty currently 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 personnel with the UP vision, suggest that the Faculty is well placed to contribute significantly to the strategic goals of the University. The Faculty's research publication output increased from 55.3 units in 2006 to 81.52 units in 2015 which represent 214 ISI-accredited journals.

In terms of postgraduate students the Faculty currently has 196 Masters, 105 PhD and 16 Post-doctorate students. The number of postgraduate graduations continues to increase compared to previous years. Challenges to sustaining these increases include the clinical nature of academics' work in some departments, the percentage of academics with doctoral degrees or NRF ratings and the percentage supervising postgraduate students. Plans are underway to increase these percentages over the next one to three years and to recruit additional postdoctoral researchers and research fellows. In 2015 the total number of postgraduate student candidates registered in the Faculty were as follows: 97 PhD, 146 MSc, 30 MMedVet, and 36 Hons candidates.

The Faculty is training professionals who are able to protect animal health, which also impacts on human health, thereby stimulating economic growth and food security. An efficient research programme must remain relevant to the needs of South Africa, but also to a constantly changing international environment. Therefore, a strong research platform will be explicitly pursued as the basis for faculty growth and development. Its vision is thus to have strong internationally recognized research groups in wildlife, infectious diseases, One Health, epidemiology and veterinary public health in support of UP's 2025 Vision and current research clusters. At the same time, it must have the potential to generate high-impact publications, attract more postgraduate students nationally and internationally, and escalate the research status of the Faculty. Fundamental to these visionary requirements, the Faculty currently operates within the following research focus areas:

Molecular studies on infectious and parasitic diseases of animals

A research-focus utilising biotechnology for the development of improved diagnostic techniques and vaccines for animal diseases and for the study of their pathogenesis.

Phytomedicine and ethno-veterinary medicine

An established multidisciplinary and collaborative research programme focusing on the development of extracts from plants with antimicrobial or anti-parasitic activity purposes.

Wildlife and environmental health

An inclusive research focus with contributions from all five departments of the Faculty, including studies on tuberculosis in buffalo, immune-contraception in elephants, theileriosis in roan and sable, the toxicity of non-steroidal anti-inflammatories in vultures and endocrine disruptors in the environment.

Veterinary aspects of food safety and food security

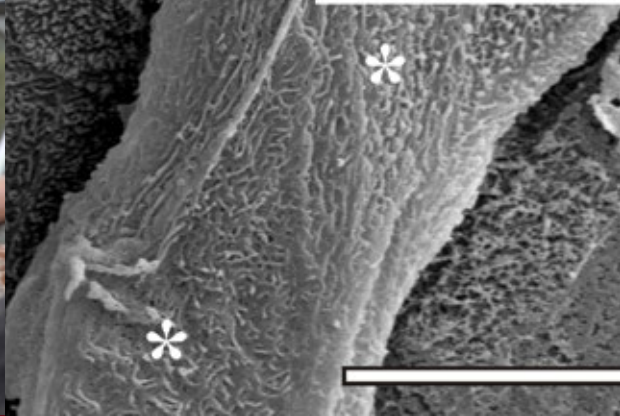
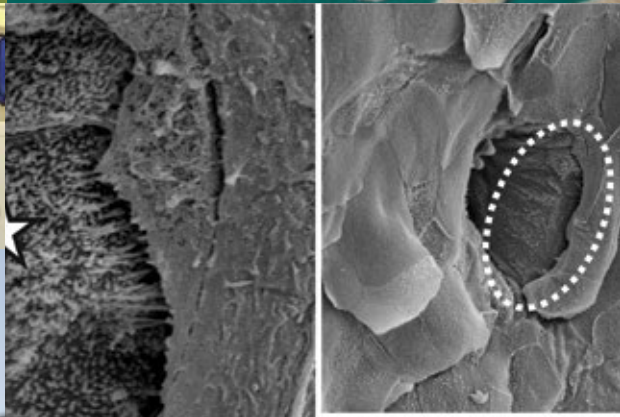
An established research focus that includes, *inter alia*, programmes in veterinary public health, community development, epidemiology and risk assessment, and poultry health.

Equine and companion animal health and welfare

A focus on infectious and other diseases of horses and other companion animals with an important impact on trade and sports medicine (the racing industry) or on the welfare and management of these animals.

The Exotic Leather Research Centre (ELRC) of the faculty established to serve as technical collaborator for the Department of Trade and Industry (dti)-approved sub-National Exotic Leather Cluster, is responsible for research, postgraduate training and services to improve the global competitiveness of the South African exotic leather and leather goods industry. The Centre also focuses on research pertaining to crocodile and ostrich health, and production, and exotic leather and leather goods marketing and trade. A recent successful IUCN/CSG (Crocodile Specialist Group) working meeting took place at Skukuza which was judged as one of the best Crocodile Specialist Group (CSG) working meetings held thus far. More than 340 delegates from 42 countries attended this prestigious event with the theme "*Crocodiles, Communities and Livelihoods*". For the first time an expo exhibiting commercial exotic leather products also formed part of the event.

The RhODIS® ground-breaking technique of the Veterinary Genetics Laboratory (VGL) to collect and catalogue DNA from rhinos and rhino horn is leading to more convictions of poachers and reduce rhino poaching in Southern Africa. Early in 2016 it led to the VGL being selected as one of the 16 winners of the Wildlife Crime Tech Challenge, an initiative of USAID, in partnership with National Geographic, the Smithsonian Institution and the TRAFFIC wildlife trade monitoring network. More importantly though the VGL was instrumental in the announcement of the international expansion of rhino horn DNA testing at the recent RhODIS® Rhino DNA Scientific workshop which took place in the Kruger National Park and at the Onderstepoort Campus.



Research Summary: 2015 – 2016 (continued)

Research output and growth

Measures to increase the Faculty's research output could, inter alia, be achieved by establishing a research ethos, increasing the numbers of postgraduate students and encouraging teaching staff to submit themselves to 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.

The number of permanent staff members with doctorates increased from 44% in 2014 to 53% in 2016. There was a dramatic upsurge in the combined number of master's and doctoral students, and the Faculty more than doubled its postgraduate output and number of postdoctoral students. A total 13 PhDs were awarded in 2015, 3 at the Spring Graduation and a further 10 candidates at the Autumn graduation. In addition to this representing the highest number of doctorates awarded by the faculty in a given year, the faculty is extremely proud that three of these candidates were staff members from the Department of Companion Animal Clinical Studies (CACS). A further 2 will follow at the September 2016 graduation.

The number of NRF-rated researchers in the Faculty's staff complement has shown a steady growth, reaching 36 at the end of 2015, compared to 27 in 2014, and nine in 2005. The Faculty now has nine B-rated, twenty C-rated and seven Y-rated staff members with Prof Christo Botha, head of the Department of Paraclinical Sciences rated at B1 level which makes him the highest NRF-rated researcher in the Faculty.

The Faculty's Research Committee (Rescom) Fund is aimed at assisting young promising new academic staff to develop their research skills and productivity. In 2015, two new grants of

R50 000 each were allocated to two academic staff members. About 104 new research protocols were approved in 2015, compared to 100 in 2014.

Faculty Day 2015 and research awards

The annual Faculty Day on 20 August 2015 provided an opportunity for our researchers to once again showcase the research activities in the Faculty to colleagues and peers, and was well attended by staff members, visitors and sponsor companies alike. Excellence in research performance was recognised at the event by the identification of the Faculty's Top 10 researchers and the allocation of the following research awards:

Researcher of the Year

Prof Geoffrey Fosgate
(Department of Production Animal Studies)

Young Researcher of the Year

Dr Carolynne Tarr
(Equine Research Centre)

Nine top researchers in the Faculty

Prof Koos Coetzer
Dr Dayo Fasina
Prof Andre Ganswindt
Prof Anita Michel
Prof Vinny Naidoo
Prof Johan Schoeman
Prof John Soley
Prof Peter Thompson
Prof Estelle Venter



The PhD graduates and their supervisors celebrate after their graduation. Prof Vinny Naidoo, Deputy Dean: Research and Postgraduate Studies (front, far left) was also present

Research Programme: Oral Presentations



Pathogenesis of kidney disease in captive cheetahs (*Acinonyx jubatus*): preliminary findings

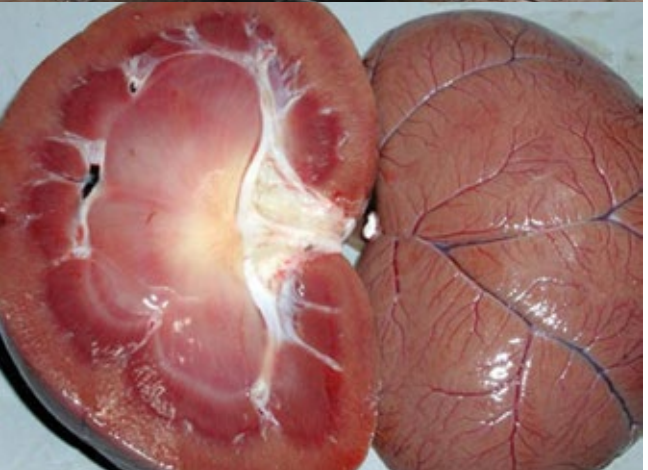
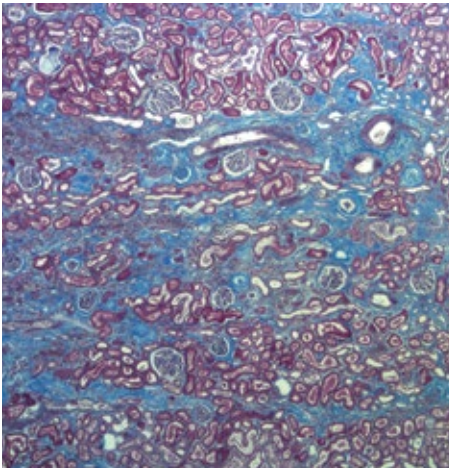
Lane E.P.¹, Prozesky L.², Dalton D.¹, Kotze A.¹, Lawrence J.²

¹ Department of Research and Scientific Services, National Zoological Gardens of South Africa, Pretoria, South Africa, e-mail:emily@nzg.ac.za

² Department of Paraclinical Studies, Faculty of Veterinary Science, University of Pretoria, Pretoria, South Africa

Captive cheetahs suffer from chronic renal disease (CRD) including AA-amyloidosis (RA) and glomerulosclerosis (GS). The birth, death, parentage and king coat status of 670 cheetahs from one facility were compared with the presence or absence of inflamed palatine clefts (IPC), gastritis, enterocolitis (EC), RA, renal cortical and medullary fibrosis and GS at death using best-fit models adjusted for small sample sizes. All the parameters measured, except for EC, were significantly associated with age at death; however, severe disease was seen in a few subadult cheetahs suggesting that very early intervention may be necessary to prevent these conditions. Survival probabilities for all age groups increased after 2001 when intensive disease management was instituted, but this could not be linked to differences in lesion prevalence. Renal medullary fibrosis was a key contributor to CRD and may precede RA since

a few of the cheetahs with no RA at death had CRD; more cheetahs had RMF than RA; and all cheetahs in which death was caused by CRD had RMF. Gastritis and EC were positively associated with each other, but neither was associated with any of the renal lesions suggesting that gastritis may not be a primary trigger for RA. Chronic renal infarction (CRI) was positively associated with renal cortical fibrosis (RCF). GS and IPC were not significantly related to any other lesion measured. These results support prior studies that RMF/RA is a separate syndrome from CRI/RCF and GS. Extrinsic factors such as diet, stress and infectious disease control likely play a more important role in the pathogenesis of CRD than genetic factors since many cheetahs that died without RMF and RA had parents with these lesions (67% and 54% respectively) and king cheetahs were not more susceptible than normal coat cheetahs to any of the lesions measured.





Phytochemicals – an alternative to synthetic chemical acaricides?

Adenubi O.T.¹ Sakong B.M.¹, Dzoyem J.P.², McGaw L.J.¹, Eloff J.N.¹, Naidoo V.^{1,2}

- 1 Phytomedicine Programme, Department of Paraclinical Sciences, Faculty of Veterinary Sciences, University of Pretoria, Onderstepoort 0110, South Africa, e-mail: bukiadenubi@gmail.com
- 2 Biomedical Research Center, Faculty of Veterinary Sciences, University of Pretoria, Onderstepoort 0110, South Africa.

Infections of domestic and wild animals that are transmitted by ticks are major causes of morbidity and mortality. Chemical treatment of host animals with synthetic acaricides is the preferred method to reduce the influence of parasites on animal and human health. Awareness of the environmental health hazards posed by these acaricides, development of tick resistance leading to recurrent ectoparasitism, danger of misuse and presence of toxic residues in food, water and animal by-products has led to the search for safe and environmentally-friendly alternatives. One of these alternatives, is the use of medicinal plants.

