



SIR ARNOLD THEILER BUILDING



2022 Faculty Day



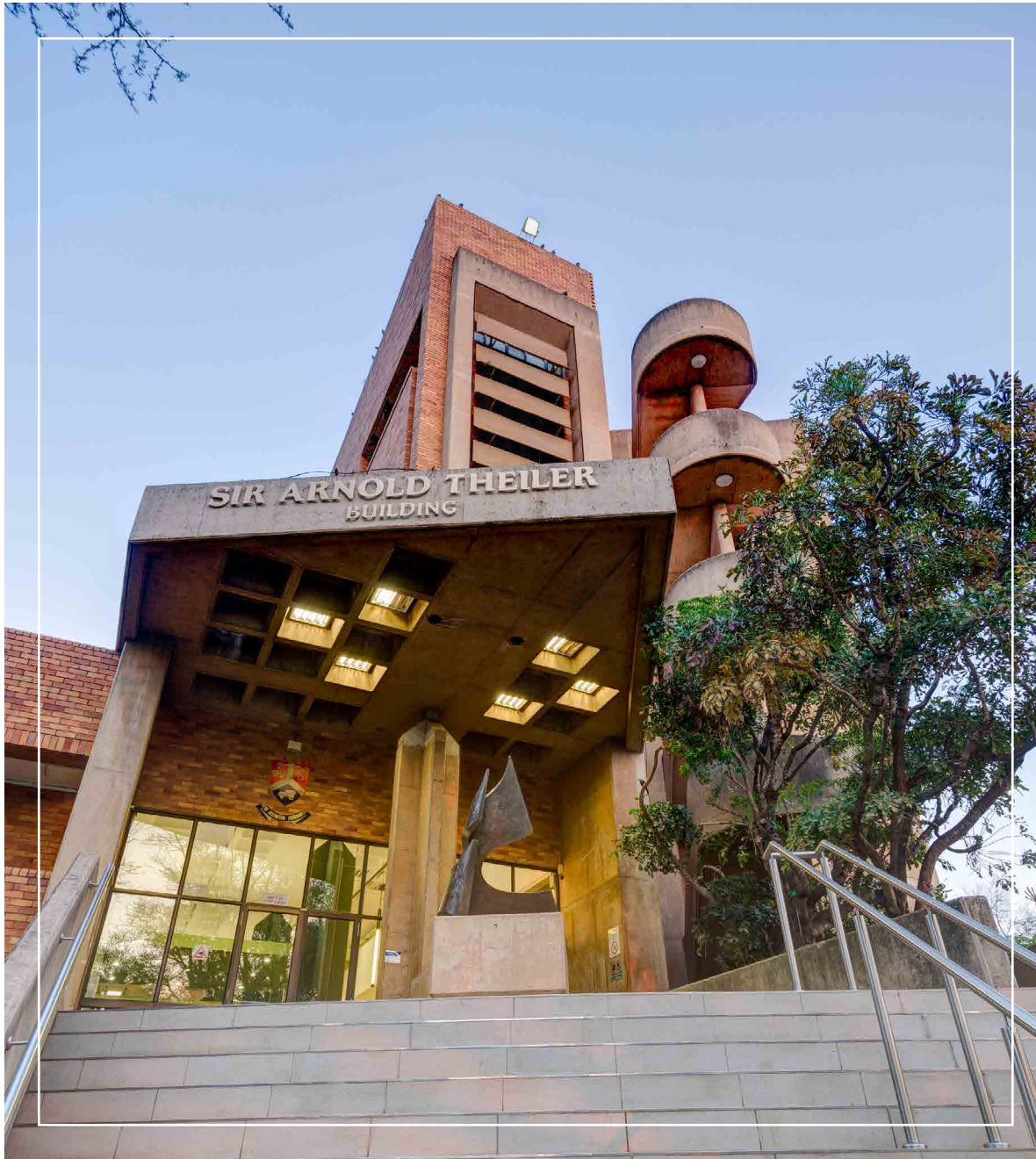
UNIVERSITEIT VAN PRETORIA
UNIVERSITY OF PRETORIA
YUNIBESITHI YA PRETORIA

Faculty of Veterinary Science

Fakulteit Veeartsenykunde
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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 the Medical University of South Africa (Medunsa) and the University of Pretoria, of showcasing the research activities of staff and students on a special, dedicated occasion.

