

Faculty Day

7 September 2017

Research Overview



Prof Robert Gilbert

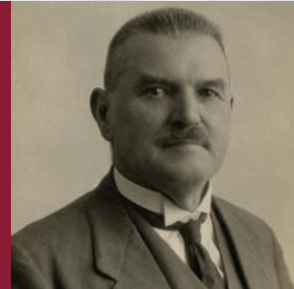
Head: Department of Clinical Sciences
School of Veterinary Medicine
Ross University
St. Kitts



UNIVERSITEIT VAN PRETORIA
UNIVERSITY OF PRETORIA
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Faculty of Veterinary Science

Fakulteit Veeartsenykunde
Lefapha la Diseanse tša Bongakadiriwa



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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 2017 Faculty Day.



Faculty Day

Faculty of Veterinary Science
University of Pretoria

7 September 2017



Contents/Programme



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

Master of Ceremonies: Prof Ken Pettey

08:30 – 08:45 **Welcoming address: Prof Darrell Abernethy, Dean of the Faculty of Veterinary Science**

08:45 – 10:00 **First Session Chairperson: Dr Takula Tshuma**

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10:00 – 10:45 **Tea (Cafeteria)**

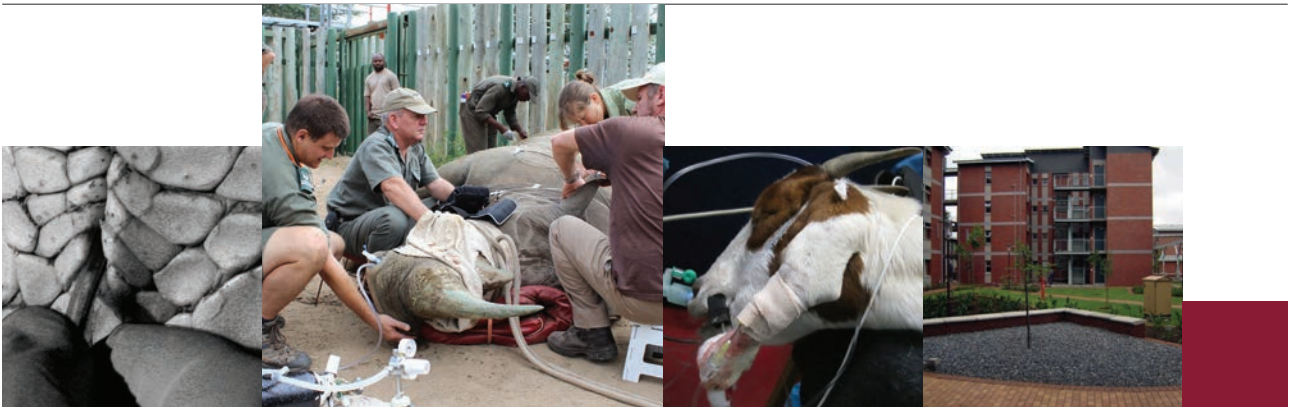
10:45 – 12:00 **Second Session Chairperson: Prof Darrell Abernethy**

Sir Arnold Theiler Memorial Lecture: Prof Robert Gilbert

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

Poster session

12:30 – 13:00 **Lunch (Cafeteria)**



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14:45 – 15:00

Interesting research development showcase from Production Animal Studies: Prof Henk Bertschinger

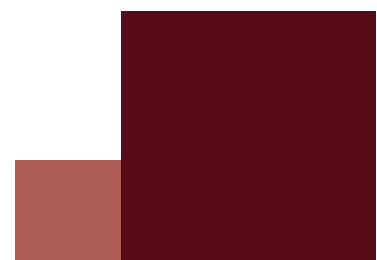
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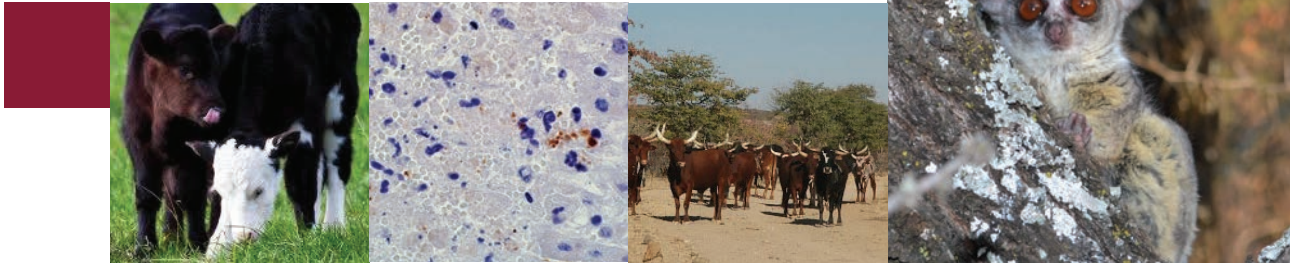
Faculty Day Awards

Researcher of the year
 Young researcher of the year
 Best oral presentation
 Best poster

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Message from the Dean

Welcome to Faculty Day 2017, where we showcase current research in the Faculty through oral or poster presentations and hear from top researchers. We also welcome Professor Robert Gilbert who will deliver a keynote address entitled: "The Research Imperative". I trust you find the day enjoyable and profitable.

Universities worldwide compete against each other to be included in a "ranking" or list of the top institutions. A high position is prestigious and important in attracting funders, researchers and students. In the last two years, veterinary faculties have also been ranked and in the latest one - the Shanghai rankings - our Faculty was placed 30th among the top 200 institutions worldwide. This is an outstanding result and strong recognition of the quality and stature of the Faculty. Research outputs count significantly towards this placing and further growth is essential to maintain – or improve! - our current placing. We must therefore strive to not only grow the volume of research but also its impact, as measured by citations, and must ensure it remains relevant, both nationally and globally.

Other recent markers of success include Veterinary Science being ranked the highest subject-specific discipline in the university in the 2016 QS rankings, the increasing proportion of academics with doctorates (53% in 2016 v 44% in 2014), a record number of PhDs being awarded in 2017 (20 v 11 in 2014) and two academics being recently recognized as exceptional researchers: Prof Johan Schoeman (Exceptional Academic Achiever) and Dr Martina Crole (Exceptional Young Researcher). The Faculty is now ranked fourth in the university in respect of NRF-rated scientists and has six B-rated scientists (Professors Christo Botha, Geoff Fosgate, André Ganswindt, Alan Guthrie, Ivan Horak, Anita Michel and Johan Schoeman), four of which were conferred or renewed in 2016. Further recognition of Faculty performance was the award of the 2016 African Union Kwame Nkrumah Scientific Award to Prof Celia Abolnik.

Such accolades are important to encourage and motivate, as well as to measure progress, but we continue to develop new strategies in order to

facilitate further growth. This we have done through a range of initiatives including new research themes, centres and chairs, as well as improving the postgraduate environment and processes. We have recently launched four new research themes (African Wildlife Health and Management; Translational Medicine; Sustainable Livelihoods and Wellbeing; Pathobiology of Diseases), appointed research coordinators and provided seed-funding to incentivize strong research. We are in the process of creating two new research centres (Molecular and One Health) and will be restructuring all our centres to better coordinate research outputs and links to departments. We have applied for new research chairs (Risk Analysis, Antimicrobial Resistance and Poultry Health), will be providing new facilities for our postgraduate students (refurbishment of the old computer facility) and have introduced guidelines to supervisors and students to improve postgraduate student throughput and outputs.

We have always had a strong international focus in research collaboration and value our overseas partners, particularly the University of Utrecht in the Netherlands and the Institute of Tropical Medicine, Belgium. However, we are also aware of the pressing needs in South Africa and our pivotal role in addressing key local challenges of animal health, poverty and food security in southern Africa. We have thus placed great emphasis on the role of the Faculty in addressing local needs – seen, for example, in the Exotic Leather Research Centre, which serves as technical collaborator for DTI in improving the global competitiveness of the South African exotic leather industry, the Equine Research Centre – essential in African horse sickness diagnosis and control, and Poultry Chair (Prof. Celia Abolnik), with a pivotal role in the current avian influenza epidemic. Another example is the Faculty's Mnisi



Prof Darrell Abernethy,
Dean: Faculty of Veterinary Science

One Health Platform, which functions in partnership with the Mnisi Traditional Authority, Peace Parks Foundation, Mpumalanga Veterinary Services and the Mpumalanga Tourism and Parks Agency. A new pilot project with great potential, Herding for Health, is a One Health, pro-poor, rural development project, which is researching an integrated way of managing livestock and improving rural livelihoods in areas at the wildlife-livestock interface.

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 more than 30 years. I hope that Faculty Day 2017 will further provide impetus to this 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 Robert Gilbert. Born and educated in South Africa, Prof Gilbert graduated from our Faculty in 1977. In 2017 he was recognized as a Fellow of the Royal College of Veterinary Surgeons for Meritorious Contributions to Knowledge. In 2016 Prof. Gilbert retired from Cornell University as Emeritus Professor and joined Ross University School of Veterinary Medicine on the Caribbean Island of St. Kitts as Professor and Head of the Department of Clinical Sciences

Again, we 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 Organizing Committee for making this event possible. May Faculty Day once again serve as an inspiration in pursuit of the Faculty's predominant research goals by sharing new scientific results, new ideas and innovative concepts.

Curriculum Vitae: Robert Gilbert



Prof Robert Gilbert

Professor Robert Gilbert was born and educated in South Africa. After graduating from the UP Faculty of Veterinary Science at Onderstepoort in 1977 he briefly entered private practice before joining Taurus Livestock Improvement Cooperative. In 1981 he returned to Onderstepoort where he taught and completed a MMedVet degree in reproduction. After two years in Wisconsin he returned to Onderstepoort as Associate Professor but soon left for Cornell University, where he spent most of his career. Over nearly three decades at Cornell Prof. Gilbert served in a variety of administrative positions (including department chair, hospital director, associate dean and senior associate dean) as well as developing a productive research programme. He has published about 140 refereed papers in many different areas of reproduction but is best known for his contributions to understanding postpartum uterine disease in dairy cows. Amongst many accolades, Prof. Gilbert was recognized as the Pfizer

Distinguished Teacher at Cornell in 2010, was DLT Smith Visiting Scientist at the University of Saskatchewan, Distinguished Academic Visitor to Queens' College Cambridge, received the Youth Advocate Award from the New York Horse Council in 2015 and in the same year was recognized as Theriogenologist of the Year by the American College of Theriogenologists. In 2017 he was recognized as a Fellow of the Royal College of Veterinary Surgeons for Meritorious Contributions to Knowledge. In 2016 Prof. Gilbert retired from Cornell University as Emeritus Professor and joined Ross University School of Veterinary Medicine on the Caribbean island of St. Kitts as Professor and Head of the Department of Clinical Sciences, allowing him to indulge his love for the ocean while still teaching veterinary students. Still active in bovine uterine disease investigation, he has embarked on new projects on aspects of reproduction in donkeys and monkeys.

“The Research Imperative”

Professor Robert Gilbert

Human population growth continues unabated and is expected to reach about 11 billion by the end of the century, of whom about 4 billion will live in Africa.

The pressure to feed the population in a sustainable way is challenged by limited and decreasing land availability, severely constrained water sources, political instability and unique disease challenges. Currently over 2 billion people lack food security, 6 million children die of malnutrition every year and over a quarter of children in the developing world are malnourished. Meeting the demand for food in Africa will require all the creative talent of our scientists in the coming decades and will place special demands on veterinary scientists to enhance animal health and productivity and especially to do so within prevailing resource constraints and without contaminating air, soil or water. These expectations may appear overwhelming but if we all contribute in our own areas of expertise I remain confident that innovation and creativity in the profession will prevail to meet these challenges.

My own investigation of uterine disease was rooted in my frustration by the lack of commonly accepted diagnostic and therapeutic approaches that prevailed 25 years ago; some people argued that endometritis was not detrimental to reproduction and the evidence on both sides was poor. We set out to define the condition and measure its impact, establishing that, amongst dairy cows in North America it was both highly prevalent and severely detrimental. We went on to examine the epidemiology of the condition and investigate bacterial

pathogens that played a role, culminating in the development of a vaccine that reduced incidence of metritis and improved reproduction. We also focused on periparturient immune and metabolic status in the pathogenesis of uterine disease.

Apart from the obvious benefit of research in enhancing animal health, welfare or productivity and adding to the arsenal of veterinary diagnostic, therapeutic and preventative tools, research is personally and intellectually satisfying. Research enriches the educational environment by encouraging a deeper understanding of subject material, by developing enthusiasm for discovery and a sense of excitement. However, research is not the exclusive preserve of academia – veterinary practitioners should be an integral part of the overall research effort and the barriers between practice and academia for research collaboration should be removed. Practitioners often have access to more cases of a specific kind than academic hospitals, and the advent of electronic records and the ability to manipulate and analyze vast databases has facilitated investigations involving many patients, herds or veterinary practices. Some practicing veterinarians find participation in research provides an outlet for their creative faculties. The vital ingredient is curiosity and an enthusiasm for the subject.

Our ability to advance veterinary practice, to feed a growing population in a healthy and sustainable way, to advance health, welfare and productivity of animals, and indeed whole populations and ecosystems, and to educate veterinarians able to adapt and flourish for 50 years after they graduate, depends on a vigorous and productive research enterprise that engages the whole profession.

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 Guillette Jr | "Predisposition for health or disease: the 'new' genetics of environmental health" |
| 2015: | Prof Graham J Louw | "Mummification – a glimpse into the sociocultural aspects of the preservation of the bodies of domesticated animals." |
| 2016: | Prof Lucille Blumberg | "One Health: a decade of shared experiences and benefits" |

* 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: 2016–2017

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 staff members 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 85.85 units in 2016 which represent 208 ISI-accredited journals. In terms of postgraduate students the Faculty currently has 181 Masters, 108 PhD and 17 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 try and further increase these percentages over the next one to three years and to recruit additional postdoctoral researchers and research fellows. In 2017 a total number of 12 PhD candidates graduated during the University's Autumn graduation week while a further 8 PhD candidates will graduate during September 2017.