The acaricidal activities of the acetone, ethanol, ethanol/water and hot water extracts of seventeen plants used by traditional healers and livestock farmers in South Africa for the control of ticks was evaluated using the contact method on *Rhipicephalus turanicus* ticks at an initial concentration of 200 mg/ml. Where

the percentage mortality after 24 hours was $\geq 80\%$, the extracts were also tested at six graded decreasing concentrations using the Adult Immersion Test. Cytotoxicity was determined by evaluating the viability of Vero African green monkey kidney cells in the presence of the extracts using the tetrazolium-based colorimetric assay. *Schkuhria pinnata*, *Calpurnia aurea* and *Senna italica* showed 83-93% efficacy, comparable to cypermethrin, a currently used acaricide. Further dose-response assays showed LC_{50} values of 60, 100 and 50 mg/ml respectively. All the extracts were relatively non-toxic on Vero cells with LC_{50} ranging from 30.58 $\mu\text{g/ml}$ to 553.61 $\mu\text{g/ml}$. This study is the first report of the acaricidal activity of *Schkuhria pinnata*, and further studies on the isolated compounds to determine the mechanism of action of these plants are ongoing. It is imperative to explore the bioactive principles of the phytochemicals or their derivatives to diversify the base of effective acaricides in the field of veterinary and human medicine.





Osteology of the carpal region of the southern white rhinoceros (*Ceratotherium simum simum*)

Meusel E.G.¹, Crole M.R.¹, Marais J.²

¹ Department of Anatomy and Physiology and ²Department of Companion Animal Clinical Studies, Faculty of Veterinary Science, University of Pretoria, Onderstepoort, 0110, South Africa; esthermeusel86@gmail.com

The southern white rhino (SWR) is a near threatened southern African subspecies which is aggressively poached for its horn. In addition to the face, the thoracic limb is often injured by bullets and snares. The carpus is a composite, weight-bearing joint and knowledge of the osteology will aid in diagnostics and surgical approaches for veterinarians faced with treating survivors of poaching.

Six SWR thoracic limbs were obtained from individuals that succumbed to poaching or natural causes. Three limbs were scanned with a 128 slice SOMATOM Definition Edge Computed Tomography (CT) scanner. The soft tissue from three limbs was removed and the limbs boiled to obtain the bones. The carpus was digitally recorded and described for all specimens.

The *Trochlea radii* and *Caput ulnae* were well-developed. Nine carpal bones were present arranged into proximal and

distal rows. From medial to lateral the *Os carpi radiale* (RCB) / *intermedium* (ICB) / *ulnare* (UCB) / *accessorium* (ACB) were present in the proximal row. The distal row comprised the *Os carpale I-V* (CB1-5) from medial to lateral. The metacarpus consisted of *Os metacarpale II-IV* (MC2-4). All nine carpal bones were uniquely and irregularly shaped. The *Trochlea radii* articulated with the RCB and ICB and the *Caput ulnae* with the ICB, UCB and ACB. MC2 articulated with CB2-3, MC3 with CB3-4 and MC4 with CB4. Prominent palmar processes were present on the ICB and CB3-4.

Similar to the elephant the *Trochlea radii* and *Caput ulnae* are prominent in the SWR. The irregularly and uniquely shaped carpal bones of the SWR are unlike the regular cubiform bones reported in the equine. A distal row of five carpal bones may be unique to the SWR. The numerous palmar carpal processes may complicate surgical access in this region of the SWR.





Histomorphology, fatty acid composition and molecular characterization of healthy adipose tissue and pansteatitis tissue in the Nile crocodile (*Crocodylus niloticus*)

Azeez O.I.^{1,4}, *Myburgh J.G.*³, *Meintjes R.A.*¹, *Bosman A.M.*², *Featherstone J.*⁵, *Oosthuizen M.C.*², *Chamunorwa J.P.*¹

1 Anatomy and Physiology Dept., Faculty of Veterinary Science, University of Pretoria, Onderstepoort 0110, Pretoria, South Africa, email: odunayoazez@gmail.com.

2 Veterinary Tropical Diseases Dept., Faculty of Veterinary Science, University of Pretoria, Onderstepoort 0110, Pretoria, South Africa.

3 Paraclinical Science Dept., Faculty of Veterinary Science, University of Pretoria, Onderstepoort 0110, Pretoria, South Africa.

4 Dept. of Veterinary Physiology, Biochemistry and Pharmacology, University of Ibadan, Ibadan, Nigeria.

5 Biotechnology Platform, Agricultural Research Council, Onderstepoort 0110, Pretoria, South Africa.

Pansteatitis is the widespread inflammation of adipose tissue, the common lesion responsible for the death of Nile crocodiles along the Olifant River, Kruger National Park, Mpumalanga, South Africa in 2008/9. In an attempt to unravel the pathophysiology of pansteatitis; a study of the histomorphology, ultrastructure, molecular biology, and long chain fatty acid composition of adipose tissue from healthy farm bred crocodiles and from pansteatitis samples from those that had died of pansteatitis was undertaken.

Adipose tissue samples were collected from the subcutaneous, visceral, intramuscular fat and the abdominal fat body of ten 4 years old juvenile crocodiles from Izinhaba Crocodile Farm (Pty) Ltd, Pretoria, while pansteatitis samples were collected from visceral and intramuscular fat of crocodiles that had died of pansteatitis at the Olifant River, Mpumalanga.

Histomorphology revealed regional variations in adipocytes shape, collagen and blood vessel distribution in adipose tissue from the subcutaneous, visceral, intramuscular adipose tissue and the abdominal fat body in healthy animals while pansteatitis samples showed considerable

inflammatory cell infiltration and fibrosis. Fatty acid methyl ester analysis showed higher composition of saturated and monounsaturated but lower polyunsaturated fatty acid in pansteatitis samples.

Molecular characterization involved Truseq stranded total RNA library preparation and sequencing on the Illumina Hiseq 2500. From denovo assembly of the transcripts from the sequenced reads using Bridger (<https://sourceforge.net/projects/rnaseqassembly/files/?source=nabar>), were characterized 42,201 transcripts out of which 37,835 mapped successfully with salt water crocodile (*Crocodylus porosus*) genes while 70 genes were differentially expressed in pansteatitis samples when mapped with our denovo assembled data using RSEM (<http://deweylab.github.io/RSEM/>). Annotation of the differentially expressed genes revealed genes involved in lipid metabolism, apoptosis, and cell migration during inflammation.

The study provides an insight into the mechanism(s) of pansteatitis and data for further study of this condition, and a platform for complete genome sequence in the near future.





Single-incision laparoscopic sterilization of the cheetah (*Acinonyx jubatus*)

Hartman M.J.¹, Monnet E.,² Kirberger R.M.¹, Schoeman J.P.¹

¹ Department of Companion Animal Clinical Studies, Faculty of Veterinary Science, University of Pretoria, Onderstepoort, South Africa, e-mail: marthinus.hartman@up.ac.za

² Department of Clinical Sciences, Colorado State University, Fort Collins, Colorado, USA..

Namibian legislation now requires all large carnivores in captivity to be permanently sterilized. Laparoscopic sterilization may provide a safe and efficient minimally invasive surgical solution to this requirement. The objective of the study was to describe laparoscopic ovariectomy and salpingectomy in cheetahs with single portal access system using a single incision laparoscopic surgery (SILS) port. A prospective randomized study was designed. Twenty one nulliparous female cheetahs (median age 11.5 years) were randomly divided into 2 groups: ovariectomy (n=11) and salpingectomy (n=10). The use and complications of a (SILS) port was evaluated in all patients. Total surgical time (TST) and CO₂ volumes were compared between the two procedures. The weight and age did not differ significantly between groups. Laparoscopic ovariectomy and salpingectomy were performed without complications using a SILS port. The poorly-developed mesosalpinx and ovarian

bursa of the cheetah facilitated access to the uterine tube for salpingectomy. TST for salpingectomy was 19.5 IQR 17-20 mins and for ovariectomy 24 IQR 23-26 mins (P = 0.005). A significantly higher total volume of CO₂ was required for ovariectomy (11.25 IQR 3.08 L) versus salpingectomy (9.0 IQR 2.52 L) (P = 0.001). All patients returned to normal behavior and appetite within 18 h of surgery. One cheetah engaged in self-mutilation of the tail and a leg during recovery from anaesthesia and another cheetah developed mild inflammation at the site. In conclusion, laparoscopic ovariectomy and salpingectomy could be performed in cheetahs using a SILS port without significant perioperative complications. Salpingectomy was faster and used less CO₂ compared with ovariectomy. Additionally salpingectomy will allow normal cyclical activity to continue implying less possible long term side effects that could be associated with ovariectomy.





Effects of thiopentone, propofol and alfaxalone on laryngeal motion during oral laryngoscopy in healthy dogs

Smalle T.M.¹, Hartman M.J.¹, Bester L.¹, Buck R.K.¹, Fosgate G.T.², Zeiler G.E.¹

¹ Department of Companion Animal Clinical Studies, Faculty of Veterinary Science, University of Pretoria, Onderstepoort, South Africa, e-mail: teshsmalle@gmail.com

² Department of Production Animal Studies, Faculty of Veterinary Science, University of Pretoria, Pretoria, South Africa.

Laryngeal paralysis is the most common disease process affecting the larynx dogs. Definitive diagnosis relies on an accurate laryngeal examination, usually under a light anaesthetic plane. However, laryngeal function can be influenced by anaesthetic agents.

The purpose of our study was to estimate the effects of thiopentone, propofol and alfaxalone on laryngeal function during oral laryngoscopy in non-premedicated healthy adult dogs. Additionally, we attempted to establish appropriate dose rates to achieve an accurate laryngeal examination by oral laryngoscopy. Six healthy adult beagle dogs were enrolled in a randomised crossover study. Each dog was randomly administered three induction agents with one week washout period between treatments. Thiopentone, propofol or alfaxalone were administered at 7.5mg/kg, 3mg/kg and 1.5mg/kg, over one minute, for induction of anaesthesia, respectively. If deemed inadequately anaesthetised then top-up boluses of 1.8mg/kg, 0.75mg/kg and 0.4mg/kg were administered, respectively. Continual examination

of the larynx, using a laryngoscope, commenced once an adequate anaesthetic depth was reached until recovery from anaesthesia. The number of arytenoid motions and vital breaths were counted during three time periods and compared over time and among treatments. The median induction time was 2.8, 2.7 and 2.5 minutes for thiopentone, propofol and alfaxalone, respectively ($p=0.727$). The median dose rate required to achieve an adequate anaesthetic depth was 6.3mg/kg/minute, 2.4mg/kg/minute and 1.2mg/kg/minute, respectively. There was no significant difference for the total number of arytenoid motions ($p=0.662$) or vital breaths ($p=0.789$) among induction agents. The median recovery time was 14.1, 5.4 and 8.5 minutes for thiopentone, propofol and alfaxalone, respectively ($p=0.016$). We concluded that there was no significant difference in the total number of arytenoid motions among the induction agents. However, at the dose rates used in this study, propofol provided adequate conditions for evaluation of larynx with a shorter recovery time which may be advantageous during laryngoscopy in dogs.





Sensitivity and specificity of three pregnancy-associated glycoprotein ELISA tests in South African dairy cattle

Motimele B.^{1,2}, Kanengoni A.², Fosgate G.¹, Irons P.C.^{1,3}

1 Department of Production Animal Studies, Section of Reproduction, Faculty of Veterinary Science, University of Pretoria, Onderstepoort, 0110, South Africa; email: bmotimele@yahoo.com

2 Agricultural Research Council-Animal Production Institute, 0062, South Africa;

3 College of Veterinary Medicine, School of Veterinary and Life Sciences, Murdoch University, 90 South Street, Western Australia, 6150

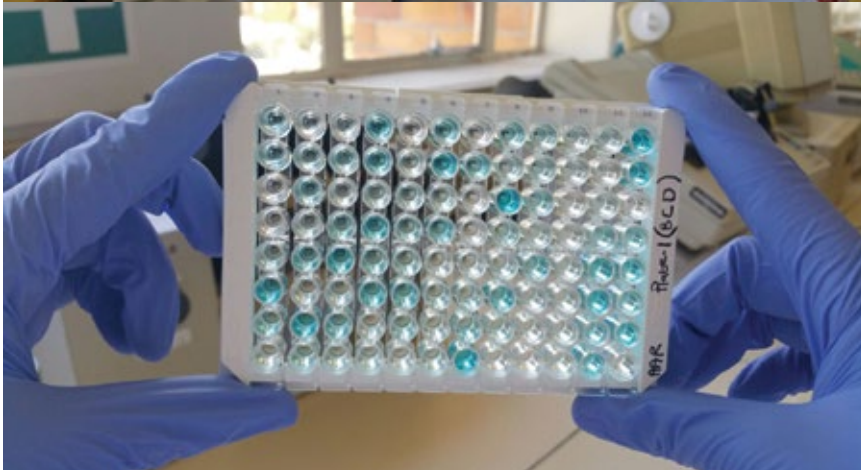
The effect of gestation stage, foetus number, foetal sex and early foetal loss on plasma pregnancy-associated glycoprotein (PAGs) have been well documented. This study was undertaken to describe the effect of days bred (28-35), days in milk, lactation number and breed on the sensitivity and specificity of three PAG ELISA.

A multicentre prospective study was conducted in five sites within South Africa. Milk and blood samples were sampled from 1250 dairy cows and heifers at 28-35 after artificial insemination (AI) and repeated two weeks later. Transrectal ultrasound (TRUS) was performed immediately after sample collection. Milk and blood were stored at 4°C and -20°C, respectively until analysis. The milk and blood samples were analysed using commercial ELISA kits (IDEXX Laboratories, Inc.). Sensitivity and specificity were estimated relative to TRUS at 42-49 days of gestation as the gold standard and confidence intervals (CI) were estimated using the mid-P exact method. Logistic regression was used to estimate the effects

of days bred, days in milk, lactation number and breed on sensitivity and specificity.