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

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

5th ARNOLD THEILER
BUILDING

FACULTY DAY

**Faculty of Veterinary Science
University of Pretoria**

20 October 2022

2022

Faculty Day



PROGRAMME



MASTER OF CEREMONIES

Dr Alfred Kgasi



08:00 - 08:20

Registration and tea (Arnold Theiler Building)
[Online Platform opens]



08:20 - 08:25

Start of proceedings and housekeeping rules



08:25 - 08:40

Welcoming address: Prof Vinny Naidoo,
Dean of the Faculty of Veterinary Science



08:45 - 09:35

Session 1: Postgraduate research [10 min each]

Session chair: *Prof Kgomotso Sibeko-Matjila*

- 08:45-08:55** The effects of nutritional stress and reproduction on stable isotope ratios of ruminants, monogastrics, and hindgut fermenters in South Africa.
GV Ringani, JP Chamunorwa
- 08:55-09:05** Prospective pathological evaluation of the prevalence of thromboemboli & associated haemostatic dysfunction in canine carcinomas & sarcomas.
P Pazzi, GT Fosgate, A Rixon, J Hanekom, AT Kristensen, A Goddard
- 09:05-09:15** Investigation of the inflammatory response in African Horse Sickness.
E-C Schlievert, EH Hooijberg, S Steyn, C Potgieter, GT Fosgate, A Goddard
- 09:15-09:25** Comparative genomics of *Bacillus anthracis* strains from anthrax outbreaks in Kruger National Park, South Africa.
SP Mokgokong, A Hassim, WC Turner, KE Lekota, H van Heerden
- 09:25-09:35** **Question & Answer session**



09:40 - 10:15

Session 2: Undergraduate research (VRE 600)
[5 min each]

Session chair: *Dr Darshana Morar-Leather*

- 09:40-09:45** Pelvic limb myology of the South American tapir (*Tapirus terrestris*).
M. Duwe, C. Steyn
- 09:45-09:50** Diagnostic accuracy of cytological examination for the identification of neoplasia in the OVAH Clinical Pathology laboratory.
N Tar, EH Hooijberg
- 09:50-09:55** Characterisation of bacterial isolates located in the oral cavity of free-ranging hippopotami (*Hippopotamus amphibius*).
M Engelbrecht, A Jonker, A Michel
- 10:00-10:05** The use of client-created video clips in testing the effectiveness of an over-the-counter product in the management of thunderstorm phobia in dogs.
S Baxter, Q Sonntag
- 10:05-10:10** Mycoplasmosis in black-footed cats (*Felis nigripes*): diagnosis, treatment, and prevention.
JD Clark, KN Koepfel
- 10:10-10:20** **Question & Answer session**
- 10:20-11:00** **Tea/coffee break (Arnold Theiler Building)**



11:00 - 11:50

Session 3: Arnold Theiler Memorial Lecture:
Prof Juergen A Richt (Kansas State University, USA)
'In the footsteps of Arnold Theiler: Vaccines and Diagnostics then, now and in the future'

Introduction and session chair: *Prof Tshepo Matjila*



11:55 - 12:45

Session 4: Postgraduate research
[10 min each]

Session chair: *Dr Raksha Bhoora*

- 11:55-12:05** Prevalence and characterization of Shiga toxin-producing *Escherichia coli* in dairy cattle in South Africa.
A Olawole, M Malahlela, MC Marufu, A Kalake, M Karama
- 12:05-12:15** The effect of glycine supplementation on the identified urinary metabolites in captive cheetahs (*Acinonyx jubatus*).
KM van Boom, L Adamson, TA Kohn, ASW Tordiffe
- 12:15-12:25** Bacterial and fungal causes of domestic ruminant abortion in South Africa.
A Jonker, PN Thompson, A Michel
- 12:25-12:35** The production of a plant-expressed virus-like particle vaccine against infectious bronchitis coronavirus and efficacy in chickens.
KM Sepotokele, C Abolnik
- 12:35-12:45** **Question & Answer session**



12:45 - 13:55

Lunch and viewing of posters
(Arnold Theiler Building and Cafeteria)



14:00 - 15:15

Session 5: Postgraduate research
(funded by AgriSETA or HWSeta)
[10 min each]

Session chair: Prof Tshepo Matjila

- 14:00-14:10** Effects of developmental genistein exposure on anxiety and depressive-like behaviour in rat offspring.
J.D Chetty, C Newton, Z Mohamed-Moosa
- 14:10-14:20** Neutrophil gelatinase associated lipocalin and symmetrical dimethylarginine in dogs with carcinoma or sarcoma.
AJ Rixon, E Meyer, S Daminet, A Goddard, A Celliers, T Kongtasai, P Pazzi
- 14:20-14:30** Identification of *Anaplasma* species in wild animal species in the Kruger National Park and surrounding game reserves using a bacterial microbiome approach.
SM Makgabo, KA Brayton, MC Oosthuizen, NE Collins
- 14:30-14:40** Anti-cancer, anti-inflammatory and antioxidant activity extracts, fractions and compounds from *Ptaeroxylon obliquum* leaves.
ET Khunoana, JN Eloff, TE Ramadwa, SM Nkadimeng, MA Selepe, LJ McGaw
- 14:40-14:50** Monitoring and controlling aspiration fluid temperature during ovum pick-up in southern white rhinoceros (*Ceratotherium simum simum*).
L Marais, BS Durrant, MP Smuts, M de la Rey, C Herbst, DE Holm
- 14:50-15:05** **Question & Answer session**
- 15:10-15:15** **Final words: Representatives from AgriSETA and HWSeta**