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

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

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

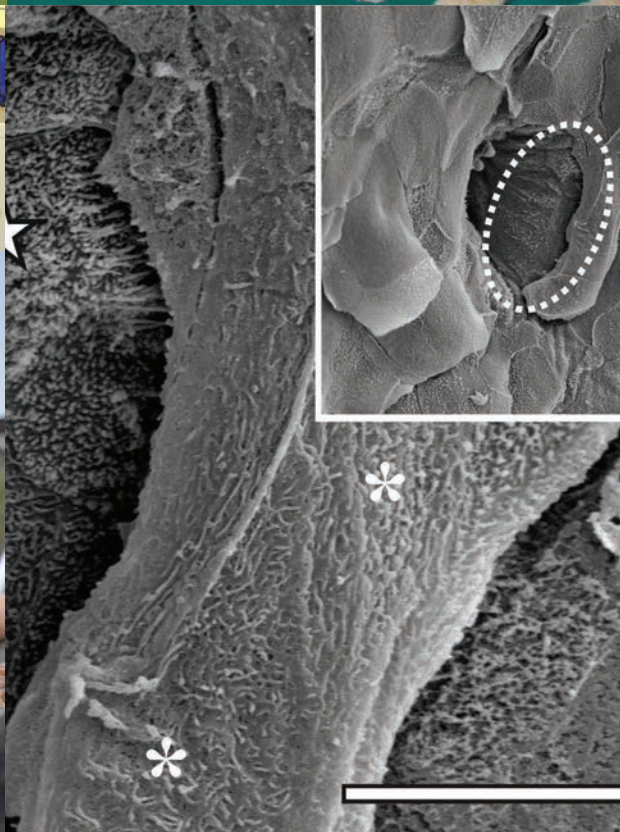
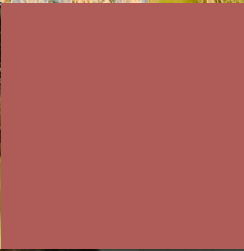
An inclusive research focus with contributions from all five departments of the Faculty, including studies on tuberculosis in buffalo, immune-contraception in elephants, theileriosis in roan and sable, the toxicity of non-steroidal anti-inflammatories in vultures and endocrine disruptors in the environment.

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.



Research Summary: 2016 – 2017 (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.

Currently the Faculty has 48 permanent staff members with doctorates. Since 2014 there was also a dramatic upsurge in the combined number of master's and doctoral students, and the Faculty more than doubled its postgraduate output and number of postdoctoral students. A total 20 PhDs will be awarded in 2017 of which 12 candidates obtained their degrees during the Autumn graduation with a further 8 to follow during the Spring graduation ceremony. This represents the highest number of doctorates awarded by the faculty in a given year.

The number of NRF-rated researchers in the Faculty's staff complement has shown a steady growth, reaching 29 in 2017. The Faculty now has nine B-rated, 14 C-rated and 6 Y-rated staff members with Prof Christo Botha, head of the Department of Paraclinical Sciences rated B1 which makes him the highest NRF-rated researcher in the Faculty.

Faculty Day 2016 and research awards

The annual Faculty Day on 25 August 2016 provided an opportunity for our researchers to showcase the research activities in the Faculty to colleagues and peers, and was well attended by staff members, visitors and sponsor companies alike. Prof Lucille Blumberg of the National Institute for Communicable Diseases delivered the annual Sir Arnold Theiler Memorial Lecture entitled *One Health: a decade of shared experiences and benefits*. Excellence in research performance was recognised at the event with the identification of the Faculty's Top 10 researchers and the allocation of the following research awards:

Researcher of the Year

Prof Estelle Venter
(Department of Veterinary Tropical Diseases)

Nine top researchers in the Faculty

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



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Research Programme: Oral Presentations

Occurrence and characterization of “big seven” Shiga toxin-producing *Escherichia coli* serotypes in healthy cattle from cow-calf operations in Gauteng and Northwest provinces, South Africa

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³ Dipartimento di Medicina Veterinaria, Laboratorio di Ispezione degli Alimenti di Origine Animale, University of Perugia, Perugia, Italy

Shiga toxin-producing *Escherichia coli* (STEC) are foodborne pathogens which cause infections in humans characterized by mild watery to severe bloody diarrhea, hemorrhagic colitis (HC) and the hemolytic uremic syndrome (HUS) as complications. Shiga toxins (*stx*₁ and *stx*₂), intimin (*eaeA*) adhesin and a number of plasmid-encoded genes *katP*, *espP*, *etpD*, *subA* and *saa* are key STEC virulence factors and markers. Cattle are the main reservoir of STEC. STEC transmission to humans is mainly through consumption of contaminated food of animal origin. The “big seven” STEC (O157, O26, O45, O103, O121, O111, and O145) are commonly incriminated in severe illness and outbreaks in humans. Although STEC have been incriminated in human disease in South Africa, current data on the role played by cattle as a reservoir of “big seven” STEC is lacking. The objectives of this study were (i) to investigate the presence of “big seven” STEC in healthy adult cattle and (ii) characterise the STEC isolates in terms of major virulence genes and markers. Culture and PCR were used to detect STEC. STEC isolates (n=578), from 559 cattle, were recovered and

screened for “big seven” STEC. Confirmed “big seven” STEC (241/578) isolates were characterised for virulence genes and markers by PCR. The overall prevalence of “big seven” STEC was 16.5% (92/559). The distribution of “big seven” STEC among the STEC positive cattle was as follows: O26, 10.2% (57/559); O45, 2.9% (16/559); O145, 2.5% (14/559); O157, 1.4% (8/559); O121, 1.1% (6/559) and O103, 0.4% (2/559). The distribution of virulence factors was as follows: *stx*₁, 69.3% (167/241); *stx*₂, 96.3% (232/241); *eaeA*, 7.1% (17/241); *ehxA*, 92.5% (223/241); *stx*₁ and *stx*₂, 55.6% (134/241); *katP*, 5.8% (14/241); *espP*, 77.2% (186/241); *etpD*, 5.8% (14/241); *subA*, 66.8% (161/241) and *saa*, 88% (212/241). The majority of isolates carried *stx*₁, *stx*₂, *ehxA*, *subA*, *espP* and *saa* but lacked *katP*, *etpD* and the *eaeA* gene which is a key STEC adhesin and a major virulence factor in human disease. These findings are evidence that cattle in South Africa carry and shed “big seven” STEC. However, the absence of *eaeA* and other major plasmid-encoded virulence markers among the STEC isolates under study may be a good reason why STEC disease remains less prevalent and sporadic in South Africa.





Seroprevalence of leptospirosis in slaughtered animals in Gauteng Province abattoirs, South Africa

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

3 Bacterial Serology Laboratory: ARC-Onderstepoort Veterinary Research, Pretoria, South Africa

4 Gauteng Department of Agriculture and Rural Development, South Africa

Leptospirosis is an important re-emerging infectious zoonotic disease caused by the spirochete bacterium *Leptospira* spp., distributed worldwide, especially in developing countries. The disease causes abortion and other reproductive problems in cattle, goats, sheep and pigs. In human cases, the disease presents as a febrile illness similar to malaria, viral hepatitis, influenza, dengue fever and typhoid fever, which can lead to kidney damage, meningitis, liver failure, respiratory distress, and even death.

This study used the microscopic agglutination test (MAT) with a panel of eight serovars, to determine the seroprevalence

of *Leptospira* spp. antibodies in serum samples from slaughtered animals in ten randomly selected abattoirs within the Gauteng Province in South Africa. Out of the 256 sera samples collected and screened using MAT, 24.2% were seropositive for *Leptospira* antibodies. The serovars detected were Bratislava, Canicola, Icterohaemorrhagiae, Pomona, Tarassova, Swajizak, Grippotyphosa and Hardjo. The data from this study provide an update of the prevalence of leptospirosis and implicated serovars in livestock in the province, which may contribute to the prevention and control of leptospirosis in livestock and humans in the country.





Interaction of *Tritrichomonas foetus* infection with the vaginal bacterial microbiota of heifers

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³ Agriculture Research Council – Biotechnology Platform Building, Onderstepoort 0110.

Bovine trichomonosis is a venereal disease responsible for extensive economic losses. *Tritrichomonas foetus* deposited during copulation adheres to the vaginal mucosa where it proliferates. The vaginal bacterial flora protects the underlying mucosa via a mechanism known as colonization resistance. Considering the detrimental effect of the protozoa and the benefits of the vaginal microbiome, we sought to describe and demonstrate the bovine vaginal microbiome using metagenomics; contrasting between oestrus and diestrus; and the interaction between the pathogen and the vaginal bacterial flora.

A total of six heifers were divided into control and infected groups. Using an endometrial guarded swab, three cranial vaginal samples were taken on days 1, 11 and 21 of their reproductive cycle, a total of eighteen swabs. DNA extraction, amplification of the V3-V4 region of the 16S rRNA gene and next-generation sequencing provided more than a million high-quality reads.

The vaginal bacterial flora of controls in the follicular phase were dominated by the phyla Tenericutes and Proteobacteria (59% and 36% respectively), represented by the families Mycoplasmataceae and Pasteurellaceae. The control group diestrus flora were significantly less diverse, dominated by the family Mycoplasmataceae ($P < 0.05$). The flora in the infected group were significantly more diverse than the control group during both phases, dominated by 5 different phyla: Firmicutes, Actinobacteria, Tenericutes, Bacteroidetes and Proteobacteria. Statistical analysis revealed a reduction of Mycoplasmataceae in the infected group compared to the controls ($P < 0.05$), and increases in the Bacillaceae ($P < 0.05$), Ruminococcaceae ($P < 0.05$), Propionibacteria ($P < 0.05$), Lachnospiraceae ($P < 0.05$).

The use of a culture-independent method expanded the knowledge of the bovine vaginal microbiota. The introduction of *T. foetus* caused a dramatic change of the vaginal microbiota and an increase in the bacterial diversity.





Prevalence of *Clostridium difficile* and *Salmonella* spp. in juvenile dogs affected with parvovirus enteritis

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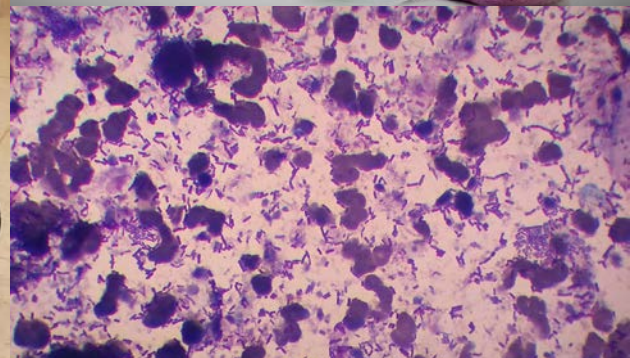
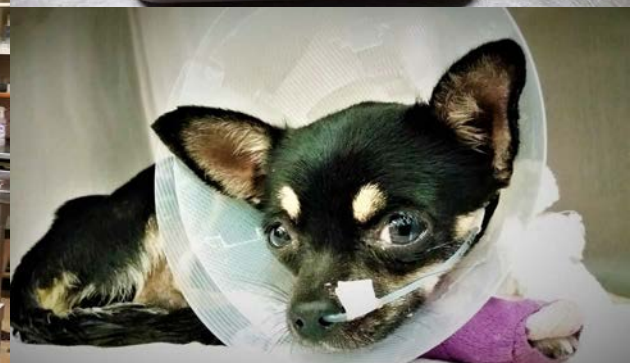
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- 4 Onderstepoort Veterinary Academic Hospital, University of Pretoria, Pretoria, South Africa

Clostridium difficile (CD) commonly causes hospital-acquired diarrhoea in humans and is associated with diarrhoea in dogs. Salmonellosis is a zoonotic disease with an unclear transmission pathway. It is thus important to evaluate risk factors that increase the likelihood of infection. Canine parvovirus (CPV) causes fatal canine enteritis, which is exacerbated by concurrent enteropathogenic infections. Persistent isolation of *Salmonella* spp. during hospital environmental surveys of the isolation ward prompted further investigation. This study aimed to determine the comparative prevalence of CD and *Salmonella* spp. in dogs with CPV and healthy dogs.

Faecal samples were collected from dogs aged 6 weeks to 9 months admitted with CPV infection, and from healthy dogs presented for routine hospital visits. CD was detected via commercial faecal antigen enzyme immunoassay. Faeces was submitted for isolation, antimicrobial susceptibility and serotyping of *Salmonella* spp.

Seventy-five dogs with CPV and 41 healthy dogs comprised the study. The prevalence of CD was 2.7% and 5% in CPV and healthy dogs, respectively, whereas the prevalence of *Salmonella* spp. was 21.3% and 32.5% in CPV and healthy dogs, respectively. No statistically significant associations between *Salmonella* infection status, possible risk factors and continuous variables were identified. Statistical analysis was not performed on CD positive cases due to limited number of cases. All *Salmonella* spp. isolates (n=32) were resistant to penicillin G, lincomycin and tylosin. Nine different serotypes of *Salmonella* spp. were identified.

In conclusion, the prevalence of *Salmonella* spp. in dogs with CPV was not statistically different from that in a healthy cohort. The prevalence in both groups was considerably higher than those previously reported (0 – 3.6%), yet similar to that reported for shelter dogs or dogs fed a raw diet (30 – 69%). This is the first report of the prevalence of CD and *Salmonella* spp. in dogs in South Africa.