Sensitivity (95% CI) of the serum, milk and visual ELISA were 99.8% (99.1-100%), 99.4% (98.5-99.9%) and 99.6% (98.8-99.9%) respectively. Specificity for the serum, milk and visual ELISA were 57.3% (52.4-62.0%), 55.3% (50.4-60.1%) and 55.0% (50.1-59.9%), respectively. Days bred, days in milk, lactation number and breed did not affect sensitivity of any PAG ELISA. However, cow breed and days bred had significant effects on the specificity of all three tests.

In conclusion, the PAG ELISA was a sensitive test for detecting pregnancy relative to TRUS from days 28-35 after AI but poorly specific in identifying non-pregnant cows. The specificity of the three PAG ELISA tests varied by breed and days bred. Pregnancy loss during early gestation might be a cause of the low specificities estimated in this study.





The effects of injectable trace mineral supplementation on semen quality of bulls

Ferreira G.M.^{1,2}, Smuts M.³, De Bruyn S.³, Hamman R.³, Durel L.⁴, Rose C.⁴, Van Wyk L.², Holm D.E.³

¹ Morvet Herd Health Practice, Potchefstroom, South Africa, e-mail: martinferreira@morvet.co.za

² Virbac South Africa, Centurion, South Africa

³ Department of Production Animal Studies, Faculty of Veterinary Science, University of Pretoria, Onderstepoort, 0110, South Africa

⁴ Virbac Carros, France

The aim was to assess the impact of a commercially available trace mineral injection containing 40mg/ml Zn, 15mg/ml Cu, 10 mg/ml Mn, and 5 mg/ml Se (Multimin Cattle, Virbac SA) on semen characteristics of 4-6 year old Brahman and Simmentaler bulls (n = 10) managed at the Faculty of Veterinary Science, University of Pretoria. Bulls were breeding sound at the onset and were fed ad lib *Eragrostis* hay supplemented with a commercial trace mineral lick prior to, and throughout the study period. Bulls were randomly allocated to group M (Treated) or group C (Control), each group consisting of 2 Brahmans and 3 Simmentalers. Group M bulls were supplemented subcutaneously with the trace mineral injection while group C animals received a sterile saline injection at 1ml/100kg. Semen was collected twice weekly for 3 months after treatment and immediately analysed to assess its volume and consistency, mass motility score (MMS), individual spermatozoa (SPZ) motility, and proportion of SPZ without morphological defects. Computer-

assisted semen analysis (CASA) was used to evaluate semen concentration (Spermaque), and proportions of motile and progressive motile SPZ (Hamilton-Thorne). The ejaculate was the statistical unit and the threshold of statistical significance was set to 0.05. Results indicate that MMS (range 0-5), proportions of ejaculates with > 70% morphologically normal SPZ and semen volume collected didn't differ between treatment groups. Mean SPZ concentration ($\times 10^6/\text{ml}$) ($P < 0.05$), proportion of ejaculates $> 1.2 \times 10^6$ SPZ/ml and $> 2.0 \times 10^6$ SPZ/ml ($P < 0.01$), and mean proportion of ejaculates with more than 50% spermatozoa with progressive motility ($P < 0.05$) was higher in treated bulls. Supplementation seemed to have a positive effect on the proportion of SPZ without nucleus defects but this requires further investigation. Injectable trace mineral supplementation increases the proportion of ejaculates with high SPZ concentration and percentage of ejaculates with more than 50% spermatozoa with progressive motility.





Host immune response profiles of calves following vaccination with live BCG and inactivated *Mycobacterium bovis* vaccine candidates

Van der Heijden E.M.D.L.^{1,2*}, *Chileshe J.*^{1*}, *Vernooij J.C.M.*³, *Gortazar C.*⁴, *Juste R.A.*^{5,6}, *Sevilla I.*⁵, *Vordermeier H.M.*⁷, *Crafford J.E.*¹, *Rutten V.P.M.G.*^{1,2}, *Michel A.L.*¹

1 Department of Veterinary Tropical Diseases, Faculty of Veterinary Science, University of Pretoria, Onderstepoort, Pretoria, South Africa

2 Department of Infectious Diseases and Immunology, Faculty of Veterinary Medicine, Utrecht University, Utrecht, the Netherlands

3 Department of Farm Animal Health, Faculty of Veterinary Medicine, Utrecht University, Utrecht, the Netherlands

4 SaBio IREC (CSIC-UCLM-JCCM), Ciudad Real, Spain

5 NEIKER-Tecnalia, Animal Health Department, Derio, Bizkaia, Spain

6 SERIDA. Villaviciosa, Asturias, Spain

7 Department of Bacteriology, Animal and Plant Health Agency, Addlestone, United Kingdom

* EvdH and JC contributed equally to this study

Bovine tuberculosis (BTB) caused by *Mycobacterium bovis* (*M. bovis*) occurs endemically in livestock and wildlife in parts of southern Africa and is detrimental to socio-economics and conservation efforts. Test-and-slaughter schemes to control BTB are inefficient in many developing countries with a human-wildlife-livestock interface and limited surveillance of animal diseases. Vaccination is considered an alternative control strategy. The safety of the use of live BCG in cattle, in an area of high human immunodeficiency virus (HIV) prevalence, is disputed. Inactivated vaccine candidates may provide a safer alternative.

The aim of this study was to investigate immune response profiles of cattle vaccinated with live *M. bovis* BCG compared to inactivated *M. bovis* vaccine candidates. Twenty-four calves, aged 4–6 months, were randomly divided into four groups and vaccinated subcutaneously with either: live BCG; formalin-inactivated BCG; heat-killed *M. bovis* or PBS/Montanide™ (control). Animals receiving inactivated vaccines were boosted after three weeks. Interferon-gamma

responsiveness and antibody production were measured prior to vaccination and at weekly intervals for nine weeks post-priming, using the BOVIGAM assay and IDEXX TB ELISA, respectively. At week nine, the animals were skin tested using bovine and avian tuberculin (PPD-B and PPD-A).

The animals in the heat-killed *M. bovis* group showed significantly higher cell-mediated and humoral immune responses compared to those in the control group as well as the groups that received live or inactivated BCG ($p < 0.05$). Treatment groups vaccinated with live and inactivated BCG did not show significantly raised levels of cell-mediated or humoral immune responses. In the tuberculin skin test, the live BCG and the heat-killed *M. bovis* vaccinated animals showed significant responses (PPD-B minus PPD-A).

In conclusion, the heat-killed *M. bovis* vaccine was shown to elicit strong and sustained cell-mediated as well as humoral immune responses in calves and warrants further investigation in experimental and/or field challenge studies.





Identification and characterisation of *Ehrlichia ruminantium* vaccine candidate epitopes

Thema N.^{1,2}, *Pretorius A.*^{1,2}, *Tshilwane S.I.*¹, *Liebenberg J.*¹, *Faber F.E.*¹, *Steyn H.*¹, *Van Kleef M.*^{1,2}

1 Agricultural Research Council-Onderstepoort Veterinary Institute, Private Bag X5, Onderstepoort 0110, South Africa, email: theman@arc.agric.za

2 Department of Veterinary Tropical Diseases, University of Pretoria, Private Bag X04, Onderstepoort 0110, South Africa

E. ruminantium recombinant subunit vaccine development strategies have had little success in the past. What must be considered is that pathogen proteins may contain epitopes which can inhibit protective immune responses or induce immunopathology. These negative effects can be avoided if T-cell epitopes that specifically stimulate immune responses are identified. This study aimed to identify and characterise the individual epitopes that induce IFN- γ by CD4+ Th1 or CD8+ T cells using *E. ruminantium* proteins previously shown to stimulate Th1 immunity. Overlapping peptides (16-mer) spanning the whole proteins were synthesised and each assayed with immune lymphocytes from tick-challenged sheep, to evaluate their ability to induce recall T-cell responses and cytokine production *in vitro*. From eight vaccine candidates, 55 peptides that induced IFN- γ and other cytokines were

identified. Most peptides activated CD4+ T cells but only a few peptides induced CD8+ T cells that were shown to be cytotoxic. Selected peptides were used to construct a multi-epitope DNA vaccine in a mammalian expression vector designed for dual expression of CD4+ and CD8+ specific peptides. Each multi-epitope construct contained specific tags for CD4+ and CD8+ T cell targeting. Two groups of sheep were immunised: 1) with a total of 50 μ g of the multiepitope DNA vaccine using a gene gun (gg) and 200 μ g pDNA delivered im, 2) with the DNA vaccine as in (1) and adding adjuvant that was administered topical (gg) and im. Empty vector control groups were included. Sheep were inoculated three times with a three-week interval and were challenged five weeks after the last inoculation. For the first time, 3/5 sheep of group 2 survived against natural tick challenge.





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Research Programme: Poster Presentations

Etorphine-ketamine-medetomidine total intravenous anaesthesia in wild impala (*Aepyceros melampus*) of 120 minute duration

Zeiler G.E.¹, Stegmann G.F.¹, Fosgate G.T.², Buck R.K.¹, Kästner S.B.R.³, Kummrow M.⁴, Gerlach C.³, Meyer L.C.R.⁵

1 Department of Companion Animal Clinical Studies, Faculty of Veterinary Science, University of Pretoria, Private Bag X04, Onderstepoort, 0110, South Africa, e-mail: gareth.zeiler@up.ac.za

2 Department of Production Animal Studies, Faculty of Veterinary Science, University of Pretoria, Private Bag X04, Onderstepoort, 0110, South Africa

3 Small Animal Clinic, University of Veterinary Medicine Hannover, Foundation, Bünteweg 9, 30559 Hannover, Germany

4 Zoo Wuppertal, Hubertusallee 30, 42117 Wuppertal, Germany

5 Department of Paraclinical Studies, Faculty of Veterinary Science, University of Pretoria, Private Bag X04, Onderstepoort, 0110, South Africa

Abstract: The need for performing long-term anaesthesia in wildlife, especially antelope, is becoming a growing necessity. The costs and logistics of transporting wildlife to veterinary practices make surgical intervention a high-stakes operation. Thus, there is a need for a field-ready total intravenous anaesthesia (TIVA) infusion to maintain anaesthesia in antelope. This study explored the feasibility of an etorphine-ketamine-medetomidine TIVA for field anaesthesia. Ten wild-caught, adult impala (*Aepyceros melampus*) were enrolled in the study. Impala were immobilized with a standardized combination of etorphine (2 mg) and medetomidine (2.2 mg), which equated to a median (interquartile range; IQR) etorphine and medetomidine dose of 50.1 (46.2-50.3) and 55.1 (50.8-55.4) µg/kg, respectively. Recumbency was attained in a median (IQR) time of 13.9 (12.0-16.5) minutes. Respiratory gas tensions, spirometry and arterial blood gas were analysed over a 120 minute infusion. Once instrumented the TIVA was infused as follows: etorphine at a variable rate initiated at 40 µg/kg/hour (adjusted according to intermittent deep-pain testing); ketamine and medetomidine at a fixed rate

of 1.5 mg/kg/hour and 5 µg/kg/hour, respectively. The etorphine had an erratic titration to clinical effect in four impala. Arterial blood pressure, respiratory and heart rates were all within normal physiological ranges. However, arterial blood gas analysis revealed severe hypoxemia, hypercapnia and acidosis. Oxygenation and ventilation indices were calculated and highlighted possible co-etologies to the suspected etorphine-induced respiratory depression as the cause of the blood gas derangements. Impala recovered in the boma post atipamezole (13 mg) and naltrexone (42 mg) antagonism of medetomidine and etorphine, respectively. The Etorphine-ketamine-medetomidine TIVA protocol for impala may be sufficient for field procedures of up to 120 minute duration. However, hypoxemia and hypercapnia are of paramount concern and thus oxygen supplementation should be considered mandatory. Other TIVA combinations may be superior and warrant further investigation.

Key words: *Aepyceros melampus*, etorphine, impala, ketamine, medetomidine, TIVA

Evaluation of two different doses of butorphanol-medetomidine-midazolam for anaesthesia in free-ranging versus captive black-footed cats (*Felis nigripes*)

Eggers B.¹, Meyer L.², Tordiffe A.², Sliwa A.³, Wilson B.⁴, Lawrenz A.⁵, Lamberski N.⁶

1 Post-graduate student (MMedVet Fer.)

2 Department of Paraclinical Science, Faculty of Veterinary Science, University of Pretoria, Onderstepoort, 0110, South Africa

3 Cologne Zoo, Riehler Str. 173, 50735 Cologne, Germany

4 Zoology Department, McGregor Museum, P.O. Box 316, Kimberley 8300, South Africa

5 Wuppertal Zoo, Hubertusallee 30, 42117 Wuppertal, Germany

6 Veterinary Services, San Diego Zoo Animal Park, 15500 San Pasqual Valley Road, Escondido, CA 92027-7017, USA; blackegg@mweb.co.za

The safety and efficacy of the reversible butorphanol-medetomidine-midazolam (BMM) anaesthetic combination, administered intramuscularly, was evaluated in captive and free-ranging black-footed cats (*Felis nigripes*) and any physiological side effects resulting from the different dose requirements of the two populations were evaluated and compared. Initial target dosages in the captive cats were butorphanol 0.2mg/kg, medetomidine 0.05mg/kg and midazolam 0.1mg/kg. In free-ranging cats the dosages were twice as high. Eight of the 23 animals anaesthetised required higher dosages.

Nine captive and fourteen free-ranging black-footed cats were anaesthetised. Due to the differing physiological stressors associated with the capture methods, the free-ranging animals were divided into two groups: those that were dug out of burrows, and those that had been caught after a short intense chase. Inductions, anaesthetic quality and recoveries were evaluated. Cardiorespiratory variables and rectal temperatures were recorded every 10 minutes and three central venous blood samples taken at 20-minute intervals were analyzed for acid-

base variables and glucose concentrations. Anaesthesia was reversed at 45 minutes with naltrexone (2x the butorphanol dose), atipamezole (3x the medetomidine dose) and flumazenil (0.01-0.02x the midazolam dose).

Anaesthetic inductions and recoveries were quick and the quality of anaesthesia was optimal for minor sampling procedures. Cardiac variables remained within normal physiological limits. Mild hyperventilation, moderate hypoxaemia and acidosis were initially recorded in the chased free-ranging animals and oxygen supplementation and fluid therapy are advised. A gradual decrease in body temperature was recorded in all the cats and thus an effective means to mitigate this heat loss is recommended.

The reversible BMM combination proved to be safe and effective, enabling the cats to resume their hunting as quickly as possible and reducing their vulnerability to predation. The use of the BMM combination, which does not compromise renal function is of major benefit in a species prone to developing renal amyloidosis.

The immunomodulatory and intracellular activity of the acetone extract of *Oxyanthus speciosus* against human (U937) and mouse (RAW 264.7) macrophage cell lines

Aro A.O., Dzoyem, J.P., Eloff, J.N., McGaw L.J.

Phytomedicine Programme, Department of Paraclinical Sciences, Faculty of Veterinary Science, University of Pretoria, South Africa, email: aroabimbola@yahoo.co.uk

Tuberculosis (TB), a disease caused by *Mycobacterium tuberculosis*, remains a significant cause of death due to challenges associated with present chemotherapy. Co-infection with HIV also greatly increases the risk of latent TB infection (LTBI) progressing to active disease due to the fact that HIV suppresses the immune system, thereby allowing infected individuals to become more susceptible to TB infection. Medicinal plants are used in many parts of southern Africa to treat TB-related symptoms including chest pain and coughing.

The acetone extract of *Oxyanthus speciosus* was screened for its immunomodulatory effect against LPS-stimulated U937 macrophage cells using a cytometric bead array (CBA) technique. Human TH1/TH2 kits consisting of a mixture of six cytokines were used for the assay and analysed using flow cytometry. The intracellular efficacy of the *O. speciosus* extract against *Mycobacterium*-infected macrophages was

investigated using RAW 264.7 mouse macrophage cell line. Mouse macrophages were infected with *M. fortuitum* with a multiplicity of infection at 10 mycobacteria per cell. The result obtained from this study revealed that the acetone extract of *O. speciosus* increased the expression of IL-2 at 0.1 mg/ml while rifampicin suppressed the expression of this pro-inflammatory cytokine. At the tested concentration the crude extract of *O. speciosus*, inhibited the stimulation of IL-4 and IL-5 while it markedly increased the expression of IL-10. The acetone extract of *O. speciosus* did not show cytotoxicity to RAW 264.7 macrophages at the highest tested concentration (1 mg/ml). On day 6 post-infection, the intracellular antimycobacterial activity of the acetone crude extract of *O. speciosus* at 1X to 4X MIC was superior to that of rifampicin, showing more than 90% reduction in colony forming units. In conclusion, the extract of *O. speciosus* had a mixed Th1/Th2 effect. The bactericidal activity observed was both dose and time-dependent.

Investigations of medicinal plants used traditionally in southern Africa to treat helminth infections in sheep

Sakong B.M.¹, Ahmed A.S.¹, Adamu M.², Naidoo V.^{1,3}, Eloff J.N.¹, McGaw L.J.¹

¹ Phytomedicine Programme, Department of Paraclinical Sciences, Faculty of Veterinary Sciences, University of Pretoria, Onderstepoort 0110, South Africa

² Department of Pharmacology, Faculty of Veterinary Medicine, University of Agriculture, Makurdi, Benue State, Nigeria

³ Biomedical Research Centre, Faculty of Veterinary Sciences, University of Pretoria, Onderstepoort 0110, South Africa

Haemonchus contortus is a major gastrointestinal helminth parasite causing haemonchiasis in production animals. Infections are characterized by fever, anaemia, weight loss and ultimately death. Haemonchiasis is a serious economic challenge globally resulting in poor reproductive efficiency, increased susceptibility to bacterial and fungal infections, as well as low production of wool, milk and meat. Treatments with synthetic drugs are currently the only effective way to control the infestation. Albendazole is a drug of choice against this parasite; it acts by binding to tubulins and causes depolymerisation of microtubules. No other anthelmintic compounds of comparable efficacy are available yet and there is a critical need to find alternative drugs. An alternative strategy is to investigate ethnoveterinary plants with putative anthelmintic properties.

The aim of this study was to evaluate selected plant extracts for anthelmintic activity *in vitro* against *Haemonchus*

contortus, as well as antifungal activity to determine correlations in these activities. Cytotoxicity of the plant extracts was also tested, and isolation of compounds was performed. The crude extracts of *Diospyros whyteana* and *Peddiea africana* showed low cytotoxicity with LC₅₀ greater than 1 mg/ml. Good antifungal activity was observed in *D. whyteana*, *Peddiea africana* and *Schotia brachypetala* with MICs ranging from 40-160 mg/ml against *Candida albicans* and selectivity index (SI) values from 6.25-12.5. In the egg hatch inhibition assay, *D. whyteana* had good results with EC₅₀=0.7377 mg/ml for acetone while water extracts were 1.508, thus the SI ranged from 0.66 to 1.35. Water extracts showed better anthelmintic activity compared to acetone extracts of *D. whyteana*, *P. africana* and *Schotia brachypetala*. The extracts had good activity in the egg hatch assay, good antifungal activity, and they were non-toxic to Vero cells. Four bioactive compounds have been isolated from *Diospyros whyteana* with excellent activity against *Candida albicans* and the structures of these are being determined.

Investigation of antimicrobial activity and safety of two *Newtonia* spp. with potential for alleviating infectious diarrhoea symptoms

Motlhatlego K.E., Ahmed A.S., Eloff J.N., McGaw L.J.

Phytomedicine Programme, Department of Paraclinical Sciences, Faculty of Veterinary Science, University of Pretoria, Private Bag X04, Onderstepoort 0110, South Africa

Diarrhoeal pathogenesis is multifaceted, including infection by a wide range of microbial pathogens, intestinal inflammation, food allergy and drug intolerance. Diarrhoea causes high mortality and morbidity especially in children and immunocompromised patients, and is becoming a neglected disease. *Newtonia* species are medicinal plants used traditionally in southern Africa to treat stomach ache and infectious diseases. *Newtonia buchananii* and *N. hildebrandtii* are two major species used as components of remedy formulation, and were selected for intensive study on their antimicrobial and other properties.

The aim of this study is to develop a product used to address diarrhoea through evaluating antimicrobial activities, phytochemical composition and safety of the selected plant extracts. The serial microdilution method was used to determine minimum inhibitory concentration (MIC) and bioautography was used to detect number of antimicrobial components against three Gram-positive bacteria

(*Staphylococcus aureus*, *Bacillus cereus* and *Enterococcus faecalis*), three Gram-negative bacteria (*Escherichia coli*, *Pseudomonas aeruginosa* and *Salmonella typhimurium*) and two fungi (*Candida albicans* and *Cryptococcus neoformans*). Cytotoxicity against Vero cells using the MTT assay and DPPH antioxidant potential of the extracts were also evaluated. *Newtonia buchananii* had the strongest antimicrobial effect against *P. aeruginosa* with MIC = 20 µg/ml and moderate MIC of 40 µg/ml against *B. cereus* while *N. hildebrandtii* had the lowest antibacterial activity against *B. cereus* with MIC = 80 µg/ml. The MIC values of *N. buchananii* and *N. hildebrandtii* against both fungi was 160 and 310 µg/ml respectively. The two plant extracts were relatively non-toxic with IC₅₀ values of 30-1000 µg/ml. The extracts had good antioxidant activity against the DPPH radical with EC₅₀ values as low as 0.24 mg/ml. The acetone extracts therefore have promising antimicrobial activities against microbes implicated in diarrhoea. Bioguided isolation of the active component(s) and evaluation of mechanism of action is ongoing.

Cutaneous neoplasms in dogs in South Africa: a retrospective study of 2553 cases

Tompkins S.C.¹, Fosgate G.², Williams J.³, Clift S.J.⁴

¹ Vetdiagnostix, Veterinary Pathology Services, Midrand, South Africa, email: tompkins@vetdx.co.za

Much literature has emanated from numerous countries in the world regarding the incidence and prevalence of canine cutaneous neoplasms. These include studies from Europe and North America, as well as Australia, South Africa, Zambia and Zimbabwe. However, the most recent report on canine cutaneous neoplasms in South Africa was reported by Bastionello in 1983; that study documented neoplasia in general across all domestic species from 1935 to 1974.

In addition to differing environmental conditions between countries (e.g. exposure to UV radiation), breeds and breed popularity also varies between countries. There are certain breeds, whose origins are specific to South Africa (e.g. the Boerboel), for which there is little information regarding their predisposition to certain neoplasms. Histopathology reports (specifically pertaining to skin tumours in dogs) were assimilated from the 2013 records (01 January to 30 June only) from the two largest private veterinary diagnostic laboratories in South Africa (Vetdiagnostix and Idexx Laboratories). These two laboratories receive the vast majority of canine skin tumour samples from all nine provinces in South Africa.

A total of 2553 cutaneous neoplasms were submitted during the study period, excluding all non-neoplastic or inflammatory

lesions. We excluded non-neoplastic skin lesions due to the vast amount of data that would have had to be processed and the limited additional information that would have been provided if we were to add non-neoplastic lesions to the data set.

This study updates the South African data and has highlighted the most significant associations between certain dog breeds and types of skin tumour. Also significant was the association of certain age groups and the development of skin tumours with breed, body location, specific tumour diagnosis, broad tumour category, tumour malignancy and gender.

Round cell tumours constituted 42% of all neoplasms recorded in the study, with low grade mast cell tumours recorded most frequently, followed by histiocytomas. The 10 most common neoplasms from most to least common were mast cell tumours, histiocytomas, haemangiosarcomas, squamous cell carcinomas, haemangiomas, sebaceous adenomas, melanocytomas, lipomas, spindle cell tumours, melanomas and perianal adenomas, and this was largely consistent across both laboratories. These findings concurred with earlier studies conducted elsewhere in the world, although breed predispositions documented for certain common skin tumours were different in South Africa.

Important trace element concentrations in ovine liver as determined by energy dispersive handheld X-ray fluorescence spectrometry

Van Loggerenberg D.E.¹, Myburgh J.G.¹, Laver P.², Botha C.J.¹

¹ Department of Pharmacology and Toxicology, Faculty of Veterinary Science, University of Pretoria, Onderstepoort, South Africa, e-mail: bernardusvl@gmail.com

² Department of Anatomy and Physiology, Faculty of Veterinary Science, University of Pretoria, Onderstepoort, South Africa

Certain trace elements are involved in a variety of biochemical processes essential to life. These trace elements essential to normal health and function of animals are required in minute amounts defined as less than 1000ppm. There are no prior data available considering the use of handheld X-ray fluorescence spectrometry to determine concentrations of important trace elements in ovine livers or any other animal of veterinary importance. A gap therefore exists in the current knowledge about the use of handheld X-ray fluorescence spectrometry in the determination of important trace elements in veterinary diagnostics. The aim of this study is to ascertain if the handheld X-ray fluorescence spectrometer will provide reliable concentrations of certain essential trace elements in the livers of sheep. 35 Sheep livers will be obtained from abattoirs. Wet liver samples taken from 30 liver specimens will be blended until satisfactorily homogeneity is achieved. Wet liver samples taken from 30 liver specimens will be oven dried at 60°C until constant mass is reached and then pulverised using a mortar and pestle

until a fine homogenous powder is formed. Wet liver samples taken from 30 liver specimens will be submitted for dry ashing. The remaining 5 livers will be subjected to standard addition ('spiking'). These samples will be analysed using a handheld X-ray fluorescence spectrometer to determine concentrations of copper, iron, manganese, molybdenum, selenium and zinc. A reference laboratory (control) will analyse samples from the same livers via ICP-MS to determine the concentrations of the abovementioned trace elements. Data sets collected from trace element concentrations obtained from ovine liver samples measured by handheld X-ray fluorescence spectrometry will be compared to trace element concentrations obtained from ovine liver samples determined via ICP-MS by the reference laboratory (control). The advantage of handheld X-ray spectrometry is that the turnaround time of samples are reduced by a great deal. Instead of sending samples away for laboratory analysis and waiting for results, samples can be analysed more rapidly with the use of a handheld X-ray fluorescence spectrometer.