Artificial insemination trial with frozen-thawed semen on exogenous hormone induced oestrus in African lion (*Panthera leo*)

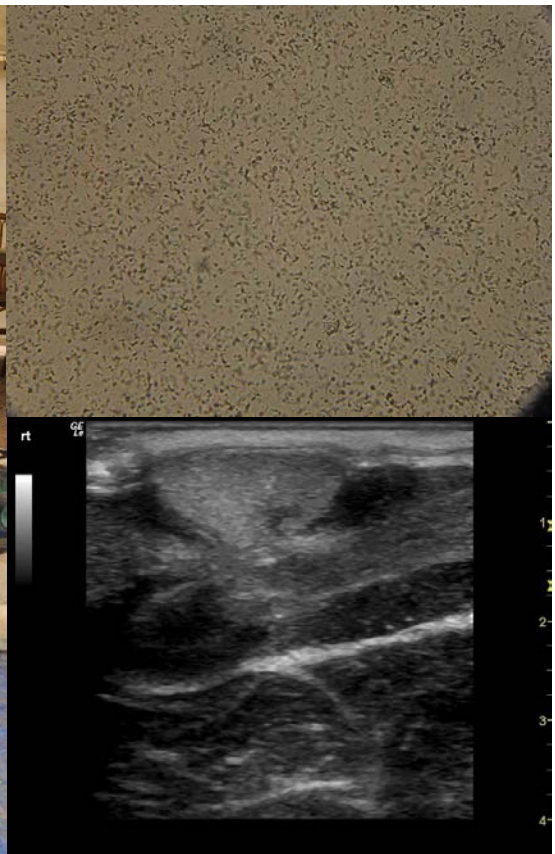
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- 5 Westfälischer Zoologischer Garten Münster GmbH, Sentruper Str. 315, 48161 Münster, Germany.
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- 7 Department of Genetics, University of the Free State, PO Box 339, Bloemfontein, South Africa.
- 8 Leibniz Institute for Zoo and Wildlife Research, Alfred-Kowalke-Straße 17, 10315 Berlin, Germany.

Assisted reproduction techniques (ART), such as artificial insemination (AI), have incredible potential in wildlife conservation, but are vastly unexplored. African lions represent an accessible model for the study of the reproductive biology of large, non-domestic felids, and the applicability of ARTs within their conservation programs. Here we describe an AI trial with frozen-thawed semen on exogenous hormone induced oestrus in two 5-year-old African lionesses. After regression of corpora lutea with PGF_{2α}, eCG was used to stimulate follicular growth, followed by induction of follicular maturation with eCG, hCG and/or GnRH analogues. Both females were inseminated transcervically with a urethral catheter (1.3 x 160 mm) and 1 ml of diluted semen with 40% post-thaw motility. One female received sperm obtained by electro-ejaculation, while the other received sperm obtained by a new collection method (urethral catheterization, UC). Mean sperm concentration

of semen obtained by this method was 1.94×10^9 /ml with a motility of $88.83 \pm 13.27\%$. After collection, aliquots of 300 mL containing 20×10^6 sperm were frozen in cryovials and in 0.5mL straws. Vital ($22.7 \pm 7.8\%$ for vial and $19.8 \pm 8.5\%$ for straw) and progressively motile ($10.0 \pm 7.9\%$ for vial and $10.0 \pm 6.4\%$ for straw) sperm after washing and 1 hour incubation at 38°C were of similar magnitude, velocity, and linearity for both packaging options. Ovulation was induced with a GnRH analogue after AI. Ultrasound scans and vaginal smears were performed on days 0, 7 and 9.

No lioness conceived following this procedure, however, the UC method for sperm collection, as well as the results of the successful ovarian stimulation protocol will be presented. There is a need to achieve a better understanding of the African lion's reproduction physiology and to develop an accurate non-surgical AI protocol during both natural and induced oestrus for this species.





Seroprevalence and factors associated with Rift Valley fever in domestic ruminants in the Free State and Northern Cape provinces

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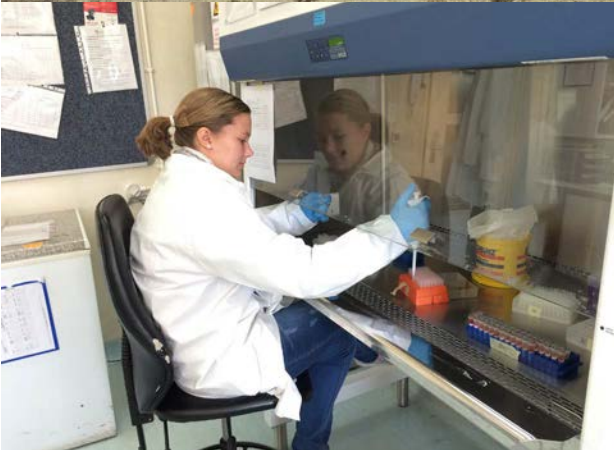
4 Centre for Emerging and Zoonotic Diseases, National Institute for Communicable Diseases, Sandringham, South Africa.

Rift Valley fever (RVF) is a mosquito-borne viral zoonosis largely confined to Africa but of major international interest due to its potential to spread to new regions. This study aimed to estimate the prevalence of antibodies against RVF virus (RVFV) in domestic ruminants in the central part of South Africa, near the 2010-2011 outbreak epicentre, and to identify factors associated with seropositivity.

A cross-sectional study was conducted during 2015-2016 within a ~40,000 km² region between Bloemfontein and Kimberley. Farms were selected using random geographic points with probability proportional to density of livestock-owning households. Livestock (cattle, sheep and goats) were sampled on the closest farm. A questionnaire was used to collect information on animal, management, and environmental factors. Sera were screened for RVFV antibodies using a RVF recombinant N Protein IgG indirect ELISA and positive tests were confirmed using RVF inhibition ELISA. Data were analyzed using multilevel logistic regression.

On 232 farms, 3,001 cattle (n=956), sheep (n=1,525) and goats (n=520) were sampled. RVF seroprevalence, adjusted for clustering and sampling weights, was 30.5% (95%CI: 24.6-37.0%) in cattle, 14.2% (95%CI: 9.7-20.3%) in sheep and 8.8% (95%CI: 4.1-18.1%) in goats. Compared to animals <2 years of age, seroprevalence was higher in animals 2-4 years (OR=2.1, *P*=0.017) and >4 years old (OR=19.7, *P*<0.001). Seroprevalence was also higher on private vs. communal land (OR=6.3, *P*=0.009), on farms that purchased animals in the previous year (OR=1.6, *P*=0.017), and in animals not kraaled at night (OR=2.6, *P*=0.010). Seropositivity was positively associated with the presence of perennial rivers (OR=2.2, *P*=0.004) and seasonal pans (OR=2.0, *P*=0.012).

The low seroprevalence likely indicates a largely susceptible population. Seropositivity in animals born after the most recent outbreak, as well as associations with known risk factors for RVF, raise the possibility that viral circulation occurred during the inter-epidemic period; this requires further investigation.





Seroprevalence and associated risk factors of West Nile virus in equine populations in South Africa

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West Nile virus (WNV) is the most prevalent mosquito-borne flavivirus species occurring in horses in South Africa. The virus is known to cause encephalomyelitis in infected horses. The risk factors associated with the spread and transmission of the virus have not been well described in South African horses.

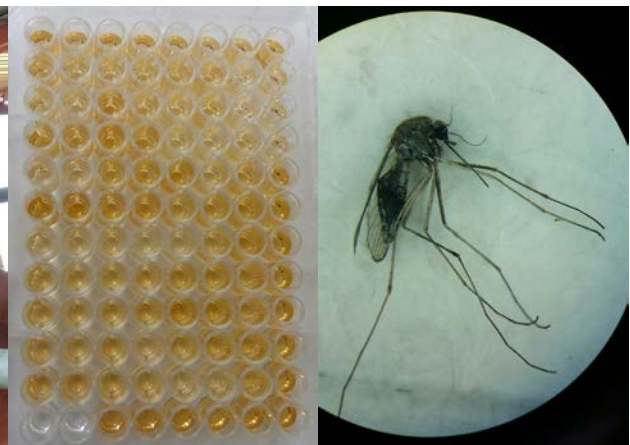
Blood samples were collected from 1219 horses from all nine provinces of South Africa and tested for antibodies specific to WNV by the serum neutralization test. A selected group of positive and negative samples was then tested with a capture IgG sandwich ELISA. The percentage of positive samples was highest in the Free State Province (78%), whilst the lowest was in North West Province (42%). There was a total of 57% (700/1220) seropositive samples, illustrating an above average seropositivity of individuals within the tested equine population.

Mosquitoes were caught using mosquito traps in two provinces: Mpumalanga and Gauteng. The pooled mosquito species were

tested for the presence of WNV using a SYBR green nested real-time reverse transcriptase-polymerase chain reaction.

All horse owners completed epidemiological based questionnaires, associated with infection and transmission of WNV. Multiple logistic regressions indicated significant associations between the various risk factors and seropositive horses. The significant risk factors associated with seropositive horses were agricultural activities of each region, contact with other horses, presence of standing water, relevant water sources, pest control methods, presence of rodents and whether or not the individuals were stabled. The main clinical symptoms significant for infection were fever and stiffness of the limbs and lower back.

There was a high seropositivity of WNV within equine populations, implying large exposure rates and the possibility that a high proportion of cases may be misdiagnosed as a result of asymptomatic presentation in infected individuals.





Evaluation of haemostatic changes in dogs with parvoviral enteritis before and after fluid resuscitation using thromboelastography

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Canine parvovirus (CPV) enteritis is associated with severe inflammation and sepsis, which can activate systemic haemostasis and cause hypercoagulability, previously described in nine dogs with CPV enteritis. The effect of crystalloid fluid resuscitation on haemostasis in CPV has not yet been investigated.

The aims were to prospectively a) confirm a hypercoagulable state in dogs with CPV (27) at admission, compared to healthy control dogs (15); b) assess changes in haemostatic status after crystalloid fluid resuscitation; and c) determine if haemostatic changes before and after fluid resuscitation are correlated to inflammatory markers and specific coagulation variables.

Blood samples were collected in all dogs at admission and immediately after fluid resuscitation. The volume of resuscitation fluid was recorded. Thromboelastography (TEG), antithrombin (AT) activity, fibrinogen concentration, haematocrit (HCT) and platelet count were measured.

For the TEG variables, the median maximum amplitude (MA) was significantly increased ($P<0.001$), reaction time (R) and clotting time (K) significantly longer ($P<0.05$) and the angle significantly smaller ($P<0.05$) in the CPV group compared to controls. Fibrinogen concentration was significantly increased in the CPV group compared to controls ($P<0.001$). There was a significant increase in MA, angle and platelet count and a significant decrease in R, K, HCT and AT after fluid resuscitation ($P<0.05$ for all). MA was moderately correlated to the platelet count at admission ($r_s=0.464$, $P<0.05$), as well as after fluid resuscitation ($r_s=0.448$, $P<0.05$) in the CPV group. R was moderately correlated with AT activity at admission ($r_s=0.405$, $P<0.05$). The decrease in HCT after fluid resuscitation correlated moderately ($r_s=0.431$, $P<0.05$) with the volume of fluid administered. The decrease in AT activity correlated strongly with the volume of fluid administered ($r_s=0.754$, $P<0.001$).

In conclusion, based on MA, CPV enteritis is associated with a hypercoagulable state at admission compared to healthy control dogs, that is exacerbated after crystalloid fluid resuscitation.





Leopards and land use: using glucocorticoid metabolites to measure stress

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Although protected areas are important for leopard conservation, the majority of suitable leopard habitats lie beyond protected area boundaries. Leopards utilizing areas under different anthropogenic influences may therefore be exposed to different environmental, physiological and psychosocial stressors. To date a test system has not yet been established to monitor stress in this elusive species nor have the behavioural and physiological responses to stress been examined in free-ranging leopards

The study aimed to examine the suitability of five different enzyme-immunoassays (EIA) for monitoring adrenocortical function in the leopard based on faecal glucocorticoid metabolite (fGCM) analysis. After performing an adrenocorticotrophic hormone (ACTH) stimulation test, examining gastrointestinal transit time under different feeding regimes and investigating the stability of fGCM post-defecation, faecal samples were collected from free-ranging leopards in a peri-urban and then a conservation area.

Our results indicate that a 5 α -pregnane-3 β ,11 β ,21-triol-20-one EIA is most suitable for assessing adrenocortical function in

male and female leopards with fGCM concentrations post-defecation remaining stable for up to 6 days. Using 100% increase as a minimum target, a 331% increase from baseline concentration (0.39 μ g/g DW) was measured in the male, and a 203% increase from baseline concentration (0.29 μ g/g DW) measured in the female. Although not statistically significant, gastrointestinal transit time (GIT) differed between males (31.2 hours) and females (40.8 hours) corresponding roughly with gut passage time. GIT appears to be dependant on food availability, quality and quantity.

Faecal GCM concentrations differed between free-ranging males (0.06 - 1.79 μ g/g DW) and females (0.04 - 6.09 μ g/g DW), with differences possibly linked to reproductive status in females.

The ability to reliably assess endocrine responses to recognised stressors in this iconic African species can help to address some of the local wildlife management, conservation and human-predator mitigation measures for free-ranging leopards under different land use practices.





Could diclofenac's toxicity in vultures have been predicted?

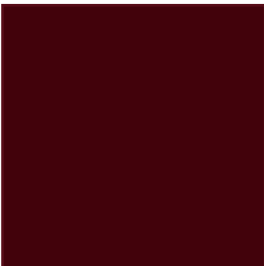
Hassan I.Z.¹, Duncan N.M.¹, Adawaren E.O.¹, Naidoo V.^{1,2}

1 Department of Paraclinical Sciences, Faculty of Veterinary Science, University of Pretoria, Onderstepoort, South Africa; e-mail: aburo001@gmail.com

2 University of Pretoria Biotechnology Research Centre

Diclofenac was responsible for the death of millions of *Gyps* vultures in the Indian sub-region with the safety of the other members of non-steroidal anti-inflammatory drugs (NSAID) being questionable. This has resulted in calls to test all the available NSAIDs for their vulture toxicity potential. Unfortunately due to the cost of testing, the time taking to establish toxicity reliably and the questionable ethics of repeat toxicity testing in an endangered species, an alternate method of testing is needed. For this study, we evaluate an OECD recommended method for determining the avian toxic potential of environmentally applied pesticides. We exposed young-adult quails (*Coturnix japonica*), ducks (*Cairina moschata*) and pigeons (*Columba livia domestica*) as per model requirements to diclofenac at various doses. This was coupled to the evaluation of the plasma toxicokinetics of the mentioned drug. Toxicity was noted in quails and ducks which appeared to be identical to clinical signs previously

reported in vultures, viz. depression and death within 48 h to 92 h of dosing; while the pigeons were insensitive. For birds that died, necropsy revealed signs of nephrosis with resultant urate deposits in kidney, spleen and liver, again as previously seen in the vulture. The toxicokinetic profile in quails showed that toxicity was related to metabolic capacity, with a $T_{1/2}$ and MRT above 6 h and 8 h, respectively, being associated with toxic signs. Despite evidence of toxicity, the estimated oral LD_{50} was very high at 405.42 mg/kg and 189.92 mg/kg in quails and ducks respectively. The latter was also substantially higher than the LD_{50} of 0.08 mg/kg extrapolated for *Gyps* vultures. We therefore conclude that these bird species are not suitable as surrogates for NSAID toxicity testing. More importantly, the results suggest that the toxicity of diclofenac in vulture is idiosyncratic and thus completely unpredictable using current pharmaceutical testing methodology prescribed by the OECD.





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Research Programme: Poster Presentations

Effect of usage of antibiotics on virulence profiles of *Escherichia coli* in pig production

*Abubakar R.H.*¹, *Madoroba E.*², *Fasina F.O.*¹

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Pathogenic *Escherichia coli* pathogens are responsible for acute profuse diarrhoea in growing pig with resultant high morbidity and mortality. Enterotoxigenic *E. coli* (ETEC) encoding STa, STb, EAST1 and LT enterotoxins and Shiga toxin *E. coli* (STEC) encoding Stx2e occur most commonly in pigs. This study investigated the prevalence of ETEC and VTEC virulence genes in two groups of growing pigs (five piglets per group) kept under routine farm management practices. One group was administered antibiotics and the other group received no antibiotics. A total of 241 *E.coli* strains were isolated in piglets from both groups between 0 and 70 days of age. Virulence genes were detected by PCR in 24.8% (18.2 - 32.7) of the antibiotic group isolates and 43.5% (34.5 - 52.9) of the

non-antibiotic group with a significant difference ($P = 0.002$). The proportions of the virulence genes STa, STb, EAST1 and Stx2e were 18.1% (8.61 - 34.39), 0% (0.0 - 10.43), 78.7% (62.25 - 89.32) and 3% (0.53 - 15.32) in the antibiotic group respectively, and 14.8% (7.40 - 27.68), 8.5% (3.36 - 19.93), 85.1% (72.32 - 92.59) and 12.7% (5.98 - 25.17) in the non-antibiotic group respectively. AIDA1 was the most dominant non-fimbrial adhesion factor while F6 was the only fimbrial factor detected. Twelve pathotypes were identified, with pathotype EAST1 being the most prevalent. The study showed that usage/non-usage of antibiotics in growing pigs does not prevent occurrence of disease causing virulence genes and other factors may be involved.

Exploring next generation sequencing on the ION S5 as a basis for investigating the potential role of the CYP 2 gene family in the metabolism of diclofenac in Cape vulture (*Gyps coprotheres*)

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Diclofenac, a non-steroidal anti-inflammatory drug (NSAID), is responsible for the death of millions of vultures (*Gyps* species) in the Asian subcontinent. Within the space of a decade about 96.7 to 99.9% of the *Gyps* species of vulture were eradicated by the consumption of diclofenac contaminated carcasses. Thus far researchers have managed to establish the pathognomonic signs of toxicity and the pharmacokinetic parameters of diclofenac in Cape vulture (*Gyps coprotheres*) and other related *Gyps* species. In addition, it has been postulated that toxicity of diclofenac to *Gyps* vultures may be due to saturation of the metabolic enzymes which is known as zero order kinetics as well as possible pharmacogenetic defects in one or more cytochrome P450 enzymes (CYP) genes, possibly CYP2C9 which has

been reported to be responsible for the metabolism of diclofenac in humans. Based on current literature on the topic there appears to be an important role for cytochrome P450 enzymes in this process. In light of this, the ION S5 platform was used for the next generation sequencing (NGS) and subsequent analysis of preliminary genome data for the Cape vulture as a starting point to identify and characterize the CYP family of genes in this organism with the view to use this as a platform for the design of transcriptome based assays of the involvement of these genes in NSAID metabolism. The initial data, which focused on identifying putative Cape vulture CYP genes, based on the reference genome of the turkey vulture (*Cathartes aura*), have yielded promising results which are currently further being explored.

PacBio Circular Consensus Sequencing uncovers the haemoparasite microbiome in South African wild and domestic felids

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In a preliminary study that amplified near full-length 16S or 18S rRNA genes, samples were sequenced using a circular consensus proofreading approach, opening a window to the microbiome in felids. Samples collected from lion, cheetah, African wild cat, caracal and tigers bred in captivity in South Africa were amplified and barcoded libraries were sequenced. Post binning, reads were converted into fasta files for analysis using a 16S rDNA database and RDP classifier. A custom database was established to analyse the 18S rRNA data. Altogether 21 data sets were obtained: nine from samples amplified with the 16S rDNA primer set and twelve from samples amplified with the 18S rDNA primer set. These data revealed a number of previously described haemoparasites in felids and a number of blood-borne bacteria and parasites

not reported previously in felids. We detected sequences with similarity to *Rickettsia* spp., *Babesia odocoilei*, *B. rodhaini* and *Hepatozoon* spp., but which could not be assigned definitively. In addition, sequences were detected that were assigned as *Hepatozoon felis*, *Anaplasma phagocytophilum* and *Babesia microti*. These latter three species have been reported in felids before, but little is known about the occurrence and pathogenicity of these parasites in domestic and wild felids. This is the first report on the felid bacterial and protozoan blood microbiome. These preliminary findings indicate which blood parasites can occur in felids in South Africa, and also that there are at least four (*Rickettsia*-like, *B. odocoilei*-like, *B. rodhaini*-like, and *Hepatozoon*-like) novel haemoparasites circulating in South African felids.

Methods for repeatable separation of the components of infertile and fertile Nile crocodile eggs for the determination of inorganic elemental constituents

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Nile crocodiles are apex predators in African aquatic environments, and are farmed commercially for their meat and skin. The crocodile farming

industry is annually worth approximately R500 million to the South African economy, a figure which is steadily increasing. Captive-produced crocodile eggs have a lower hatching rate than those from wild populations, which could reflect issues with husbandry, including sub-optimal feeding of parent stock.

Little is known of the normal amounts and distribution of macro- and microelements in the various compartments (shell, shell membrane, yolk and albumen) of the infertile egg. Nothing has been published on the amount of variance between and within clutches and the effect of time within laying season and egg size on the concentration and total amount of the various biologically important inorganic elements. Furthermore, the movement of biologically essential elements to the foetus from stores in the various compartments has not been well described for this species.

To determine the concentration of inorganic elements using sensitive spectroscopic techniques such as atomic absorption spectroscopy (AAS) or induction-coupled plasma optical emission spectroscopy (ICP-OES) necessarily requires that the compartments of each egg can be cleanly and reliably separated.

To this end, techniques and equipment were devised to open the eggshell, separate the yolk and albumen and freeze- and oven-dry the resulting components. A scoring system was developed to gauge the amount of contamination among compartments, which could aid in subsequent sample collection.

A total of 968 infertile and 34 fertile eggs were collected and processed during the 2016 laying season. This presentation demonstrates methodology used, highlights pitfalls and issues encountered, and suggests possible areas of improvement.

Cardiopulmonary effects of anaesthesia maintained by propofol infusion versus isoflurane inhalation in cheetah (*Acinonyx jubatus*)

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The continuing decline in wild populations of cheetah (*Acinonyx jubatus*) places emphasis on captive populations for species maintenance, thus highlighting a need for suitable field anaesthetic protocols. This study compared cardiopulmonary function in zoletil-medetomidine immobilised cheetah undergoing propofol total intravenous anaesthesia (TIVA) to those maintained with isoflurane to evaluate feasibility for a field anaesthetic protocol.

Twenty four captive adult cheetah were immobilised with zoletil (1.2 mg/kg) and medetomidine (40 µg/kg) delivered intramuscularly by darting. A maintenance protocol of either propofol TIVA (Group-P) or isoflurane inhalation anaesthesia (Group-I) was randomly assigned to each cheetah. Cardiopulmonary parameters were recorded at five minute intervals and three arterial blood gas samples were analysed. General anaesthesia was maintained for at least 60 minutes. Following maintenance, the medetomidine was antagonised with atipamezole (5:1) and recovery was observed.

Recumbency and lack of responsiveness to manipulations was maintained in all cases (end tidal isoflurane $1.1 \pm 0.1\%$,

propofol rate maintained at 0.1 mg/kg/min). Heart rates and respiratory rates were 82 ± 10 beats/minute and 14 ± 4 breaths/minute, respectively, with no significant differences between groups. End tidal carbon dioxide tension increased slowly for all cheetah but remained within acceptable limits (44.0 ± 5.0 mmHg) with no differences between groups.

All cheetah were initially markedly hypertensive (mean arterial pressure 163.3 ± 17 mmHg). Blood pressure normalised for cheetah in Group-I (125 ± 30 mmHg) but remained high throughout maintenance for Group-P (161.0 ± 17 mmHg) ($P < 0.001$). Arterial pH indicated an acidaemia (7.25 ± 0.09). Arterial carbon dioxide tension (48.9 ± 14.6 mmHg) did not differ significantly between groups at any point. The recovery time was 10.8 ± 5.0 and 51.9 ± 23.5 minutes for Group-I and Group-P, respectively.

Both isoflurane and propofol provided acceptable cardiopulmonary values throughout maintenance. Propofol may be an alternative to isoflurane for field use, but the long recovery time is concerning and requires further investigation.

A comparison between manual count, flow cytometry and qPCR as a means of determining *Babesia rossi* parasitaemia in naturally infected dogs

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Parasite quantification is crucial to understand disease pathogenesis. An automated method of determining parasite density would facilitate higher throughput and provide results that are more objective.

The study objectives included: a) validating the use of flow cytometry to detect and quantify *Babesia rossi* nucleic acid; b) comparing *B. rossi* parasite density in venous blood quantified by manual count, flow cytometry and quantitative real-time PCR (qPCR) in the same dog; and c) comparing the parasite density of *B. rossi* in capillary blood (quantified by manual count), with the parasite density in venous blood, as determined by manual count, flow cytometry and qPCR in the same dog.

Peripheral capillary and central venous blood was sampled from 40 naturally *B. rossi*-infected dogs and 10 healthy control dogs. Samples were analyzed by reverse line blot to confirm a mono-*B. rossi* infection. Capillary blood parasite density was

quantified using light microscopy (manual counts) and venous blood parasitaemia quantified using manual counts, flow cytometry and qPCR.

Flow cytometry, using SYBR Green I staining, showed promise in quantifying *B. rossi* nucleic acid in venous blood. Non-parametric methods were used for statistical analysis. Spearman's rho revealed a significant correlation between the venous manual counts and both flow cytometry ($r_s = 0.467$; $P = 0.001$) and qPCR ($r_s = -0.813$; $P < 0.001$), as well as a significant correlation between the capillary manual counts compared to venous manual counts ($r_s = 0.793$; $P < 0.001$), flow cytometry ($r_s = 0.400$; $P = 0.004$) and qPCR ($r_s = -0.760$; $P < 0.001$).

Preliminary results suggest that both flow cytometry and qPCR may be of value as an alternative to the gold standard (manual count) for quantifying *B. rossi* parasitaemia in canine whole blood.

Preliminary assessment of seven under-investigated South African plants from the Myrtaceae family for activity against *Bacillus anthracis*

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Anthrax, caused by *Bacillus anthracis* infection, is a severe acute disease. Apart from its zoonotic importance and potential as a bioterrorism agent, the economic losses associated with morbidity and mortality of animals infected with *B. anthracis* makes the control of the disease a high priority globally. Against the backdrop of antimicrobial resistance, there is motivation to develop new anti-*B. anthracis* products especially from natural sources to provide alternative or complementary remedies. Recent empirical evidences show that plants hold potential for anthrax control. Few South African plants have been tested for their efficacies against *B. anthracis*. This preliminary study reports the growth inhibitory activity of acetone leaf extracts of plants from the myrtaceae family against *B. anthracis* Sterne vaccine strain. A two-fold serial microdilution assay was used to determine the minimum inhibitory concentration (MIC) of the extracts against *B. anthracis*. Anti-oxidant activities of the extracts of the plants were determined using the 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging

method. The total antibacterial activities (yield/MIC) were obtained. The MIC of the plant extracts ranged from 39 to 156 µg/ml. Excellent MIC values were observed for the following plants: *Syzygium legatii* (39 µg/ml), *Eugenia natalitia*, *Syzygium masukuense* subsp. *masukusense* (78 µg/ml) and *Eugenia erythrophylla* (150 µg/ml) compared to gentamicin (3.9 µg/ml). The total antibacterial activity (the maximum volume to which 1 g of plant's extract can be diluted and still inhibit microbial growth) of the extracts ranged from 242 to 2368 ml/g. *Eugenia erythrophylla* and *Syzygium legatii* had the best total activity (2368 and 2364 ml/g). *Syzygium legatii* had the best antiradical activity (mean IC₅₀=8 µg/ml), compared to Vitamin C (IC₅₀=1 µg/ml). The crude acetone extracts of the selected plant species, especially *Syzygium legatii*, have promising anti-*B. anthracis* activity and good antiradical potential. Further research is on-going to determine the safety of extracts, and isolate the compounds responsible for anti-*B. anthracis* activity.

Evaluation of several tree species for activity against salmonella

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Handling and consumption of contaminated foods (meat, milk and eggs) are considered a major source of infection in humans. This public health risk reinforces the need for close monitoring and collaboration between public health and veterinary authorities to mitigate the risk at the human-animal interface necessary for health and food security. Gastroenteritis that is not self-limiting is mostly treated with a wide range of antibiotics. The indiscriminate use of these antibiotics has resulted in the upsurge of resistant and multi-resistant strains of bacteria. This complicates treatment, especially in patients with human immunodeficiency virus (HIV) infection, necessitating the search for novel, cheaper, safer and efficacious antibacterial products. Recent *in vitro* studies have revealed that indigenous South African plants possess antimicrobial properties against gastrointestinal disorders and diarrhoea-causing organisms.