Anthelmintic, antifungal and cytotoxic activities of acetone leaf extracts, fractions and isolated compounds from *Ptaeroxylon obliquum*

Ramadwa T.E., McGaw L.J., Adamu M., Eloff J.N

Phytomedicine Programme, Department of Paraclinical Sciences, Faculty of Veterinary Science, University of Pretoria, Private Bag X04, Onderstepoort 0110, South Africa, e-mail: ramadwa.te@gmail.com

Ptaeroxylon obliquum is traditionally used in South Africa for ethnoveterinary purposes to treat parasitic infections. In this study the antiparasitic, antifungal and cytotoxic activities of acetone leaf extracts of *P. obliquum*, fractions and isolated compounds were determined. *In vitro* anthelmintic activity was investigated against *Haemonchus contortus* ova and larvae using egg hatch inhibition and larval development assays. Antifungal activity was determined by a serial microplate dilution method against *Aspergillus fumigatus*, *Cryptococcus neoformans* and *Candida albicans*. The cellular toxicity of the crude extracts, fractions and isolated compounds was determined against Vero African green monkey kidney and human liver (C3A) cell lines.

Compounds were isolated from the chloroform fraction using silica gel open column chromatography and were characterized as obliquumol, eranthin and a mixture of lupeol and β -amyryn. The anthelmintic activity of the extract and obliquumol against *H. contortus* ova and larvae

indicated that the compound was more effective than the crude extract with an EC_{50} as low as 95 $\mu\text{g/ml}$ against the larvae. Obliquumol and eranthin had good antifungal activity against the fungi with minimal inhibitory concentration (MIC) values ranging from 2-16 $\mu\text{g/ml}$. Obliquumol was more active against *Candida albicans* than amphotericin B, the positive control, confirming previous studies done on the compound. The acetone crude extract was toxic with an IC_{50} value as low as 14.2 $\mu\text{g/ml}$ against the Vero cells. However, the isolated compounds were not toxic against either Vero or C3A cells and had a good selectivity index against fungal pathogens which commonly cause opportunistic infections in HIV patients. The results of the anthelmintic activity demonstrated potential of *P. obliquum* in the management of *H. contortus* which may explain the ethnoveterinary use of this species in South Africa. The activity of the isolated compounds may be important sources of remedies against opportunistic fungal pathogens in immuno-compromised patients.

Is being with girls stressful? Social environment influences hormone levels in male giraffes

Wolf T.E.¹, Bennett N.C.², Burroughs R.³, Ganswindt A.^{1,2,4}

¹ Endocrine Research Laboratory, Department of Anatomy and Physiology, Faculty of Veterinary Science, University of Pretoria, Onderstepoort 0110, South Africa

² Department of Zoology and Entomology, Faculty of Natural and Agricultural Sciences, University of Pretoria, Pretoria 0028, South Africa

³ Centre of Veterinary Wildlife Studies, Faculty of Veterinary Science, University of Pretoria, Onderstepoort 0110, South Africa

⁴ National Zoological Gardens of South Africa, Pretoria 0001, South Africa; u14449031@tuks.co.za

Giraffes (*Giraffa camelopardalis giraffa*) live in a fission-fusion social system, characterized by the splitting and reunion of subgroups within a larger social network. Adult bulls show a roaming tactic to search for fertile females, whereas younger bulls are often seen in all male groups.

In this study we combined behavioural observations with hormonal data. Therefore, a giraffe population of about 80 individuals (11 adult males, 28 adult females, and 32 juvenile / sub-adult animals) was monitored six days a week from dawn to dusk for a period of 12 months (Nov 2014–Oct 2015). Giraffes were individually identified by their unique pelage pattern and adult bulls assigned to age classes based on their appearance (A, B, and C), with class A bulls being the oldest and tallest. A total of 790 faecal samples were collected and analysed for faecal androgen (fAM) and glucocorticoid metabolite (fGCM) concentrations.

Class A bulls show significantly higher fAM levels compared to younger bulls, with lowest fAM levels found for the juvenile / sub-adult group. Longer periods of increased fAM levels in the oldest bulls mainly occur during summer and are associated with an observed birthing peak. Within class A, fAM levels of bulls in all male groups are significantly lower than when associated with females, however, these levels are still significantly higher compared to respective fAM levels from youngest males.

No significant differences in overall fGCM levels could be found between the different age classes. However, within class A the bulls show a trend for higher fGCM levels when sexually active compared to when associated with an all-male group. In contrast, juvenile / sub-adult bulls show highest fGCM levels when they are in all-male groups, which is possibly linked to intrasexual competition as younger bulls still need to establish their position within the hierarchy.

Thoracic limb myology of Temminck's ground pangolin (*Smutsia temminckii*)

Steyn C., Crole M.R., Soley J.T.

Department of Anatomy and Physiology, Faculty of Veterinary Science, University of Pretoria, Onderstepoort, 0110, South Africa; christine.steyn@up.ac.za

Temminck's ground pangolin is a threatened species and the only pangolin found in South Africa. It is a myrmecophagous mammal with a bipedal gait and rolls up into a ball when threatened. The manus has five clawed digits and the central three claws are enlarged for digging. The thoracic limbs are used to break open ant nests, dig for food and expand previously occupied burrows. This study thus aims to describe the myology of the thoracic limb.

Thoracic limbs from five Temminck's ground pangolins (3.0–8.3 kg), which succumbed from electrocution or natural causes, were removed and immersion fixed in 10% neutral-buffered formalin. The muscles were described and sequentially removed.

The muscles were generally well developed with fleshy bodies and short tendons. The cutaneous musculature over the

proximal limb was prominent and attached to the underlying muscles and humerus. Some muscles, such as *M. triceps brachii*, *M. pronator teres* and *M. flexor digitorum profundus*, displayed additional bellies and extensive attachments. Other muscles were absent, for example, *M. coracobrachialis*. Two new muscles were noted and named: *M. scapuloradialis* and *M. anconeus medialis*.

The features described above are indicative of the thoracic limb being adapted for digging (pronation, supination, protraction, retraction, flexion and extension) rather than locomotion. The extensive attachment of the cutaneous muscles to the thoracic limb may aid in protraction of the thoracic limbs when the pangolin rolls into a defensive ball. The large *M. triceps brachii* may render it useful as a site for intramuscular injections.

Gross morphology of *Musculi bulbi* of the ostrich (*Struthio camelus*) and emu (*Dromaius novaehollandiae*)

Kleyn E., Crole M.R.

Department of Anatomy and Physiology, Faculty of Veterinary Science, University of Pretoria, Onderstepoort, 0110, South Africa; ellene.kleyn@up.ac.za

The avian eye is uniquely adapted and reflects some structural peculiarities in comparison to mammals. *M. bulbi* consists of four rectus, two oblique and two nictitating membrane muscles. These have been described in several avian species, including the ostrich. The ostrich and emu are commercially important and a description of *M. bulbi* would aid in diagnostics and surgery.

Three sub-adult ostrich and three sub-adult emu heads were collected after slaughter in a commercial abattoir and immersion fixed in 10% neutral-buffered formalin. Enucleation involved removing extra-ocular tissues and incising the origins of *M. bulbi* in half the specimens and the insertions in the remainder. *M. bulbi* was described and compared.

In both species *M. bulbi* was composed of eight thin, sheet-like muscles; with the two nictitating membrane muscles

being more robust. *M. rectus dorsalis / ventralis / lateralis / medialis* originated peripherally to the *For. optici*. *M. obliquus dorsalis / ventralis* originated dorsally and rostrally to those above. The straight and oblique muscles inserted near the equator. Minor differences in the attachments of these muscles were noted between the species. *M. quadratus / pyramidalis membranae nictitantis* originated dorsally and ventro-medially on the equator, respectively. The pyramidal muscle inserted on the nictitating membrane via a long tendon to which the quadratus muscle also attached.

M. bulbi was similar in both species and the minor differences need to be morphometrically quantified. Functions of the muscles were comparable in both species. A similar surgical procedure for enucleation is recommended in the ostrich and emu.

The importance of faecal androgen metabolite monitoring in conservation of the endangered Southern Ground-Hornbill (*Bucorvus leadbeateri*)

Neller S.¹, Koetze A.², Bennet N.³, Kemp L.⁴, Ganswindt A.¹

¹ Endocrine Research Laboratory, Department of Anatomy & Physiology Faculty of Veterinary Science, University of Pretoria, Onderstepoort, 0110, South Africa;

² Research and Scientific Services Department, National Zoological Gardens, 232 Boom Street, Pretoria, South Africa;

³ Department of Zoology and Entomology, Faculty of Natural and Agricultural Sciences, University of Pretoria, South Africa;

⁴ Mabula Ground Hornbill Project, Mabula Private Game Reserve, Bela Bela 0480, Limpopo, South Africa; u16329572@tuks.co.za

Within South Africa, the Southern Ground-Hornbill has experienced a 20% decline in range and at least a 10% decline in population since the 1980s. A trend which seems to continue due to a number of anthropogenic threats. The species is long-lived and slow breeding with a highly complex, cooperative social structure; making the effort to reverse the decline expressly difficult. The reintroduction of new groups into areas of previous occurrence is the most effective method to rebuild the population, however, selecting a combination of both wild and hand-reared individuals to form the correct social dynamics is an on-going challenge.

The species is group-living with large territories (100km²) often containing a group of 2–12 individuals, including a single breeding alpha pair. The remaining group members are present to help with the rearing of offspring, but do not breed, and females are evicted from their natal group once their sexually dimorphic, blue throat colouration begins to show. However, it has recently been discovered that several

individuals displaying blue, originally assigned to be females, were mis-identified, and they are actually males displaying female colouration.

In such a complex species it is vital that the role of such individuals is understood, in order to optimize the future management practices for reintroduction candidates. Therefore, a thorough investigation into the suitability of enzyme-immunoassays for faecal androgen metabolite (fAM) monitoring, to characterize changes in fAM levels related to throat colouration, is currently being developed. The proposed methodology will involve the non-invasive sampling of approximately 100 captive individuals of various demographics and origin. It is anticipated that establishing baseline fAM values for all demographics (eg, male vs female vs juvenile, and dominant vs subordinate) will enable the correlation between fAM concentration and throat colouration to be distinguished. Further investigation into the behavioural consequences can then be observed.

Poo's potential – can we link different land use practices to stress-related hormone levels in leopards (*Panthera pardus*)?

Webster A.B.¹, Burroughs R.E.J.², Laver P.N.¹, Ganswindt A.¹

¹ Endocrine Research Laboratory, Department of Anatomy and Physiology

² Centre for Veterinary Wildlife Studies, Faculty of Veterinary Science, University of Pretoria, Onderstepoort, 0110, South Africa; andi.webster@gmail.com.

The leopard's versatility as a generalist predator facilitates occupation of a wide range of habitats. Although protected areas are important for the conservation of this species, the majority of suitable leopard habitat lies beyond protected area boundaries. Leopards utilizing areas under different anthropogenic influences may therefore be exposed to different environmental, physiological, and psychosocial stressors.

This study aims to examine the suitability of enzyme-immunoassays for monitoring adrenocortical function in the leopard based on faecal glucocorticoid metabolite (fGCM) analysis by performing an adrenocorticotrophic hormone (ACTH) challenge test. Subsequently the study will investigate variations in fGCM concentrations of animals under different anthropogenic influences to identify the potential extrinsic and intrinsic stressors linked to different land use types.

The ACTH challenge will be performed on one adult male and one adult female housed at Predator World in the

Northwest Province. In addition, gastrointestinal transit time will be determined in 10 animals housed at different captive facilities in the North West, Mpumalanga, and Gauteng Provinces. A potential aging effect of the hormone matrix will be investigated by determining the rate of metabolism of fGCMs post-defaecation. Finally, faecal samples from free-ranging leopards in protected and unprotected areas of Mpumalanga and Limpopo provinces including the Sabi Sands Game Reserve, Guernsey, the Hoedspruit Wildlife Estate and surrounds, will be collected over a period of 6 months.

The ability to reliably assess adrenocortical function in leopards will provide a solid foundation from which to further examine endocrine responses to recognised stressors in this iconic African species. Specifically, our study will help to untangle some of the wildlife management, conservation, and human-predator conflict mitigation challenges faced by free-ranging leopards at the human-domestic-wildlife interface.

Clinical signs, synovial fluid cytology and growth factor concentrations after intra-articular use of a platelet-rich product in horses with osteoarthritis

Smit Y.¹, Goddard A.¹, Mahne A.T.¹, Thompson P.N.², Marais H.J.¹

¹ Department of Companion Animal Clinical Studies, University of Pretoria, Onderstepoort, South Africa, e-mail: Yolandi.smit@up.ac.za

² Department of Production Animal Studies, University of Pretoria, Onderstepoort, South Africa.

Osteoarthritis (OA) is a common cause of lameness in equines. Platelet rich plasma can deliver a collection of bioactive molecules that play important roles in conditions such as OA. This study aimed to report on the clinical and synovial changes after intra-articular injection of normal and OA joints with a platelet-rich product (PRPr). Serum amyloid A (SAA) and total protein (TP) concentrations on serum and synovial fluid, as well as synovial fluid growth factor concentrations i.e. platelet-derived growth factor (PDGF-BB) and transforming growth factor (TGF- β 1) were measured. A PRPr was prepared from 5 horses with naturally occurring OA, affecting either carpus or metacarpophalangeal joints, or 5 normal horses using a gravity filtration system. Clinical evaluation, synovial fluid analysis, and determination of SAA and TP concentrations were performed on Day 0 (PRPr treatment), Day 1, Day 2, Day 5, Day 21 and Day 56. Synovial fluid PDGF-BB and TGF- β 1 were determined on Day 1 and

Day 5. PRPr composition was also analyzed. Platelets were successfully concentrated by the system (4.7-fold increase compared to blood). PRPr caused a significant increase in synovial nucleated cell count, consisting predominantly of intact neutrophils, within groups on Day 1 and Day 2 ($P < 0.001$ for both), but returned to normal levels by Day 21. Mean PDGF-BB concentrations were not significantly different between groups on Day 1 and remained elevated on Day 5 in both groups. Mean TGF- β 1 was significantly decreased in the OA group (2792 ± 615 pg/mL) compared to normal group (4021 ± 783 pg/mL) on Day 1 ($P = 0.017$), but declined in both groups on Day 5 with no significant difference. It was concluded that the gravity filtration system produced a moderately concentrated PRPr. Intra-articular treatment with PRPr caused a synovial inflammatory reaction of short duration and growth factor concentrations were elevated intra-articularly after PRPr administration.