In a preliminary screening, the antibacterial activities of acetone, ethanol, methanol and water extracts of

the leaves were determined using a two-fold serial microdilution method against a range of pathogenic bacteria (*Staphylococcus aureus*, *Enterococcus faecalis*, *Bacillus cereus*, *Escherichia coli*, *Salmonella Typhimurium* and *Pseudomonas aeruginosa*). The plant species investigated were *Protosia longifolia*, *Searsia leptodictya*, *Carissa macrocarpa*, *Combretum bracteosum*, *Kirkia wilmsii*, *Loxostylis alata*, *Brachychiton acerifolium*, *Brachychiton bidwillii*, *Noltea africana* and *Blighia unijugata*. All the extracts had activity against at least one of the test organisms over an incubation period of 24 hours. The average MIC values of the plant extracts against the different bacteria ranged from 0.2 mg/ml to 1.4 mg/ml. The Gram-positive bacteria (*S. aureus*, *B. cereus* and *E. faecalis*) were more susceptible to the plant extracts than the Gram-negative bacteria (*E. coli*, *S. Typhimurium* and *P. aeruginosa*). *P. longifolia* and *L. alata* extracts were the most active against nearly all the bacteria tested with MIC values as low as 0.02 mg/ml. *L. alata* was selected for further work to isolate compounds active against *Salmonella* species.

Shotgun genome sequence and population diversity of *Mannheimia haemolytica* isolates from sheep in South Africa

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Respiratory disease caused by *Mannheimia haemolytica* is a major economic and welfare concern in the cattle and small stock industry worldwide. Disease occurs due to the interaction of numerous factors, including weaning stress, shipment, inclement weather, and overcrowding coupled with viral and bacterial infections. The whole genome of *M. haemolytica* strain Mh10517 was analyzed using an Illumina MiSeq high throughput sequencing platform. The genome size is 2.67 Mb with 2,879 predicted gene sequences.

The molecular evolution and relatedness of *M. haemolytica* was investigated using nucleotide sequence data of seven housekeeping gene fragments from 21 ovine isolates. MEGA version 7.0 genomic workbench was used for alignment and analysis of the nucleotide data sets. For each gene fragment, the sequences were compared and isolates with identical sequences were assigned the same allele number. Results

suggested that the 21 isolates belonged to six sequence types (ST) and ST 28 accounted for 33% of the isolates. Neighbour joining method was used to produce dendrograms based on the concatenated sequences of the seven loci in multilocus allelic profile. There was significant variation between the number of synonymous and non-synonymous substitutions between each sequence pairs ($p=0.018$) based on results from the Fisher's exact test of neutrality of sequence pairs.

These preliminary data show substantial sequence variations and this supports the hypothesis that ovine isolates of *M. haemolytica* are more diverse than what has been reported for isolates from other species. These results will advance studies on various aspects of the biology of *M. haemolytica* in Africa, and the world at large.

The pathology of the spleen in canine *Babesia rossi* infection

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The spleen reacts immunologically in response to blood-borne antigens. Human malaria and canine babesiosis are both vector-borne protozoal diseases and are of importance in human and veterinary medicine, respectively. These two diseases display many similarities and have therefore been compared to one another for many decades.

Malaria studies that have evaluated the histopathology of the spleen, primarily in murine models and humans, have shown a striking loss in delineation between the white and red pulp areas. Also, there is a significant increase in plasma cell as well as macrophage densities. The intermingling of the white and red pulp is the result of leukocyte redistribution. Currently there is a paucity of information describing the pathology of canine babesiosis.

Nine *Babesia rossi*-infected dogs and four healthy control dogs were included in this study. The histomorphology of the spleens from the babesia-infected dogs revealed a diffuse blurring of the white and the red pulp as well as an increase in resident and bone marrow derived macrophage densities. Although plasma cell numbers were not significantly increased in the dogs with babesiosis, they were diffusely redistributed within the red pulp.

The splenic histopathology in dogs with babesiosis is very similar to what has been described in the spleen of humans and mice with malaria. Future studies should endeavour not only to evaluate a greater number of spleens from infected animals but more particularly spleens from healthy animals. A flow cytometric assessment of immunocyte numbers/proportions is also required. This is due to the inherent variability in the histomorphology of the normal spleen.

The effect of preconditioning on the health and production of calves in a South African feedlot

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The pressure is on production veterinarians to reduce the use of antibiotics in intensive beef production systems. This study investigated whether preconditioning, the process whereby weaned calves destined for the feedlot are prepared over a period of time, improved health and production of calves in a South African feedlot.

Preconditioned calves (n=301) and control calves (n=332) were sourced from the same origin on two occasions, and arrived at the feedlot on the same day. Bovine respiratory disease (BRD) was defined as the "pulling" of clinically sick calves from feedlot pens, followed by the standard protocol for treatment of BRD (including antibiotic treatment). Outcome variables related to health were BRD overall incidence, 45 day BRD incidence, BRD re-pulling, BRD mortality and lung lesion scores. Production outcome variables measured were carcass weight, carcass average daily gain (ADG) and days on feed (DoF).

Initial carcass weight was estimated from live weight in order to estimate the effect of preconditioning on carcass gain, the most economically relevant outcome. Statistical analyses were done using multiple linear regression. Predictor variables were preconditioning vs control, gender, starting weight and DoF.

A lower proportion of preconditioned calves were pulled and re-pulled for BRD compared to control calves (8% vs 17% and 8% vs 16%, respectively, $P < 0.01$). A higher proportion of preconditioned calves compared to control calves were market ready at 90 DoF (89% vs 67%, $P < 0.01$). In the multivariable models preconditioning was associated with a 197 g/d increase in carcass growth rate ($P < 0.01$) and with a 17.7 kg increase in overall carcass gain ($P < 0.01$) after adjusting for DoF.

It is concluded that a positive impact of preconditioning can reduce the use of antibiotics, reduce feedlot standing time and improve production of calves in South African feedlots.

Towards a yellow tulp vaccine: preliminary studies exploiting the potential for cross-reactivity with related bufadienolides

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Poisoning by *Moraea pallida* Bak. (yellow tulp) is the most important of all cardiac glycoside induced toxicoses which collectively account for 33% and 10% of deaths in large and small stock due to plant poisoning, respectively, in the Republic of South Africa. This study was conducted to investigate the potential for developing a vaccine against epoxyscillirosidine, the toxin contained by yellow tulp. Epoxyscillirosidine was extracted, isolated, purified and confirmed using ¹³C NMR spectroscopy. Bufalin and proscillaridine were purchased and together with epoxyscillirosidine were coupled to BSA and KLH to render them immunogenic. The immunogens (4 mg/ml) were emulsified with an equal volume of Montanide ISA, as an adjuvant. Adult male New Zealand White rabbits (n=15) were randomly assigned to 5 equal groups. Rabbits in groups I, II, III and IV were immunized

with proscillaridine-BSA, bufalin-BSA, epoxyscillirosidine-KLH and epoxyscillirosidine-BSA conjugates, respectively. Group V served as control where animals were administered BSA only. The rabbits were immunized on Days 0, 21 and 42 by intradermal injection of 0.1ml of the vaccine at four sites on the dorsum. Blood was collected prior to each vaccination and on Day 67. An ELISA was performed to determine antibody response. Antibodies were raised against proscillaridine, bufalin and epoxyscillirosidine. Furthermore, the antibodies synthesized by Group I and II rabbits also cross-reacted with epoxyscillirosidine. However, the degree of cross-reactivity was low. This may be enhanced by optimizing the vaccine to induce stronger antibody response. The antibodies will be evaluated to determine the neutralization efficacy against epoxyscillirosidine.

Gross morphology and basic histological structure of the *Apparatus lacrimalis* of the ostrich (*Struthio camelus*) and emu (*Dromaius novaehollandiae*)

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The avian eye is uniquely adapted and reflects structural peculiarities which protect the eye against environmental and microbial insult. The avian lacrimal apparatus consists of Harderian and lacrimal glands. Ostriches and emus are commercially important and a comprehensive description of the *Apparatus lacrimalis* would be invaluable in ophthalmological studies.

Sub-adult ostrich and emu heads were collected from a commercial abattoir and immersion fixed in 10% neutral-buffered formalin. After enucleation, the lacrimal apparatus was isolated and gross morphological features described before preparing the material for histological examination.

The lacrimal gland was positioned at the lateral canthus, on the anterior sclera and the Harderian gland in the ventro-medial orbit. Both glands consisted of a body, neck and main duct. Harderian and lacrimal gland secretions emptied via a single duct, at the inner margin of the nictitating membrane and lower eyelid, respectively. Both glands were compound

tubular in nature and the parenchyma was subdivided into lobes by connective tissue septae extending from the gland capsule. Each lobe consisted of lobules, each of which effectively represented a simple branched tubular gland that opened, via a tertiary duct, into one of the secondary branches of the main duct. Lymphoid tissue, fibroblasts, blood vessels and myoepithelial cells were evident in the glandular interstitium. The Harderian gland of both ostrich and emu seems to have a greater infiltration of lymphoid cells compared to the lacrimal gland.

The gross morphological and histological structure of the lacrimal apparatus in the ostrich and emu follow the basic avian pattern. The observation that the lacrimal apparatus, particularly the Harderian gland, due to its content of lymphoid tissue, plays an important role in local ocular immunity was confirmed in the present study. The minor species variations in glandular morphology should be considered during diagnostic or surgical procedures in the ostrich and emu.

Genetic variability of *Anaplasma phagocytophilum* strains circulating in wild rodents in Bushbuckridge, Mpumalanga, South Africa

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Anaplasma phagocytophilum is a zoonotic, tick-borne, obligate intracellular bacterium capable of causing disease in diverse hosts, including humans, dogs, cattle and horses. It has not been detected often in Africa, but recent research suggests its presence in the Mnisi community, a rural community located in the heart of a human/livestock/wildlife interface in Bushbuckridge Municipality, Mpumalanga Province, South Africa.

It has been documented that wild rodents are reservoir hosts for many tick-borne pathogens; however, it is not known if wild rodents play a role in the transmission of tick-borne zoonoses in the community. Recent research in the area indicated 76% of households sampled reported seeing rodents in and around their homes, hence the aim of this study was to explore the genetic diversity

of *A. phagocytophilum* in wild rodents in order to better understand its circulation in the study community. To achieve this, DNA extracted from blood samples from 282 wild rodents collected from five different habitat areas in Bushbuckridge, were screened for *A. phagocytophilum* using a quantitative real-time polymerase chain reaction (qPCR) assay that targets the *msp2* gene. Results revealed that 59% of wild rodents sampled were positive for *A. phagocytophilum*. Characterization of different strains by targeted sequencing of the 16S rRNA, and *msp2* genes from positive samples revealed the presence of different variants of *A. phagocytophilum* circulating within the community. This is the first detailed report of *A. phagocytophilum* in wild rodents in South Africa and highlights its possible cause of acute febrile illness in the country.

Prevalence of brucellosis in slaughter animals in Gauteng province abattoirs, South Africa: food safety implications

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Brucellosis is a neglected zoonotic disease: a highly infectious and contagious disease of humans and a wide range of domestic animals, especially ruminants. To date, there have been limited empirical data or published reports on the prevalence of brucellosis in the livestock industry in South Africa. Globally, abattoirs are used for passive and active surveillance of diseases of both economic and public health significance.

The aims of this study were to determine the prevalence of *Brucella* spp. in slaughter livestock in abattoirs in Gauteng Province, to assess risk factors that predispose abattoir workers to zoonotic infections, and to use a 'One Health' multidisciplinary approach to educate the abattoir workers on how to reduce the risk of contracting zoonoses while working at the abattoir.

The Rose Bengal test (RBT) and indirect ELISA (iELISA) were used to determine the prevalence of brucellosis antibodies in

serum samples, and PCR was used to detect *Brucella* DNA in the lymphoid tissue of seropositive animals. Eleven consenting abattoirs were visited and 174 cattle sampled, comprising 81 females and 93 males. The seroprevalence of brucellosis was 12.1% (21/174) and 5.2% (9/174) by the RBT and iELISA respectively, with 43% (9/21) agreement in seropositive animals between both tests. PCR detected *Brucella* DNA in the lymphoid tissues of 6 (67%) of 9 iELISA-seropositive cattle.

Brucella-positive cattle were found at all of the 11 abattoirs sampled, indicating a zoonotic risk at all the facilities. Of the 143 abattoir workers interviewed, 78.3% were males and 21.7% were females. Thirty-seven (37.1%) believed that they could not contract zoonoses from working in the abattoir. However, 83.9% of all workers interviewed had cut their hands at least once while performing their duties at the abattoirs, and 88.1% of the workers that experienced sickness had not sought medical attention.

Effects of chemical and mechanical stimulation on laryngeal motion during anaesthetic induction with alfaxalone, thiopentone or propofol in healthy dogs

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Anaesthetic drugs commonly used for laryngeal examination have been reported to alter the intrinsic laryngeal function. The challenge arises to accurately diagnose laryngeal paralysis and note subtle changes in laryngeal function that could be masked. Either mechanical stimulation of the arytenoids or administration of a respiratory chemical stimulant can be proposed as a corrective measure. This study compared the effects of chemical and mechanical stimulation on arytenoid cartilage motion in healthy dogs using three different anaesthetic induction drugs: alfaxalone, thiopentone and propofol, in attempt to minimise all factors contributing to an inaccurate diagnosis. Eight 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 7.5 mg/kg, 3 mg/kg and 1.5 mg/kg, over one minute, for anaesthetic induction, respectively. If deemed inadequately anaesthetised, then top-up boluses of 1.8 mg/kg, 0.75 mg/kg and 0.4 mg/kg were administered,

respectively. Continual examination of the larynx commenced once an adequate anaesthetic depth was reached until recovery from anaesthesia. Both chemical and mechanical stimulation were randomly performed at a fixed time period during examination. The chemical stimulant doxapram HCl was administered intravenously at 2.5 mg/kg. Mechanical stimulation was administered to the right *cricoarytenoid dorsalis* process. The number of arytenoid motions and vital breaths were counted during three time periods and compared over time and among treatments. The data was analysed on R Statistical Software using non-parametric tests. A significant difference was observed in the examination time among treatments ($P=0.001$). No significant difference was observed among treatments for number of vital breaths recorded. We concluded that chemical stimulation, combined with the three anaesthetic induction agents, displayed significantly higher arytenoid motions, shorter examination times and faster recoveries in healthy dogs that may be advantageous in the accurate diagnosis of laryngeal paralysis.