Case report: Routine inflammatory markers in an injured white rhinoceros

Hooijberg E.H.¹, Cray C.², Du Preez J.P.¹, Steenkamp G.¹, Goddard A.¹

¹ Department of Companion Animal Clinical Studies, Faculty of Veterinary Science, University of Pretoria, South Africa, e-mail: amelia.goddard@up.ac.za

² Department of Pathology and Laboratory Medicine, University of Miami Miller School of Medicine, Florida, USA.

The white rhinoceros (*Ceratotherium simum*) is currently threatened by poaching and survivors of attacks have large wounds which require long-term intensive veterinary care. The objective of this investigation was to explore whether routine inflammatory markers were helpful in monitoring recovery in a 4-year old female rhinoceros that suffered extensive wounds to the facial region. Haematology, serum protein electrophoresis, fibrinogen, serum amyloid A, C-reactive protein and iron concentrations were determined on day 11, 32, 47, 77, 139 and 144 post-poaching. The rhinoceros improved clinically over the time period apart from showing lethargy and inappetence around day 139, which had improved by day 144. There was

an initial leukocytosis with mature neutrophilia which resolved by day 47; a band neutrophilia with toxic change was seen on day 139. Gamma globulins and iron showed an increasing trend apart from a decrease on day 139. C-reactive protein decreased slightly from 9.2 to 4.0 mg/L and serum amyloid A was consistently below the detection limit of the assay used. There was a mild decrease in fibrinogen. In conclusion, the leukogram was most consistent with clinical progress; other markers examined here such as serum iron may have potential but need to be investigated further. The automated serum amyloid A assay routinely used for horses lacks analytical sensitivity for use in the white rhinoceros.

Prevalence of and risk factors for feline hyperthyroidism in South Africa

McLean J.L.^{1,2}, Lobetti R.G.¹, Thompson P.N.³, Schoeman J.P.²

¹ Bryanston Veterinary Hospital, Box 67092, Bryanston, South Africa, email: joanne@fuseprop.co.za

² Department of Companion Animal Studies, Section of Small Animal Medicine, Faculty of Veterinary Science, University of Pretoria, Onderstepoort, South Africa.

³ Department of Production Animal Studies, Section of Epidemiology, Faculty of Veterinary Science, University of Pretoria, Onderstepoort, South Africa.

Feline hyperthyroidism is a disease of middle-aged to older cats that has shown a marked increase in worldwide incidence within the last three decades as well as a marked geographic variation in prevalence. The exact pathogenesis of the disease still remains obscure and despite a plethora of epidemiological studies, clear risk factors have not been identified. The purpose of this study was to determine the prevalence of feline hyperthyroidism in South Africa and to identify potential risk factors. Serum total thyroxine (tT4) and thyroid stimulating hormone (cTSH) were measured in 302 cats aged 9 years and older that were presented at various veterinary clinics. In a few cats, serum free thyroxine (fT4) was also measured. At the time of blood sampling a questionnaire was completed regarding vaccination history, internal and external parasite control, diet and environment. The prevalence of hyperthyroidism (tT4 > 50nmol/L or tT4

between 30-50 nmol/L with cTSH <0.03ng/ml and fT4 > 50pmol/L) within this population was 7.0% with no significant difference in prevalence between healthy (5.1%) and sick (8.2%) cats. Cats ≥12years of age (OR= 4.3, p= 0.02) and cats with canned food in their diet (OR= 2.1, p= 0.1) were more likely to be diagnosed with hyperthyroidism. No significant relationship between vaccinations, parasite control or indoor environment and hyperthyroidism was observed. Hyperthyroid cats were more likely to present with weight loss (OR= 3.2, p= 0.01) and with a heart rate ≥ 200bpm (OR= 5, p= 0.01) than cats without the disease. The prevalence of feline hyperthyroidism in South Africa appears to be similar to that in cats in Japan and Portugal but less than that in cats in the UK and Germany. Risk factors for hyperthyroidism, previously found in other studies, specifically older age and eating canned food was present in this study population.

Spatial distribution of bovine Trypanosomiasis in Malawi

Chimera E.T.¹, Fosgate G.T.¹, Etter E.^{1,3}, Neves L²

¹ Epidemiology Section, Department of Production Animal, Faculty of Veterinary Science, University of Pretoria, Onderstepoort, 0110, South Africa, e-mail: u15033369@tuks.co.za

² Department of Veterinary Tropical Diseases, Faculty of Veterinary Science, University of Pretoria, Onderstepoort, 0110, South Africa

³ UR AGIRs, Department Environment and Societies, CIRAD

The epidemiology of livestock transmitted trypanosomiasis is affected by changes in the environment. The interaction of livestock, wildlife, humans and the vector also affects the epidemiology of the disease. In this study we report on the spatial distribution of bovine trypanosomiasis in Malawi. A cross sectional study was conducted to establish the prevalence of bovine trypanosomiasis. Multistage sampling was performed at dip tanks in 13 selected districts distributed among the three regions of the country. A total of 444 blood samples were collected from cattle between January 2016 and February 2016. Samples were screened for trypanosomiasis using an indirect ELISA and PCR. Sample to positive ratios were calculated as, $S/P = (\text{sample optical density (OD)} - \text{negative control OD}) / (\text{positive control OD}$

$- \text{negative control OD})$. Distribution of the S/P ratios were tested for normality by plotting histograms and performing a Shapiro-Wilk normality test. S/P ratios were transformed using the natural logarithm and averaged over all cows sampled in each village. Serological results were then linked with their geographical locations. Risk maps were created by interpolation of the average village-level S/P ratios using ordinary kriging. The Moran's I was used to estimate the spatial autocorrelation of bovine trypanosomiasis based on the natural logarithm of the S/P ratio.

The identification of high risk areas for trypanosomiasis will help in implementing effective prevention, control and mitigation measures of bovine trypanosomiasis in Malawi.

Serological responses of cattle inoculated with inactivated trivalent foot-and-mouth disease vaccine

Lazarus D.D.^{1,7}, Fosgate G.T.¹, Burroughs R.E.J.², Van Schalkwyk O.L.³, Rikhotso O.⁴, Heath L.⁵, Maree F.F.⁵, Blignaut B.^{1,5}, Thomson G.R.⁶

¹ Department of Production Animal Studies, Faculty of Veterinary Science, University of Pretoria, Onderstepoort, 0110, South Africa, e-mail: lazdav2003@yahoo.co.uk

² University of Pretoria, Faculty of Veterinary Science, Centre for Veterinary Wildlife Studies, Onderstepoort, 0110, South Africa

³ Office of the State Veterinarian, Kruger National Park, South Africa

⁴ Mpumalanga Veterinary Services, Department of Agriculture, Land and Environmental Affairs, South Africa

⁵ Onderstepoort Veterinary Institute, Transboundary Animal Disease Programme, Onderstepoort, South Africa

⁶ TAD Scientific, Pretoria, South Africa

⁷ National Veterinary Research Institute, FMD Research Laboratory, Vom Nigeria

Tuberculosis (TB) is an infectious and deadly disease that infects about one-third of the world's population, with approximately 1.4 million deaths and 8.4 million new cases recorded annually. Many plant species contain antimycobacterial compounds which may possibly serve as template molecules for new anti-TB drugs. The Rubiaceae family is the largest family of trees in southern Africa and preliminary evidence has shown some antimycobacterial activity in of several species. The acetone extract and 5 fractions from *Psychotria zombamontana* were screened for antimycobacterial activity against three non-pathogenic mycobacteria, namely *Mycobacterium aurum* (NCTC 10437), *M. fortuitum* (ATCC 6841) and *M. smegmatis* (ATCC 1441), as well as pathogenic *M. tuberculosis* (8104) using a serial microdilution assay. Cytotoxicity was determined using a tetrazolium-based colorimetric cellular assay (MTT) against C3A human liver cells and Vero monkey kidney cells. The selectivity index (SI) values of the extracts were calculated.

Experiments to investigate synergistic activity of the extract and fractions with rifampicin were also conducted. The acetone extract of *P. zombamontana* had significant activity against the four mycobacteria. The hexane and chloroform fractions had excellent activity with MIC values ranging from 0.039 to 0.16 mg/ml. The crude extract and fractions had relatively low cytotoxicity against C3A liver cells with LC_{50} values ranging from 2.28-0.36 mg/ml. The chloroform fraction had the highest SI values of 0.625, 2.56 and 0.16 while the 35% water fraction had the lowest SI values of 0.024, 0.02 and 0.048 against *M. aurum*, *M. fortuitum* and *M. smegmatis* respectively. The combination of *P. zombamontana* with rifampicin and its fractions with rifampicin against the tested organism showed synergistic to additive effects with ΣFIC values ranging from 0.31-0.75 and 0.078-0.531 respectively. It can be concluded that the chloroform fraction contains one or more compounds with antimycobacterial activity, thereby justifying further research on isolating the active compounds.

Puppy survival and vigour associated with the use of a low dose medetomidine premedication, propofol induction and maintenance of anaesthesia using sevoflurane gas-inhalation for caesarean section in the bitch

De Cramer K.G.M.¹, Joubert K.E.², Nöthling J.O.³

¹ Rant en Dal Animal Hospital, 51 Cecil Knight Street, Mogale City, Gauteng, South Africa, email: kdcramer@mweb.co.za

² Department of Paraclinical Sciences, Faculty of Veterinary Science, University of Pretoria, Onderstepoort, 0110, South Africa

³ Department of Production Animal Studies, Faculty of Veterinary Science, University of Pretoria, Onderstepoort, 0110, South Africa

The safety of an anaesthetic protocol that included the alpha2-adrenergic agonist medetomidine hydrochloride at 7 µg/kg iv as premedicant, propofol (1 to 2 mg/kg iv) as induction agent and sevoflurane, at 2% in oxygen for maintenance of anaesthesia was studied in 292 caesarean sections (CS) and 2232 puppies delivered. The effects of the medetomidine was reversed using atipamezole at 50 µg/puppy sc immediately following delivery and in the bitch at 20 µg/kg iv immediately following surgery. The safety was expressed using survival of puppies immediately after, 2 h after and 7 d after delivery, as well as Apgar scores of the puppies. Maternal survival was established after delivery of the last puppy, at 2 h and at 7 d post CS. The 292 CSs included 148 Boerboel bitches, 84 English bulldog bitches and 60 other purebred bitches, which delivered a total of 2232 puppies. The live delivery percent for the Boerboel,

English bulldog and other purebred bitches was 97.39%, 96.67% and 96.69%, respectively. After correction for malformed euthanized puppies and fetuses discovered dead on ultrasound examination, the 2 h puppy survival was 98.21%, 95.60% and 94.30%, respectively. The Apgar scores averaged 9.66 for all the breeds combined. The results of the current study compare favourably with those published of other widely used anaesthetic protocols. The average Glasgow pain scale for bitches at discharge was (6.40 ± 0.65, S.E.M. = 0.04, n = 292). This study concludes that the use of medetomidine hydrochloride at 7 µg/kg iv is safe as premedicant in bitches prior to caesarean sections and is associated with good puppy vigour, 2 h and 7 d puppy survival. The use of medetomidine as premedicant permitted the use of less than ½ the dose of propofol usually required as an induction agent.

Identifying high risk areas for foot-and-mouth disease (FMD) outbreaks in South Africa

Sirdar M.M.¹, Blignaut B.^{1,2}, Gummow B.^{1,3}, Fosgate G.T.¹

¹ Epidemiology Section, Department of Production Animal Studies, Faculty of Veterinary Sciences, University of Pretoria, Onderstepoort, 0110, South Africa, e-mail: sirdar44@hotmail.com

² Trans-boundary Animal Diseases Programme, Onderstepoort Veterinary Institute, Agricultural Research Council, Onderstepoort, 0110, South Africa

³ College of Public Health, Medical and Veterinary Sciences, James Cook University, Townsville, 4811, Queensland, Australia

Foot-and-mouth disease (FMD) is a controlled (notifiable) disease in accordance with the South African Animal Diseases Act (Act 35) of 1984. In 1996, the International Committee on FMD of the OIE endorsed South Africa's FMD free status without vaccination. According to the OIE status, the areas excluded from the free zone were the endemically infected Kruger National Park and the FMD protection areas. During the previous 15 years, at least one FMD outbreak has occurred per year in the protection zone with the exceptions being 2005, 2007, and 2014. Continuing outbreaks threaten the FMD-free status of the country; outbreaks raise concerns about the efficiency and sustainability of FMD control measures within the protection areas. The objective of the study was to develop isopleth risk maps in effort to identify high risk areas in Mpumalanga and Limpopo Provinces. Data were collected

from provincial veterinary services and World Animal Health Information Database (WAHIS) Interface for the period 2005-2015. Cattle affected by the Southern African Territories serotypes 1 and 2 of FMD Virus were selected for modelling. The proportion of affected cattle at the dip-tank level was calculated and used as the dependent variable. Data were assessed for normality by plotting histograms, calculating descriptive statistics, and performing the Anderson-Darling test for normality. Data for each serotype and a combined analysis were interpolated using ordinary kriging of reported outbreaks. Moran's I was used to estimate the spatial autocorrelation of FMD outbreaks in cattle within the protection zone of South Africa. Data presented can assist with strengthening current FMD control measures and subsequently contribute to the development of further quantitative models.

Prevalence and risk factors of enterotoxigenic *Escherichia coli* in diarrhoeic neonatal and post weaning piglets and weaner pigs

*Ogundare S.T.*¹, *Fasina F.O.*², *Karama M.*³

¹ Department of Production Animal Studies, Faculty of Veterinary Sciences, University of Pretoria, Onderstepoort, 0110, South Africa, e-mail: stogundare@gmail.com

² Department of Veterinary Tropical Diseases, Faculty of Veterinary Sciences, University of Pretoria, Onderstepoort, 0110, South Africa

³ Section of Veterinary Public Health, Department of Paraclinical Sciences, Faculty of Veterinary Sciences, University of Pretoria, Onderstepoort, 0110, South Africa

Diarrhoea in piglets and weaners is a multifactorial condition associated with enterotoxigenic *Escherichia coli* (ETEC) and other pathogens that colonise piglet intestines. ETEC attach to the intestinal microvilli and produce enterotoxins that activate the enterocytes to secrete water and electrolytes. ETEC strains can also produce a shiga toxin (Stx2e), which causes oedema disease in pigs. The objective was to determine the prevalence and risk factors associated with ETEC diarrhoea in piglets in Gauteng and Northwest Provinces of South Africa. 280 rectal swabs from diarrhoeic and non-diarrhoeic pigs were collected from 8 piggeries of different sizes (16 to 650 sow units) in the 2 provinces. ETEC were detected and identified by multiplex polymerase chain reaction (mPCR) that targeted the four toxin-encoding genes carried by ETEC: *STaP*, *STb*, *LTb* and *Stx2e*. In addition, animal and management-related variables were collected simultaneously through the use of a checklist to determine risk factors associated with ETEC. Laboratory results were matched with the collected variables and data were

analysed using logistic regression. The prevalence of ETEC was 67.1% (188/280) (95% confidence interval: 61.48-72.46). PCR revealed the following frequencies of toxin-encoding genes in ETEC: *estB* (44.15%), *estA* (2.66%), *eltB* (3.72%), *estB/estA* (25.53%), *estB/eltB* (14.89%), *estB/stx2e* (2.66%), *estB/estA/stx2e* (2.13%) and *estB/estA/eltB* (4.26%). Risk factor analysis showed that a reduced risk of diarrhoea was associated with the use of antibiotics (OR = 0.534; P=0.092) but continuous use showed significant increase in the odds of diarrhoea (OR=5.636; P=0.038). Likewise, leaving a sick piglet in pen with other litter mates increased the risk of ETEC diarrhoea (OR=9.207; P<0.0001). Wet cleaning of pen (OR=7.785; P=0.013) and dirty feed troughs (OR=2.564; P=0.023) were also associated with increased risk of diarrhoea in piglets. More attention should be paid to the identified variables in order to prevent/mitigate ETEC-associated diarrhoea in piglets because of the negative impacts on production. piglets because of the negative impacts on production.

Initial blood urea nitrogen concentration predicts subsequent blood urea nitrogen in beef cows

*Tshuma T.*¹, *Fosgate G.T.*¹, *Hamman R.*¹, *Holm D.E.*¹

¹ Department of Production Animal Studies, Faculty of Veterinary Science, University of Pretoria, Onderstepoort, 0110, South Africa, e-mail: takula.tshuma@up.ac.za

This study was performed to determine whether beef cows have an inherent ability to maintain their relative blood urea nitrogen (BUN) concentration when cattle are exposed to varying levels of dietary nitrogen supplementation. Ten Hereford and 11 Nguni cows, aged between 2 and 16 years, were utilised in two crossover designs. In the first design, cows were exposed to diets containing normal and high crude protein (CP) levels. At the end of the first crossover design, cows received a normal diet for one week before commencement of the second design. In the second crossover design, cows were fed diets containing normal and low CP levels. Blood urea nitrogen concentration was measured 17-21 times (mean = 20) during the study. A linear mixed-effects model was used to assess whether baseline

BUN concentration (measured one week before onset of the study) was predictive of subsequent BUN concentration in individual cows. The model was also used to assess whether any of the measured variables were predictive of subsequent BUN concentrations. Baseline BUN concentration was a significant predictor of subsequent BUN concentration in individual cows ($P = 0.004$). Other variables that were significantly associated with subsequent BUN concentration were breed ($P = 0.033$), the diet that the cows received before the current treatment ($P < 0.001$), treatment ($P < 0.001$) and the week during which sampling was performed ($P < 0.001$). Beef cattle appear to have an inherent ability to maintain their relative BUN concentration within herds despite changes in levels of dietary nitrogen supplementation.

Determining the adrenocortical activity as a measure of stress in domestic pigs (*Sus scrofa domesticus*) based on salivary and faecal analysis

Mangwiro N.¹, Ganswindt A.², Fasina F.O.³

1 Department of Production Animal Studies, Faculty of Veterinary Science, University of Pretoria, Onderstepoort 0110, South Africa, e-mail: mangwiron@yahoo.com

2 Endocrine Research Laboratory, Department of Anatomy and Physiology, Faculty of Veterinary Science, University of Pretoria, Onderstepoort 0110, South Africa.

3 Department of Veterinary Tropical Diseases, Faculty, of Veterinary Science, University of Pretoria, Onderstepoort 0110, South Africa.

Domestic pigs are subjected to farm management procedures, some of which might be perceived as causing stress and therefore may have welfare concerns. When confronted with a stressor, animals display a response that consists of a suite of physiological and behavioural alterations to restore homeostasis. Physiologically, the response is usually determined using glucocorticoid concentrations (GC) albeit invasively, with the disadvantage of a possible handling-induced stress response. To date, no non-invasive method exists for determining stress-related responses in pigs. This study aimed to examine the suitability of enzyme-immunoassays (EIAs) for determining GC concentrations in saliva and faeces of domestic pigs by performing an ACTH challenge. A total of 6 animals were studied with 4 receiving 10µg/kg of Synacthen® (Novartis, South Africa Pty Ltd) and the remaining 2 receiving 0.5 ml physiologic saline. Baseline salivary glucocorticoid (sGC) concentrations of 2.38±1.83ng/ml (mean±SEM) increased

by six-fold (14.03±6.83 ng/ml) within 40-90 minutes after administration of ACTH ($P < 0.0001$) and the elevated salivary cortisol levels were maintained for up to nine hours. Administration of saline caused no significant differences in sGC concentrations ($P = 0.82$). Similarly, baseline faecal glucocorticoid metabolite (fGCM) level of 235±46µg/g rose significantly up to 393±164µg/g within 36 hours post ACTH administration ($P < 0.0001$). No significant difference was found between baseline and post saline administration ($P = 0.57$). Significantly higher sGC concentrations were found in samples collected in the morning compared to those collected in the afternoon, indicating a circadian rhythm. In terms of stability of fGCMs, post-defecation levels only changed by 4% over the course of 50h ($P = 0.76$). In conclusion, EIAs can be used to determine sGC and fGCM concentrations to assess adrenocortical function in pigs. FGCM levels are relatively stable for at least two days post-defecation, which facilitates field sample submissions.

Complete genome sequencing, characterisation and comparison of mycoplasmas isolated from South African poultry farms

Beylefeld A.¹, Wambulawaye P.², Bwala D.¹, Wandrag D.B.R.¹, Abolnik C.¹

1 Department of Production Animal Studies, Faculty of Veterinary Science, University of Pretoria, Onderstepoort, 0110, South Africa, e-mail: u25285573@tuks.co.za

2 Bacteriology laboratory of the Department of Veterinary Tropical diseases, Faculty of Veterinary Science, University of Pretoria, Onderstepoort, 0110, South Africa

Of the 23 mycoplasma species known to infect avian species, most are associated with respiratory disease. Complete genomes have been published for the four mycoplasma species considered most economically important; i.e. *Mycoplasma gallisepticum*, *M. synoviae*, *M. meleagridis* and *M. iowae*. During a study to develop a real-time PCR for the detection and differentiation of mycoplasmas found on South African poultry farms, a decline of samples identified as *M. gallisepticum* or *M. synoviae* and an increase in unidentified mycoplasma species was observed. As these samples were taken from chickens displaying clinical signs typical of pathogenic mycoplasma, it has become increasingly important to identify these mycoplasmas, opening the door for further investigation into the less known mycoplasma species. Samples that tested positive for mycoplasma infections were isolated and sequenced. A benefit of the project was the completion and annotation of genomes from the previously

less studied mycoplasmas and this led to the completion of the first *M. gallinaceum* genome. To date 35 complete genomes have been generated using ion-torrent sequencing. Using Bayesian analysis, the 16S rRNA phylogenetic tree was inferred to identify the samples. 51% were identified as *M. gallinaceum*; 11% as *M. synoviae*; 14% as *M. gallisepticum*; 9% as *M. pullorum* and 6% as *M. gallinarum*. Using de novo genome assembly methods, contigs were obtained for the *M. pullorum* samples. The pooled contigs were joined in an attempt to obtain a scaffold genome to be used in assemble-to-reference genome assembly for the first complete *M. pullorum* genome, which could then be annotated using the National Center for Biotechnology Information (NCBI) annotation tool. Our data represents the largest set of its kind and will generate novel insights into comparative genome organisation and future studies aimed at understanding these complex microorganisms.

A study of Newcastle Disease and the utilisation of social networks and market chains to enhance poultry disease surveillance in Zambia

Mubamba C.^{1,2,4}, Ramsay G.³, Dautu G.⁴, Abonlik C.¹, Gummow B.^{1,21}

1 Department of Production Animal Studies, Faculty of Veterinary Science, University of Pretoria, Onderstepoort, 0110, South Africa, e-mail: Celia.Abolnik@up.ac.za

2 Discipline of Veterinary Sciences, College of Public Health, Medical and Veterinary Sciences, James Cook University, Townsville 4811, QLD, Australia

3 School of Animal and Veterinary Science, Charles Sturt University, Australia

4 Department of Veterinary Services, Ministry of Fisheries and Livestock, P.O. Box 510016, postal code 10101, Zambia

Newcastle disease (ND) is a highly infectious disease of poultry that seriously impacts on food security and livelihoods of communities in tropical regions of the world. In Zambia, very little or no research has been done to assess the current status of the disease and its spread within and between communities. This background led to a series of studies that attempted to increase understanding of the disease in eastern Zambia that included a retrospective study and time series modelling of ND, and a questionnaire survey that assessed movement of poultry and poultry products. Major hotspots for poultry trade and movement were thereby identified, and sero-surveillance of ND among unvaccinated chickens was followed by molecular characterisation of circulating Newcastle Disease Virus (NDV) strains. The retrospective study found that ND cases followed a seasonal and cyclic pattern with peaks in March, August, September and December as well as cycles every

three years. Aid delivered through government programmes appeared to have no major impact on ND trends between 1989 and 2014 and time series modelling predicted an increasing trend in ND annual incidence over the next 25 years. Social network analysis revealed that the provincial capital and larger towns were most influential senders and receivers of poultry and its products. Trade of poultry and its products was at its peak in December and January and was associated with Christmas and New Year celebrations respectively. Sero-surveillance results revealed a provincial prevalence of 42% ranging from 0% to 97% in specific districts. Finally, typing reverse transcriptase PCR revealed that three NDV isolates belonged to the highly virulent VIII genotype that is also the current South African field outbreak strain. Results of these studies justify and form the basis for establishing more targeted ND surveillance within this region.

Neonate immunity, growth and puberty in dairy calves: Influence of dietary conjugated linoleic acid supplementation of the dam

Cardoso C.L.¹, Esposito G.^{1,2}

1 Department of Production Animal Studies, Faculty of Veterinary Science, University of Pretoria, Onderstepoort, 0110, South Africa, e-mail: dracardoso@gmail.com

2 Institute of Food, Nutrition and Well-Being, University of Pretoria, Hatfield, 0028, South Africa

Colostrum provides maturational, immune-modulatory and anti-microbial factors. Supplementation of conjugated linoleic acid (CLA) reportedly increase blood immunoglobulins (Ig) and insulin-like growth factor-I IGF-I levels.

The effect of CLA supplementation of the dam on colostrum quality and calves immune system, growth and attainment of puberty was evaluated. 34 Holstein cows, blocked by parity, body weight and body condition score were randomly assigned to two groups: control (Ca salts: 100gr/cow/day) and CLA (CLA: 100gr/cow/day). Cows received supplementation from day 20±7 prepartum to 35 days in milk. Colostrum from each cow was sampled for IgG quantification and fed to the respective calves from birth to day 3. Calves were bled every other day from 0 to 15 days for IgG and total protein (TP) levels and weekly for growth hormone (GH) and IGF-I. Body measurements and weight were recorded over the whole trial. Puberty was monitored from 5 (males) and 7 (females) months respectively Semen was obtained via

electroejaculation for evaluation of semen parameters. Puberty was declared with concentration of 50 million spermatozoa/ejaculate with >10% progressive motility. Females were bled fortnightly, and ovarian activity was monitored by ultrasonography. Puberty was declared when a corpus luteum present and progesterone level >1ng/ml. Growth rate, average daily gain, blood and colostrum were analysed by repeated measures ANOVA. Age at puberty by one-way ANOVA. Growth curves were calculated by regression of weights against time. Regression slopes were compared with the F- test.