Phylogenetic analysis of 2015–2016 Nigerian highly pathogenic avian influenza (HPAI) H5N1 viruses

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Highly pathogenic avian influenza (HPAI), characterized by sudden onset and excessively high mortality, was first reported in Nigeria in 2006 and again in 2015 to date with devastating economic consequences. The complete genomes of 100 isolates collected between 2015 and 2016, covering 16 states and the Federal Capital Territory, were analysed to provide a better understanding of the genetic characteristic and evolution of the current H5N1 viruses circulating in Nigeria. The complete genomes of 100 H5N1 viruses collected from January 2015 to June 2016 in Nigerian poultry were sequenced and submitted to the GenBank database. The deduced amino acid sequence (PQRERRRKR*GLF) at the HA cleavage sites is characteristic of an HPAI

virus strain, but possesses an amino acid deletion at position 345 compared to clade 2.2 viruses previously reported in Nigeria. The topology of the phylogenetic tree of the HA gene demonstrated that all the viruses analyzed fall within clade 2.3.2.1c and cluster with H5N1 viruses identified since 2014 in Asia, the Middle East, East Europe and other West African countries including Niger, Ghana, Burkina Faso and Ivory Coast. Specific amino acid signatures were also considered in defining sub-groups observed within this clade. The Nigerian viruses collected in 2015 are dispersed throughout the tree, indicating the possible occurrence of multiple independent introductions or the evolution of the virus into multiple genetic groups.

Effect of climatological factors on the bulk milk somatic cell count on South African dairies

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A summer rise in somatic cell count (SCC) is a phenomenon seen in cows, goats and sheep globally. This study aimed to determine the attributable effect of climatological factors on bulk milk somatic cell count (BMSCC) on South African dairy farms.

Individual cow production data from more than 60 000 cows, from January 2013 to July 2015, were accessed from a milk recording scheme (Logix, Studbook SA). Using current and prior SCC recordings, cows with a $SCC > 200 \times 10^3$ were classified as High (H) and cows with a $SCC \leq 200 \times 10^3$ cells/ml as Low (L), allowing for the transition categories HH, HL, LH and LL. Weather data were obtained from the South African National Weather Service (WeatherSA) allowing calculation of the temperature humidity index (THI). The confounders parity, breed, days in milk (DIM) and region were adjusted for. The contribution from each transitional category to the bulk tank was calculated and the change in BMSCC between winter/summer and low/high THI conditions determined.

The geometric mean SCC varied from 163×10^3 to $2,132 \times 10^3$ cells/ml. The geometric mean herd milk production ranged from 11.3 to 42.0 L/cow/day. Individual cow milk production varied between 0.3 to 94.3 L/cow/d. Maximum THI ranged from 58 to 81. HH cows showed annual fluctuation while LL cows per herd ranged from fewer than 10% to upwards of 70%. The proportion of each transitional category changed between summer and winter and across THI categories. The total contribution of cells from each group to the bulk tank showed that cells from the LL cows decrease while the cells from the HH cows increase as THI increases.

Cows with healthy udders (LL) are less affected whereas cows with chronic mastitis (HH) tend to show a marked increase in SCC as THI increases. These cows should be the focus when addressing a summer BMSCC rise.

Dietary supplementation of conjugated linoleic acids on sperm quality and freezability in bovines

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Feeding rumen-protected isomers of conjugated linoleic acid (CLA) to fresh dairy cows improves fertility by reducing the postpartum interval to first ovulation and enhancing blood IGF-I levels. To our knowledge, there are no studies on the effect of CLA dietary supplementation on semen quality and few on the effect of CLA addition to semen extender during freezing. The objective of the study was to evaluate the effect of dietary CLA supplementation and of CLA addition to semen extender on semen quality and freezability in bovines.

14 bulls blocked by breed, age, BW and BCS were randomly assigned to 2 groups: control (CTL) and CLA (50 g/day). CLA was supplemented for 10 weeks and samples of blood, seminal plasma and ejaculate were collected twice a week on -2 and -1 weeks (before supplementation), 4 and 5 weeks (during supplementation), and 11 and 12 weeks (after supplementation). Blood and seminal plasma were analyzed for IGF-I; ejaculate from each bull was frozen in 6 subgroups to which CLA isomers were added to the semen extender as follows: CTL (no addition), CLA_{9,11} 50 µM, CLA_{9,11} 100 µM, CLA_{10,12} 50 µM, CLA_{10,12} 100 µM, CLA mix. The sperm was analyzed for %motility, %progressive, average pathway velocity (VAP), straight-line velocity (VSL), curvilinear

velocity (VCL), beat cross frequency (BCF), straightness (STR), Lateral-displacement amplitude (ALH), using the CASA system; morphology through eosin-nigrosine staining; viability, mitochondrial activity and oxidative stress using the flow cytometer with live/dead viability kit, mitoprobe JC-1, 2',7'-dichlorodihydrofluorescein diacetate and TO-PRO-3 dyes. Data were analyzed with ANOVA for repeated measures using PROC MIXED (SAS 9.3; IGF-I) and nested variable effect (morphology, CASA and flow cytometry). Week -2 was used as co-variate.

Preliminary results show that dietary CLA supplementation decreased the total volume of the ejaculated ($P<0.05$), increased sperm concentration ($P<0.1$) and increased plasma and seminal plasma IGF-I levels ($P<0.001$) compared to the CTL. Sperm from CLA bulls had increased VAP, VSL, ALH and VCL and decreased BCF and STR ($P<0.05$). A numerical decrease in production of reactive oxygen species was also observed for the CLA group compared to the control. The results support the hypothesis of dietary CLA supplementation improving semen quality and bull performance. Investigation on the effect of CLA addition into the semen extender and its interaction with CLA dietary supplementation is ongoing.

Herd-level prevalence of bovine leukaemia virus infection and associated risk factors in commercial dairies in five provinces of South Africa

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The immunosuppressive nature of bovine leukaemia virus (BLV) infection is well documented and associated production losses make it one of the most important diseases of cattle, particularly in dairy cows. Any management interventions should be informed by an understanding of the herd-level and within-herd infection prevalence and of risk factors for spread of infection. Costs associated with blood tests have been a prohibitive factor in routine monitoring in domestic herds.

A regional cross-sectional study was undertaken to estimate herd-level prevalence of BLV infection in commercial dairy herds and identify associated risk factors. Bulk tank milk (BTM) from 185 dairy herds randomly selected from Limpopo, North West, Mpumalanga, Gauteng and Free State provinces was tested for BLV infection using an indirect enzyme-linked immunosorbent assay (ELISA). A questionnaire was used to collect data regarding management practices and herd-specific factors. Logistic regression was used to assess factors associated with herd seropositivity. Multiple linear regression

was used to identify factors associated with the quantitative outcome of the BTM ELISA (E_{quant}), as a proxy for within-herd prevalence of BLV infection.

Estimated herd-level prevalence was 96% (149/155; 95%CI 92-99%); it differed between provinces ($P=0.001$): 77% (17/22) in Mpumalanga, 97% (34/35) in Gauteng and 100% in North West ($n=65$), Free State ($n=26$) and Limpopo ($n=3$). Questionnaire data were obtained from 125 herds. No factors were significantly associated with herd BLV infection, likely due to the small number of negative herds. Use of communal calving camps, reuse of rectal gloves without disinfection and use of artificial insemination in cows were associated with higher E_{quant} . Regular treatment against flies was associated with lower E_{quant} .

Infection with BLV is widespread amongst commercial dairy herds in the northern part of South Africa. Various aspects of herd management have been identified where interventions can be made to control of the disease.

Prevalence of *Salmonella* spp. in slaughter cattle, the abattoir environment and meat sold at retail outlets in Gauteng province

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Salmonellosis is an important foodborne disease associated with serious public health and food safety problems. There is a scarcity of comprehensive recent information regarding the prevalence of *Salmonella* spp. in the beef production value chain in South Africa. The aim of this study was to determine the prevalence of *Salmonella* spp. in abattoirs and in beef and beef products sold at retail outlets in Gauteng province, South Africa.

In a cross-sectional study, 517 samples of various types (meat, swabs, water) were collected seasonally from a random selection of 12 abattoirs (n=252) and 31 retail outlets (n=401) between November 2015 and November 2016. The isolation and identification of *Salmonella* spp. were performed using standard microbiological techniques. The prevalence of *Salmonella* spp. was 9.9% (25/252; 95%CI 6.5-14.3%) in abattoir samples and 12.7% (51/401; 95% CI:

9.6-16.4%) in retail outlet samples. In abattoir samples, the frequency of isolation of *Salmonella* spp. was 44% for effluents, 27% for walls and floors, 10% for faeces, 13% for perineal swabs, 12% for carcass rinsates and 10% for carcass swabs. For meat samples collected from retail outlets, frequency of isolation of *Salmonella* spp. varied between sample types and was highest in minced meat (16%) and lowest in biltong (8%), although differences were not significant ($P=0.4$). There was evidence of seasonal variation, with highest prevalence in autumn (18%) and lowest in summer (10%) ($P=0.07$).

These results indicate the extent of contamination by *Salmonella* spp. in the abattoirs and retail outlets sampled in the study. This highlights the potential risk of salmonellosis posed to consumers by contaminated, improperly cooked meat sold at retail outlets in Gauteng.

Magnetic resonance imaging of the muscle tendons in the carpal region of the southern white rhino (*Ceratotherium simum simum*)

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The southern white rhino (SWR) may experience injury to the carpus resulting from bullets and snares used in poaching. Knowledge of the myology of this region is important to aid in diagnostics and surgical approaches.

Six thoracic limbs from succumbed SWR were submitted for magnetic resonance imaging (MRI) using a 1.5 Tesla MAGNETOM Symphony and Total imaging matrix (Tim; Siemens Healthcare). One limb was transversely sectioned with a band-saw at 6-8 cm intervals to correlate to the MRI.

Both methods demonstrated that the muscles for carpal and digital flexion and extension become tendinous proximal to the antebrachiocarpal joint. The five extensor tendons are cranially positioned and lie superficially under the skin and fascia, whereas the 3 flexor tendons are located caudally, with 2 of them running deep in the carpal canal. The largest

and most centrally located tendon is the *M. flexor digitalis communis* (CDF). In the carpal canal it is bordered laterally by the *Os carpi accessorium*, caudally by the *Retinaculum flexorum* and medially by the small *M. flexor carpi radialis*. The CDF has a well-developed tendon sheath. The *M. flexor carpi ulnaris* lacks a tendon sheath. The tendons of the remaining muscles possess sheaths of differing lengths. The *M. extensor carpi radialis* and the *M. abductor digiti I longus* share a tendon sheath for a small portion of their length.

The superficial and deep digital flexor muscles appear to be present as the CDF in the SWR. This has also been reported in the Asian elephant and may be a feature of some graviportal species. The topography of the muscle tendons in the SWR is similar to the equine, except for the CDF. The size of the CDF makes it an ideal landmark on MRI and the large tendon sheath is a consideration for surgery in the region.

Investigation of antimicrobial, antioxidant, and anti-inflammatory activities of two *Newtonia* spp. with potential for alleviating infectious diarrhoea symptoms

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Diarrhoea, a neglected disease, causes high mortality and morbidity especially in children and immunocompromised patients. The antimicrobial activity of *Newtonia hildebrandtii* and *N. buchananii* extracts was determined against three Gram-positive bacteria (*Staphylococcus aureus*, *Bacillus cereus*, *Enterococcus faecalis*), three Gram-negative bacteria (*Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella typhimurium*) and two fungi (*Candida albicans*, *Cryptococcus neoformans*) known to be implicated in causing diarrhoea and related gastrointestinal infections. *Newtonia buchananii* had strong antimicrobial effect against *P. aeruginosa* with minimum inhibitory concentration (MIC) of 20 µg/ml and moderate activity of 40 µg/ml against *B. cereus*. The cytotoxicity of these plant species was evaluated against Vero cells using the MTT assay. Both plants were relatively non-toxic with IC₅₀ values of 30-750 µg/ml. Selectivity index values as high as 18.75 were reached with the methanol-dichloromethane leaf extract of *N. buchananii*. The anti-inflammatory activity of extracts was determined using the nitric oxide (NO) inhibition assay in

lipopolysaccharide (LPS) activated RAW 264.7 macrophages and on the enzyme 15-lipoxygenase (15-LOX). All the extracts tested inhibited NO production in a concentration-dependent manner. The *N. hildebrandtii* methanol-DCM (1:1) leaf extract had the highest NO inhibitory activity with IC₅₀ of 76.82 µg/ml. The *N. buchananii* acetone leaf extract and the *N. buchananii* methanol-DCM leaf extract had the highest lipoxygenase potential with IC₅₀ values of 13.85 and 14.82 µg/ml respectively. Antioxidant activity was determined using DPPH and ABTS radical scavenging assays. All extracts from the two *Newtonia* species exhibited good antioxidant activity with IC₅₀ comparable to the standard compounds (trolox and ascorbic acid). Due to the highest antimicrobial effect of *N. buchananii* extracts, the active extract was subjected to bioassay-guided isolation of compounds with chemical structures being identified through NMR spectral analysis. The identification and bioassays of the isolated compounds from *N. buchananii* and the isolation of active compounds from *N. hildebrandtii* are ongoing.

A comparative study of the infundibular epithelium in laying and moulting commercial hens

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The infundibular region of the oviduct has several functions including: engulfing the ovulated oocyte; providing a site for fertilization and forming the chalaziferous components of the developing egg. At approximately 18 months of age, laying hens enter the moulting phase of the reproductive cycle, during which egg production decreases or ceases due to oviductal regression. Although extensive research has been conducted on the effects of moulting on the physiological condition of hens, relatively little is known about the histomorphological changes which occur in the oviduct during this process. The present study was undertaken to compare the histomorphology of the infundibular epithelium in laying and moulting commercial hens.

A total of 10 laying (32 weeks old) and 10 moulting (75 weeks old) commercial hens were used in the present study. Tissue samples from the infundibulum were fixed in buffered neutral formalin for 5 days. The samples were later processed routinely

for light microscopy and stained with haematoxylin and eosin, as well as Periodic acid Schiff-Alcian blue (PAS-AB).

The infundibulum was sub-divided into proximal funnel and distal tubular regions. In laying hens the funnel region was lined by a ciliated simple columnar epithelium. In moulting hens this region displayed localized areas of swollen, non-ciliated cells with pale-staining cytoplasm and nuclei. The tubular infundibular region in laying hens was lined by a pseudostratified columnar epithelium composed of ciliated and non-ciliated cells. The latter cells stained positive for PAS indicating the presence of neutral mucins. The epithelium in the tubular region of moulting birds contained cells at different stages of degeneration. Alcian blue, as well as PAS- AB cells were observed, indicating the presence of acidic and mixed mucins. The results of the study indicate that the infundibular epithelium differs histologically and physiologically during the laying and moulting phases of the reproductive cycle.

Rift Valley fever, Congo fever and brucellosis: human seroprevalence and behavioural exposure in central South Africa (2015–2016)

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Rift Valley fever (RVF), Congo fever (CCHF) and brucellosis are important, notifiable, re-emerging zoonoses in South Africa, and are non-vaccine preventable febrile illnesses in people with highly variable consequences and duration. In addition, infection with RVF virus and *Brucella* bacteria cause disease and production loss in livestock. This study aimed to better understand exposure amongst people in high-risk occupations.

A four-month cross-sectional study in 2015-2016 assessed baseline seroprevalence and behavioural determinants for seropositivity amongst livestock owning households and veterinarians within a 40,000 km² area between Bloemfontein, Free State and Mokala National Park, Northern Cape. This is a known epicentre of previous large RVF outbreaks and endemic for *Hyalomma* ticks, the vector of CCHF.

IgG seroprevalence of RVF and CCHF was 8.8% (CI95%: 6.5-11%) and 3.1% (CI95%: 1.7-4.6%) respectively, and antibody

prevalence against *Brucella* was 7.5% (CI95%: 5.4-9.6%). On univariate analysis, RVF and brucellosis seroprevalence increased with age ($p < 0.001$). No increased risk of brucellosis, CCHF or RVF seropositivity was found associated with drinking raw milk, recent or past tick bite or tick squashing, and mosquito bites, respectively. Brucellosis seropositivity was associated with reported brucellosis in livestock in the past ($p = 0.001$), but no such association was found for RVF, including those who had worked ≥ 5 years on the farm. Injecting animals, blood collection and birthing appeared to increase risk of RVF and brucellosis seropositivity. Results of multivariable regression analyses will be reported.

Understanding human knowledge, behaviour and exposure is crucial to assess what the human role can be in outbreak or case prevention.

Aspects of plant variety protection (plant breeders' rights), in relation to seed crops, that may impact on policy and legislative development in South Africa

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A plant breeder's right (PBR) is a form of intellectual property protection afforded to breeders of newly bred plant varieties that are distinct, uniform and stable (DUS). In South Africa, new plant varieties are eligible for protection in terms of the Plant Breeders' Rights Act, 1976 (Act No. 15 of 1976). The objective of this study was to explore aspects of the PBR system that may need policy interventions and legislative amendments, focusing on seed crops. A study was conducted on the trends of plant breeders' rights grants in genetically modified (GM) varieties *versus* conventional varieties; as well as on the understanding of the plant breeders' rights system, particularly the farmers' privilege provision, which allows farmers to save seed of protected varieties for their own use, by key stakeholders. The main methods of data collection involved the analysis of information captured in the official plant breeders' rights register maintained by the Registrar of Plant Breeders' Rights and semi-structured interviews conducted with

various stakeholders, including historically disadvantaged small-holder farmers in Eastern Cape, Limpopo, Free State and Western Cape provinces. The study showed that 82% of the historically disadvantaged small-holder farmers who participated in the study were not familiar with the PBR Act and how it impacted on them and their farming practices. This study also highlighted gaps in policy, particularly addressing the possible dual protection of GM varieties; as well as the shortcomings of the farmers' privilege provision in the current legislation. Through this study, amendments to the farmers' privilege as provided for in the current Act were proposed and were included in the draft PBR Bill. This study highlights the importance of engaging stakeholders from both the formal and informal sector in the development of policies and the need for effective co-operation between different government departments dealing with various forms of intellectual property protection applicable to seed crops.

Ovarian dynamics and injection site reactions associated with immunocontraceptive zona pellucida (ZP) and GnRH vaccination of domestic horse mares (*Equus caballus*)

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Determinants when utilising veterinary immunocontraceptive vaccines include ovarian function and injection site reactions. Ovarian dynamics and injection site changes were monitored following administration of zona pellucida (ZP) and GnRH immunocontraceptive vaccines in 41 domestic horse mares. Mares were assigned to five treatment groups, stratified by age, parity and body condition score. Groups 1-4 treatments incorporated Pet Gel A (6%) and Poly (I:C) (500 µg) adjuvant in sterile water, in a two or three inoculation protocol 5 weeks apart, incorporating the following specified antigens in 1 ml total volume: no antigen (Group 1, n=9); initial 100 µg pZP then 100 µg pZP booster (Group 2, n=8); initial 500 µg recombinant zona pellucida (reZP) then 500 µg reZP and finally 500 µg reZP booster (Group 3, n=8) and initial 100 µg pZP then 500 µg reZP booster (Group 4, n=8). Group 5 (n=8) received 2 ml of 400 µg GnRF-protein conjugate initially and as booster. Mares were examined by trans-rectal palpation and ultrasound of the reproductive tract at each treatment period. Injection site reactions were assessed by inspection and palpation using a

three point scale and rectal temperature was measured for 7 days following treatments.

Comparing functional ovarian status one month after final treatments demonstrated strongly significant differences between treatment groups ($P < 0.0001$). Notably, a high proportion of both Groups 3 and 5 had ceased ovarian activity. The occurrence of injection site reactions and elevated body temperatures across all groups were mild, resolving one month after treatments, without any difference between treatment groups following initial or subsequent booster treatments.

Using reZP vaccine may be indicated in mares where suppression of ovarian activity is a desirable outcome following immunocontraception. Incorporation of a Pet Gel A and Poly (I: C) adjuvant formulation provides an effective alternative to currently-utilised Freund's adjuvants reportedly associated with adverse injection site reactions.

Anti-inflammatory activity of selected southern African medicinal plants with possible application against parasitic nematode infections

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Thirteen plant species were selected in this study based on their use in traditional medicine against inflammation in southern Africa. Five extracts from different solvents (acetone, ethanol, hexane, methanol and water) were prepared from each plant and the extracts were tested for their antioxidant, anti-inflammatory and anthelmintic activities. The anti-inflammatory activity of extracts was evaluated via the 15-lipoxygenase inhibitory assay and the nitric oxide (NO) inhibition assay using lipopolysaccharide (LPS)-activated RAW 264.7 murine macrophages. The antioxidant activity of the extracts was determined using radical scavenging DPPH and electron reducing ABTS assays. The anthelmintic activity was determined against the nematode *Haemonchus contortus* using the egg hatch assay (EHA) and larval development assay (LDA). The acetone extract of *Typha capensis* exhibited the highest antioxidant activity with IC_{50} of 7.11 µg/mL and 1.91

µg/mL respectively on DPPH and ABTS assays. However, the hexane extract of the same plant (*Typha capensis*) had good lipoxygenase inhibitory effect with IC_{50} of 4.62 µg/mL which is significantly ($p < 0.05$) better than quercetin (IC_{50} of 26.60 µg/mL) used as the positive control. The same hexane extract of *Typha capensis* also had good nitric oxide inhibitory activity on LPS-activated macrophages with IC_{50} of 32.20 µg/mL and a percentage NO inhibition of 77.45% and cell viability of 97.49% at 50 µg/mL. In addition, the acetone extract from *Typha capensis* was the most efficient against *Haemonchus contortus* with IC_{50} 27.99 mg/mL. This study revealed that out of the thirteen plants tested, *Typha capensis* had good antioxidant and anti-inflammatory activities but low anthelmintic activity against *Haemonchus contortus*. This plant is therefore a potential source of compounds that could be used against inflammation.

Prevalence and characterization of Shiga toxin-producing *Escherichia coli* in beef carcasses and beef products in Gauteng province

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Shiga toxin-producing *Escherichia coli* (STEC), particularly the O157 strains, are zoonotic pathogens associated with food and waterborne diseases and have been recognized as a public health problem worldwide. Cattle are a major reservoir of STEC and foods of cattle origin have been implicated in outbreaks. However, human STEC-associated disease is likely under-reported in South Africa. During Nov 2015 to Nov 2016, we conducted a random cross-sectional survey to investigate the prevalence and molecular characteristics of O157 and non-O157 STEC on beef carcasses and in beef products in Gauteng Province.

A total of 265 swab samples of beef carcasses from 12 abattoirs and 399 beef products from 31 retail outlets were screened for STEC using a multiplex PCR. The overall prevalence in abattoir samples was 37% (55/149) in summer and 34% (39/116) in winter. The highest prevalence of 50% (28/56) was detected in

perineal samples; this decreased through the slaughter process and the final prevalence in 24 h chilled carcasses was 20% (7/35; 95% CI 8% - 37%). The difference between high and low-throughput abattoirs was present only in perineal samples ($P = 0.060$) and post-evisceration samples ($P = 0.017$), and was no longer evident in 24 h chilled samples. In beef products it was 20% (27/137) in autumn, 14% (18/130) in winter and 17% (22/132) in summer; the highest prevalence was detected in boerewors (35%) followed by mincemeat (21%). The predominant serotypes detected were O113 (19.4%) and O157 (14.9%) in beef products, and O113 (14%) from abattoirs.

Our results demonstrate that STEC is present in South African beef and beef products. This may pose a real food-borne disease threat; further investigation of the epidemiology of the pathogen is required.

Mycobacterium bovis infection in cattle at the wildlife/livestock interface in northern KwaZulu-Natal province, South Africa

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A cross-sectional study was carried out in Northern KwaZulu-Natal at the wildlife/livestock interface to determine the prevalence of *Mycobacterium bovis* infection in cattle using a modified BOVIGAM® interferon gamma assay (IFN- γ). Although cattle are known as the primary host, *M. bovis* can also affect other domestic animals, wildlife and humans. Using a random sampling technique, whole blood samples were collected from 387 cattle registered at two dip tanks (Mpempe and Nkomo) in the uMkhanyakude district, which consisted of 267 females and 120 males from a total of 100 herds. The apparent *M. bovis* prevalence rate at animal level was 13.9% (95% confidence interval (CI) 10.6 - 17.4) and the true prevalence indicated a similar prevalence rate of 13.6% (95% CI 10.2 - 16.9). The apparent and true prevalence rate at Nkomo dip tank was 7.9% (95% CI 4.4 - 13.6) and 5.7% (95%

CI 1.4 - 11.6) respectively. The apparent and true prevalence rates at Mpempe dip tank were almost similar: 17.3% (95% CI 13.1 - 22.5) and 18% (95% CI 11.9 - 24.1) respectively. At Mpempe dip tank 20/52 (39%) of the farmers had at least one test positive animal, whilst at Nkomo dip tank, this figure was 8/48 (17%). Based on sex, 11 (9.1%, 95% CI 3.9 - 14.2) of the males and 43 (16.1%, 95% CI 11.7 - 20.5) of the females tested positive with the assay.

This study forms part of a One Health project at the wildlife/livestock interface. Confirmation of infection will be done through isolation and culture of tissues from test positive animals. The isolates will be genotyped and compared with isolates from wildlife, to monitor *M. bovis* transmission at the wildlife/livestock interface.

Vaccine matching: a different pathway to foot-and-mouth disease control

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Foot-and-mouth-disease (FMD) is a highly contagious transboundary animal disease that affects cloven-hoofed animals. Vaccination is one of the most important approaches for FMD prevention and control. In the vaccinated FMD protection zone of South Africa, cattle are routinely vaccinated every four months with a trivalent vaccine (South African Territories (SAT) serotypes 1, 2 & 3). Vaccine matching is used to select the most effective vaccine to use during outbreaks by comparing the antigenic variability between field and vaccine viruses. The objectives of this study were to assess the vaccine match of 40 FMD field viruses and to develop a new vaccine matching technique that can be used when live vaccine virus is not available in the laboratory.

A diverse group of 20 SAT1 and 20 SAT2 isolates collected from 1990-2015 were selected for study. Virus neutralization tests (VNT) were performed following the method described in the OIE Manual (2012). Two sets of pooled sera were used

for each serotype; vaccinated bovine sera (4 to 16 weeks post-vaccination) and convalescent bovine sera collected 3 weeks post experimental challenge. Novel r_1 -values were calculated using a new vaccine matching technique that incorporated reference strain viruses in the absence of information concerning the homologous vaccine viruses. Ratios were also calculated for the vaccine titre compared to a standardized positive control.

Standard deviation and coefficient of variation were used to assess variability in titre measures. Scatter plots and Spearman's rank correlation coefficient were used to evaluate agreement between the two methods. The kappa statistic was also used to assess agreement based on a "adequate match" cutoff of 0.3 as stated in the OIE FMD Diagnostics Manual.

These new methods provide a feasible, rapid and reliable vaccine matching approach that will contribute to control of FMD in southern Africa.