Female CLA calves showed higher TP levels ($P<0.05$), while in males GH and IGF-I levels were lower ($P<0.05$ and $P<0.1$) compared to the control. Growth rate was higher in CLA male calves from 180 days of age compared to control ($P<0.01$). In conclusion, colostrum from CLA supplemented dams proved to have a long-term effect on calves' growth. However, it failed to alter puberty.

Msp1aS genotyping of *Anaplasma centrale* indicates a wildlife reservoir

*Khumalo Z.T.H.*¹, *Catanese H.N.*², *Liesching N.*¹, *Hove P.*¹, *Collins N.E.*¹, *Chaisi M.E.*¹, *Gebremedhin A.E.*², *Oosthuizen M.E.*¹, *Brayton K.A.*^{2,3}

¹ Department of Veterinary Tropical Diseases, University of Pretoria, Pretoria, South Africa, Email: mantungwa98@gmail.com

² Department of Electrical Engineering and Computer Science, Washington State University, Pullman, WA, USA

³ Department of Veterinary Microbiology and Pathology, Washington State University, Pullman, WA, USA

In seminal experiments in the 1900s Sir Arnold Theiler described the use of *Anaplasma centrale* as a vaccine against *A. marginale*. The vaccine strain that Theiler isolated has been disseminated throughout the world, and more than 100 years later, *A. centrale* remains in use as a live blood vaccine in many parts of the world. There has been little interest in the epidemiology of *A. centrale* as a result, there are few reports detecting natural infections of this organism. When detected in cattle, it is often assumed that it is due to vaccination and in most cases it is reported as a co-infection with *A. marginale* without characterization of the strain. In this study we tested 380 animals using a duplex qPCR assay to screen for the presence of *A. centrale* and *A. marginale* in vaccinated and unvaccinated cattle and wildlife from South Africa. Samples that tested positive using the duplex qPCR assay were

analyzed using a new test that we developed based on the homolog of *A. marginale msp1a*, *msp1aS*. The *msp1a* gene of *A. marginale* contains repeats near the 5' end which vary in sequence and number that are useful for genotyping strains. We detected 47 Msp1aS repeats corresponding to 32 different *A. centrale* genotypes that were found in cattle, buffalo and wildebeest in South Africa. We used a newly developed tool, RepeatAnalyzer, to catalogue and manage the repeat data. Our results showed that the vaccine strain is widely distributed across South Africa and is present in vaccinated cattle and in cattle and wildlife with no history of vaccination. A high degree of repeat diversity was detected amongst the repeats, suggesting that *A. centrale* strains have been circulating in nature and evolving *msp1aS* repeat sequences that are distinct from the sequenced vaccine strain.

Mortality rates and survival analysis of owned, free-roaming dogs in a resource-limited community, Bushbuckridge, South Africa

*Kolo F.B.*¹, *Conan A.*², *Henning A.*³, *Clift S.*³, *Knobel D.*²

¹ Department of Veterinary Tropical Diseases, University of Pretoria, Pretoria, South Africa, e-mail: kolofrancis@hotmail.com

² Ross University School of Veterinary Medicine, Basseterre, Saint Kitts and Nevis

³ Department of Production Animal Studies, University of Pretoria, South Africa

Canine rabies can be successfully controlled in dogs through mass vaccination. In populations of free-roaming dogs in resource-limited settings, the maintenance of herd immunity through vaccination is challenged by the high population turnover. Understanding and describing mortality in these populations may therefore assist in the control of rabies. The objective of this study was to determine the rates and causes of mortality in owned, free-roaming dogs in Hluvukani village, Bushbuckridge, South Africa, from May 2014 through July 2015.

From the Health and Demographic Surveillance System in Dogs in Hluvukani village, we followed a nested cohort of dogs one year and older over a 12-month period and puppies born to the cohort for 120 days, from May 2014. Deaths were recorded and investigated through verbal autopsy and post-mortem examination. Survival rates from enrolment (adults) or from birth (puppies) were compared using Cox proportional hazard regression models.

Of the cohort of 367 adult dogs (203 males and 164 females), 27 died during the follow-up period. The mortality rate was 78 per 1,000 dog-years in the cohort. Adult females had a shorter survival time from enrolment (mean = 341.7 days) compared to adult males (mean = 356 days; $p = 0.05$). No difference in survival was detected between age groups. Enrolled litters were 62 and 329 enrolled puppies, 135 died before 120 days of age. Mortality in puppies was high with 2,390 deaths per 1,000 dog-years recorded, and a mean survival time of 60 days. No difference in survival was observed between males and females ($p = 0.3$). In adults and puppies, causes of death were identified as natural (43%), non-natural (53%) and euthanasia (4%).

Mortality was low in adult dogs, but very high in the puppies. Despite high population turnover through births and deaths, the vaccination coverage was still sufficient to prevent rabies outbreaks in the village.

In silico functional prediction and characterization of selected *Theileria parva* hypothetical proteins

Mahlobo B.P.¹, Mokoena F.^{1,2}, Matjila T.¹, Sibeko-Matjila K.P.¹

¹ The Department of Veterinary Tropical Diseases, University of Pretoria, Pretoria, South Africa, e-mail: mahlobobongiwe@gmail.com

² Department of Life and Consumer Sciences, University of South Africa, South Africa

Cattle theileriosis is infamous for hampering the economic development of south, central and east African countries due to exorbitant numbers of cattle mortalities. The disease is caused by *Theileria parva*, an important tick-transmitted haemoprotozoan parasite that belongs to the phylum *Apicomplexa*. Infection of cattle with cattle-derived *T. parva* isolates is responsible for East Coast fever while infections by buffalo-derived isolates results in Corridor disease. A transcriptome study comparing two *T. parva* isolates, representing cattle- and buffalo-derived parasites, identified 1089 differentially expressed transcripts (DETs). Analysis of DETs revealed 593 (54.4%) hypothetical proteins (HPs). These proteins are believed that they could be crucial in understanding the diseases caused by *T. parva* infections. Thus, this study purposed to characterize these proteins. The initial screening using sequence similarity searches led to designation of sequence descriptions for 284 HPs; this report focuses on the analysis of the remaining 309. Applying

an integrated bioinformatics approach including a variety of domains discovery tools, protein family classification systems and approaches that are based on amino acid sequence characteristic, as well as 3D structures predictions, functions of these HPs were predicted. Furthermore, information of functionally characterized homologs, subcellular localization and functional partners of HPs was considered in the analysis. Overall, n = 193 HPs were successfully annotated for function and some of these were virulent proteins, significant in the survival of the pathogen in the host. Subcellular localization revealed three HPs that could be investigated as possible therapeutic targets. Secretome analysis revealed 57 HPs containing signal peptides, suggesting possible interactions with the host. The results of this study will facilitate a better understanding of the mechanism of pathogenesis of cattle theileriosis caused by *T. parva* and development of more effective disease control strategies.

Assessment of sequence descriptions of *Theileria parva* hypothetical proteins, retrieved from sequence similarity search databases

Mampa M.S.¹, Mokoena F.^{1,2}, Matjila T.P.¹, Sibeko K.P.¹

¹ Department of Tropical Veterinary Diseases; University of Pretoria, Onderstepoort, South Africa, e-mail: mmogauselina@yahoo.com

² Department of Life and Consumer Science; University of South Africa, Johannesburg, South Africa.

The protozoan parasite *Theileria parva* is the causative agent of cattle theileriosis, a disease with a destructive impact on the agricultural economy through mortality and morbidity of affected cattle. In cattle, *T. parva* infection results in varied disease syndromes depending on the parasite host of origin; cattle-derived *T. parva* causes East Coast fever while buffalo-derived parasites cause Corridor disease. The differences in the resulting disease caused by *T. parva* infection have raised an interest to understand the proteins involved in the disease manifestations. Consequently, a transcriptome study comparing the cattle and buffalo-derived *T. parva* isolates was undertaken; differentially expressed genes were detected of which 74% (867) were hypothetical proteins (HPs). Since HPs could play a vital role in the pathogenicity and host-parasite interaction, the primary aim of the study was to identify biological roles of these proteins. A combination of *in silico* analysis tools was employed to annotate HPs according to sequence descriptions, confirmed

by sequence homology in comparison with closely related species and conserved domains. Initial screening for sequence descriptions (SDs) based on sequence similarity search using Blast2GO retrieved results for 392 HPs. Comparison of this output to other databases (KEGG and KOBAS) detected consensus SDs for 229 HPs, of which 109 were further confirmed by inferring homology to related species. Sequence homology analysis also resulted in designation of SDs to 74 HPs from the remaining 163 without consensus SDs from database analyses. For HPs which did not meet the criteria employed in sequence homology analysis (209), conserved domain analysis facilitated assigning of SDs for 114 HPs. Overall, 297 (76%) HPs were successfully allocated SDs. Finally, the results from this study have showed that output from automated sequence similarity databases is not always reliable in assigning SDs for specific species, making confirmation using other approaches necessary.

Determination of innate and adaptive immune transcriptomes after infection with *Ehrlichia ruminantium*

Nefefe T.^{1,2}, Liebenberg J.¹, van Kleef M.^{1,2}, Pretorius A.^{1,2}

1 ARC-OVI, Onderstepoort, South Africa, nefefet@arc.agric.za

2 Department of Veterinary Tropical Diseases, University of Pretoria, Onderstepoort, South Africa

Heartwater (HW) is a tick-borne non-infectious fatal disease of wild and domestic ruminants caused by the bacterium *Ehrlichia ruminantium*, transmitted by *Amblyomma* ticks. A long-term aim of this study is to introduce a new systems biologically oriented approach to HW vaccine development. As a first step, it attempts to measure several different aspects of the innate and adaptive immune responses that follow infection and challenge. Unlike studies confined to measuring a single parameter, an overall analysis of which immune pathways are up- or down-regulated is expected to lead to a better understanding of the global immune response of sheep to *E. ruminantium* infection. Blood collection from four sheep before and during heartwater infected tick feeding and challenge was conducted. The blood was processed for *E. ruminantium* pCS20 detection, cytokine detection and phenotyping of circulating lymphocytes to identify the optimum time point(s) of

cytokine production. Quantitative real-time PCR showed that *E. ruminantium* could only be detected in the sheep blood a day before and during the febrile reaction. Cytokines qPCR indicated that IFN- γ , IL-2, IL-4, IL-6, IL-10, IL-12, TGF- β and TNF- α were expressed before and during febrile reaction after primary infection and challenge of immune sheep. Flow cytometry indicated an increase in the percentage of B-cells in the circulating blood during the first three days after primary infection by *E. ruminantium*, whereafter there is a gradual decrease to normal levels. CD4 and CD8 T-cells increased early after primary infection, decreased before febrile reaction and then increased during febrile reaction. After challenge the CD4 and CD8 T-cells fluctuated throughout. Cytokine expression and flow cytometry data will enable transcriptome sample selection for further analysis of innate and adaptive immune cells using transcriptome sequencing.

Transcriptome analysis of *Ehrlichia ruminantium* during the developmental stages in mammalian and tick cell culture

Tjale M.^{1,2}, Liebenberg J.^{1,2}, Van Kleef M.^{1,2}, Pretorius A.^{1,2}

1 ARC-OVI, Onderstepoort, South Africa, TjaleM@arc.agric.za

2 Department of Veterinary Tropical Disease, University of Pretoria, Onderstepoort, South Africa

Heartwater, caused by *Ehrlichia ruminantium* (ER), is the third-most economically important tick-borne disease of domestic ruminants in sub-Saharan Africa. The current method of protective immunisation confers limited protection against common virulent genotypes. Previous host-pathogen interaction studies revealed that for a pathogen to invade and multiply in the host cells, it requires life stage specific adaptations which are likely to be reflected in gene transcription and protein expression. Therefore, this study was aimed at identifying ER differentially expressed genes in the developmental stages in mammalian and tick cell culture. The ER (Welgevonden isolate) was cultivated in bovine aortic endothelial cells (BA886) and *Ixodes scapularis* embryonic cells (IDE8). The infective extracellular forms, the elementary bodies (EBs) and vegetative intracellular forms, reticulate bodies (RBs)

were collected. RNA was prepared from duplicate samples and were sent for transcriptome sequencing. Approximately 820 ER genes were expressed in mammalian and tick cells. Several genes were found to be exclusively expressed in the EBs and/or RBs in mammalian or tick cells. Quantitative real time PCR was performed and the differential expression results correlated with those obtained by transcriptome sequencing. This study reports for the first time the transcriptome analysis of ER in the tick vector and the ruminant host cells. These findings contribute towards the increasing knowledge of additional proteins/antigens expressed during ER infection. Knowledge of genes expressed in the EBs will aid in the early immune detection, thus antigens that are potentially essential for the infection of endothelial cells. Antigens expressed in the RBs might assist in elucidating putative CTL epitopes.

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Published by the Faculty of Veterinary Science, University of Pretoria
Head: Communications and Media
E-mail: chris.vanblerk@up.ac.za

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