Serum albumin level of donor cows as indicator of development competence of oocytes

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Adequate nutrition is required for maintenance of normal production and reproduction in cattle. Strydom et al (2008) showed that albumin (the most abundant plasma protein) is the best predictor of malnourishment in South African cattle. Protein supplementation of *in vitro* embryo production (IVEP) media affects oocyte development into blastocysts. The objective of this study was to determine if serum albumin level of the donor cow could be used as a predictor of the developmental competence of oocytes, and if IVEP media can be optimised by additional protein supplementation for oocytes derived from donor cows with inadequate (≤ 35.9 g/l) levels of serum albumin.

A total of 1024 oocytes were cultured during this prospective cohort study. Of these, 460 oocytes originated from cows with inadequate serum albumin levels and 564 from cows with adequate serum albumin (≥ 36.0 g/L). Oocytes of these two cohorts were randomly allocated to a control IVEP protocol or a protocol with additional protein supplementation. Mixed effects

Poisson regression was performed for the number of oocytes that developed into blastocysts by the 7th day of culture.

Adequate serum albumin level of donor cows independently resulted in 46% increased blastocyst formation in the control IVEP protocol ($P = 0.02$). Although protein supplementation of the IVEP protocol did not affect blastocyst formation in oocytes originating from cows with inadequate serum albumin, it independently reduced blastocyst formation by 30% in oocytes originating from cows with adequate serum albumin ($P = 0.02$). Other independent predictors of blastocyst outcome included higher serum urea nitrogen, lower beta-hydroxybutyric acid levels and lower fat grading of donor cows.

It is concluded that adequate serum albumin of donor cows is a significant predictor of developmental competence of oocytes, and further research is required to determine optimal IVEP protein supplementation for oocytes originating from inadequately nourished cows.

The preventive and failure costs of mastitis in South Africa

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With many mastitis prevention measures available, economic studies usually limit their research to the costs of the consequences of mastitis: milk production loss, treatment costs of clinical cases and the replacement of culled cows. These do not acknowledge the time and money invested in prevention. To optimize mastitis management, the dairy farmer must minimize costs, grouped as either failure (FC) or preventive (PC) costs. The study sought to determine these costs for South African dairy farmers in 2016.

Eighty-five farms provided milking routine information, bulk tank somatic cell count, mastitis incidence and/or cull rates due to mastitis. The major preventive costs included drying off, liner replacement, the usage of milking gloves and the usage of predip and postdip. Major failure costs included treatment costs for clinical cases, milk discardments, milk losses due to subclinical mastitis and culls due to mastitis. FC and PC were

calculated for every farm, allowing for a FC-PC intercept. FC and PC were compared for different farm sizes and systems.

The South African dairy farmer spends on average R402 ± 201 (ranging from R49 to R1,276) per cow per year on mastitis prevention. The losses due to mastitis are estimated at R1,546 ± 426 (ranging from R493 to R4,296). The total cost of mastitis per cow per year is R1,948. Excluding culling costs, the FC is R1,248 or about double the average direct cost of R685 as estimated by dairymen when they included milk loss, treatment and labour costs. There was little difference in FC and PC between farm sizes and systems.

This is the first estimation of both failure and preventive costs for mastitis in South Africa, showing FC decreasing as PC increases. Dairy farmers can use the methodology to assess the total cost of mastitis and the potential value of any preventive measure being considered.

A survey of mastitis management practices used on South African dairies

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An industry funded project, *Resistance to Available Antibiotics in Lactating Cows with Mastitis*, initiated in 2015. The study of antimicrobial resistance (AMR) must elucidate the context to differentiate treatment and prevention failure due to AMR from failure attributed to mastitis management practices. This study aimed to survey South African dairies and their management practices as they pertain to mastitis treatment and prevention.

An online survey was deployed to the Milk Producers Organization (MPO) membership of ±1,700 dairymen in April of 2016. Data collection continued through December 2016. Twenty of the surveyed farms, milking ≥200 cows, were selected randomly for on-site visits. Data were collected regarding parlour procedures and milking routines, general udder health management, mastitis and its treatment, employees and consultants.

Following vetting, 147 surveys (8.6% of the MPO) were eligible for analysis. Herd size averaged 446 ± 78 (SEM). Herds were

defined as those on pasture only (49%), those on pasture with concentrate and/or a total mixed ration (TMR) (35%) and those feeding only a TMR (16%). Holsteins (38%) and Jerseys (42%) are the most popular breeds. The survey average for milk production was 18.2 L/cow/day (ranging from 7.0 to 40.0 L/cow/day), for butterfat 4.24% and protein 3.54%. A wide variety of parlour procedures and milking routines exist with the timing between the milking routine steps erratic, between and within farms. Fifty-three percent of farms participate in a milk recording scheme. The average mastitis incidence was 38%. Milk samples for microbial identification were collected on 29.2% of farms and 20.2% requested antimicrobial sensitivity. Only 30% of dairy farmers felt they had enough knowledge about mastitis.

The South African dairy industry shows immense diversity despite the low response rate. There are mastitis management practices, if consistently executed, that could improve udder health, strengthen milk flow and help minimize AMR.

Adaptation of SAT1 and SAT2 foot-and-mouth disease virus in cattle and goats

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Foot-and-mouth disease (FMD) is a highly contagious viral disease affecting cloven-hoofed animals. Infection of susceptible livestock species with FMD virus (FMDV) causes an acute, febrile illness that is characterised by a rapid onset of clinical signs. The South African Territories (SAT) type viruses are maintained in wildlife reservoirs, especially the African buffalo (*Syncerus caffer*), which provide a potential source of infection for domestic livestock and other wildlife.

There are few data available on the adaptation and comparison of infection of the SAT1 and SAT2 viruses in domestic hosts. Therefore, a study was performed wherein cattle (n=2/serotype) and goats (n=2/serotype) were infected with SAT1 or SAT2 field isolates to characterise the development of clinical disease and to identify the genetic changes associated with adaptation of the virus

in each species. Vesicular epithelium samples from the feet and mouth were collected from all animals each day post-challenge. The development of clinical signs was faster in cattle and cattle developed more severe lesions that persisted for longer periods compared to goats. Next-generation sequencing data were analysed to evaluate the sequence differences between animal species and SAT virus populations.

FMDV infection in goats is often asymptomatic and oral lesions are observed less frequently than on the feet. Understanding the host-virus interaction is key to both understanding transmission between animals and for the rational design of intervention strategies. These data will improve our understanding of FMD viruses, which will in turn aid us in the control of FMD at the wildlife-domestic interface.

Seroprevalence of Rift Valley fever virus in domestic and wild ruminants in far northern KwaZulu-Natal, South Africa

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Rift Valley fever (RVF) is a zoonotic disease caused by the RVF virus (RVFV), a *Phlebovirus* in the family *Bunyaviridae*. It affects mainly livestock and humans and is transmitted mainly by mosquitoes, including *Aedes* and *Culex* spp. Affected livestock have high mortality rates in newborn animals and abortion storms in pregnant animals. Human infections can result in self-limiting febrile illness, with symptoms including fever, severe headache, malaise, muscle pain and nausea, but in severe cases can result in encephalopathy, haemorrhagic syndrome, retinopathy and even death.

This study determined the seroprevalence of RVFV in domestic ruminants and wildlife in the Ndumo area, KwaZulu-Natal, close to the Mozambique and Swaziland borders. ELISA and the serum neutralization test (SNT) were used to detect antibodies specific to RVFV. A total of 424 cattle were sampled in June 2016, 70 goats in February 2017 and 106 opportunistic wildlife samples, consisting mostly of impala (*Aepyceros melampus*) and nyala (*Tragelaphus angasii*) were collected. RVFV antibody-negative cattle were resampled monthly, when available,

between November 2016 and March 2017, and seronegative goats were re-sampled in March 2017.

The seroprevalence in cattle sampled in 2016 was 28.5% (121/424; 95% CI 24.3-33.1%). Of the cattle that were originally seronegative to RVFV and were re-sampled, 38.3% (72/188; 95% CI 31.3-45.7%) seroconverted between June 2016 and March 2017. The seroprevalence in goats sampled in February 2017 was 36% (25/70; 95% CI 25-48%). Of the seronegative goats, 20% (8/41; 95% CI 9-35%) seroconverted between February and March 2017. Between June 2016 and March 2017, 53% (56/106; 95% CI 43-63%) of wildlife samples tested positive for antibodies specific to RVFV.

The presence of seropositive animals in the absence of vaccination and the recent seroconversion of animals clearly indicate a high level of circulation of the virus in this region, which warrants further investigation. Collection of mosquito vectors is ongoing and these will be tested to confirm the presence of the virus.

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

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There are no data available on the use of handheld X-ray fluorescence (XRF) spectrometry to determine concentrations of important trace elements in ovine livers. The aim of this study was to ascertain if the handheld XRF spectrometer will provide reliable concentrations of certain essential trace elements in the livers of sheep. Thirty ovine livers were obtained from an abattoir and samples were prepared as wet blended, oven dried and dry ashed samples. All the prepared liver samples were then analysed using handheld XRF spectrometry to determine concentrations of copper (Cu), iron (Fe), manganese (Mn), molybdenum (Mo), selenium (Se) and zinc (Zn). A reference laboratory analysed the same liver samples using ICP-MS to determine the concentrations of the abovementioned trace elements (control). The means (mg/kg) of the ICP-MS results on a dry matter basis were: Cu (505),

Fe (351), Mn (12.3), Mo (3.8), Se (1.8) and Zn (168). The means (mg/kg) of the XRF oven-dried results were: Cu (502), Fe (289), Mn (11.7), Mo (1.6) and Zn (141.9). Bayesian correlation was used to determine the best correlation between XRF and ICP-MS data. Overall, the oven-dried preparation procedure for XRF appeared to provide the best correlation with the ICP-MS data. For Cu and Zn these correlations were strong and the XRF method may represent a suitable substitute for ICP-MS. For Mn and Fe the correlations were moderately strong and the XRF method may be suitable depending on the intended application. For Mo the correlation was moderate and XRF cannot be recommended. For Se no preparation method for XRF was suitable. The advantage of handheld X-ray spectrometry is that the turnaround time of samples is reduced.

Thromboelastographic platelet mapping findings in dogs with complicated *Babesia rossi* infection

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Despite being markedly thrombocytopenic, dogs with *Babesia rossi* infection display a normocoagulable thromboelastogram due to platelet activation. Thromboelastographic platelet mapping (TEG-PM) assesses the individual contributions of thrombin, fibrinogen and platelets to clot formation; and may provide further detail regarding mechanisms of this haemostatic alteration. This study assessed whether there were any differences between TEG-PM variables obtained in dogs with complicated *B. rossi* infection compared to healthy controls, and whether these variables correlated with platelet activation indices.

The maximum amplitude (MA) following thrombin generation (MA_{Thrombin}) was determined using kaolin-activated TEG. TEG-PM variables included MA following addition of platelet agonists arachidonic acid (MA_{AA}) and adenosine diphosphate (MA_{ADP}), and MA due to fibrin alone

(MA_{Fibrin}). Platelet indices and fibrinogen concentration were determined. Thirteen dogs with complicated *B. rossi* infection and five healthy control dogs were included.

Compared to controls, the median MA_{Fibrin} and fibrinogen concentration were significantly higher ($P < 0.01$ for both) and platelet count significantly lower ($P < 0.01$) in the babesiosis group. No significant differences were found for MA_{Thrombin} and MA_{AA/ADP}. MA_{Fibrin} was positively correlated with fibrinogen concentration ($r = 0.735$), mean platelet volume ($r = 0.517$) and mean platelet mass ($r = 0.498$); and negatively correlated with haematocrit ($r = -0.685$), platelet count ($r = -0.476$) and plateletcrit ($r = -0.479$) ($P < 0.05$ for all).

This study suggests that hyperfibrinogenaemia and concurrent platelet activation compensate for the severe thrombocytopenia associated with *B. rossi* to result in normal thromboelastograms and lack of clinical bleeding.

Carnivore population dynamics on two reserves, comparable or not?

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Small carnivore species might provide the disease link in disease transmission between domestic dogs and apex predators. The objective of this study was to determine the presence of carnivores and prey species on two wildlife reserves in Mpumalanga Province.

Carnivore presence was determined on two reserves managed by Mpumalanga Tourist and Parks Agency (MTPA). Manyeleti is contiguous with Kruger National Park (KNP), 23 000 hectares in area, and has villages on one side. Andover is not adjacent to KNP, 7 000 hectares in area, and has villages on three sides of the reserve. The presence of carnivores and determination of inter-species interactions were measured during a two-year period using randomly placed clusters of camera traps. These camera traps were placed at each selected location for a period

of 4 to 6 weeks before being moved. Permanent camera traps were also placed on the interface between reserves and villages. Data were entered into Microsoft Excel and mapped using ArcGIS 10.4.1. Ordinary Kriging and inverse distance weighting interpolation were used to determine the spatial distributions of observed wildlife. The Mackenzie model was used to determine the daily detection probability of each species. The most abundant species present in Manyeleti were hyena (*Crocuta crocuta*) and Lion (*Panthera leo*). In Andover, the only apex predator present was leopard (*Panthera pardus*), while serval (*Leptailurus serval*), caracal (*Felis caracal*), and mongoose (*Mungos mungo*) were also present. Some locations were shared among carnivore species, while other areas had no carnivore species detected at all. Areas with prey species, water and access routes to water had the highest probability of carnivore detection.

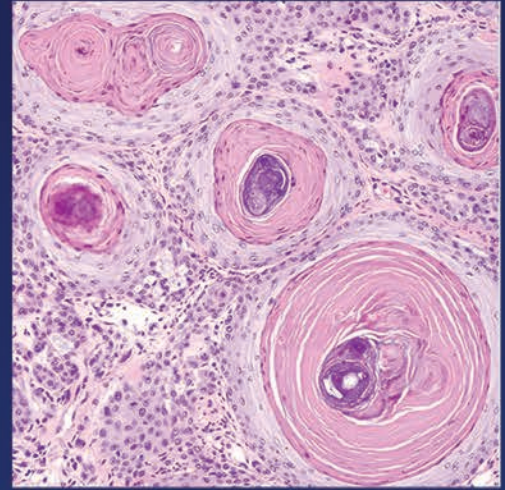
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