15:20 - 15:45

Session 6: 3-Minute Thesis (Doctoral candidates) and 1-Minute Dissertation (Masters candidates) presentations

Session chair: Dr Selaelo Tshilwane

3-minute thesis presentations: [PhD]

- 15:20-15:23** Case report: The clinical presentation and management of a naturally occurring bluetongue virus infection in a pregnant Rottweiler dog. *J Hanekom*
- 15:24-15:27** Interrelationships of warthogs, *Ornithodoros* ticks and African swine fever virus in South Africa. *A Craig*
- 15:28-15:31** Plant extracts influences surface hydrophobicity, cell adhesion, quorum sensing and extracellular matrix production involved in biofilm formation of multidrug resistant *Escherichia coli* O157:H7. *MM Lebeloane*
- 15:32-15:35** Molecular epidemiological investigation of contagious equine metritis in South Africa. *CE May*

1-minute dissertation presentations: [MSc]

- 15:36-15:37** Acute stress response in southern white rhinoceros (*Ceratotherium simum simum*) immobilized with four different drug protocols. *LS Michaelides*
- 15:38-15:39** Investigation of *Bacillus anthracis* spore survival in soils from Kruger National Park in South Africa and Etosha National Park in Namibia. *K Govender*
- 15:40-15:41** Prevalence and factors associated with *Salmonella* species in mechanically recovered poultry meat imported through a port of entry into South Africa. *TN Ndobeni*
- 15:42-15:43** A population model to investigate the drivers that cause the replacement of *Rhipicephalus decoloratus* by *Rhipicephalus microplus* on cattle. *JL Kotze*
- 15:44-15:45** Comparative antimicrobial efficacy of four surgical hand preparation procedures prior to application of an alcohol-based hand rub in veterinary students. *HS Viljoen*



15:52 - 16:00

Social media highlights of 2021 *[video]*



16:00 - 16:15

Annual Faculty Research Prize Giving



16:15 - 16:20

Closing remarks

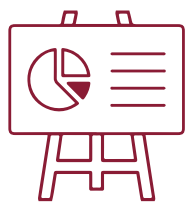
Opportunity available for additional photographs of the winners with the sponsors after the Closing Remarks



16:20

Cocktail event





POSTER PRESENTATIONS

1. The bacterial microbiome of *Rhipicephalus sanguineus* ticks in the Mnisi community, South Africa. *R Ackermann*
2. Efficacy of South African plants against drug-resistant staphylococci isolated from clinical cases of bovine mastitis. *AO Akinboye*
3. *In silico* identification and comparative analysis of *Theileria parva* vaccine candidates identified using reverse vaccinology methods. *LL Borchers*
4. Mandibular fractures in cats: a comparison of signalment, distribution, conformation and association with teeth between referral practices in South Africa and Canada. *M Conradie*
5. Farmer's knowledge, perceptions and attitudes of FMD in the control zone of Limpopo Province. *K Daddy*
6. Evaluation of the efficacy and safety of ketamine-medetomidine and ketamine-butorphanol-medetomidine for the immobilization of free-living African lions (*Panthera leo*). *AC Donaldson*
7. *In vitro* biological activities of *Combretum molle* R.Br. exG.Don (Combretaceae) against mastitis-causing organisms. *RC Erhabor*
8. Development and evaluation of an RT-qPCR assay for the detection of Rift Valley fever phlebovirus in formalin-fixed, paraffin-embedded tissues in natural cases of sheep. *S Gwala*
9. The seroprevalence of African horse sickness in dogs living in Tshwane, South Africa (2014 - 2019). *J Hanekom*
10. An Epidemiological Study of Canine Lymphoma in South Africa. *SB Harris*
11. Molecular epidemiology of pigeon paramyxovirus (PPMV) in South Africa. *MC Hayes*
12. Detection of bacterial tick-borne pathogens in two provinces of South Africa using a microbiome sequencing approach. *BL Khoza*
13. The effects of developmental genistein exposure on learning and memory function in rat offspring. *AO Kunene*
14. A survey on medicinal plants used by indigenous people of Limpopo for treatment of Covid-19 and respiratory symptoms in humans and animals. *JK Madisha*
15. Antimycobacterial activity of traditional herbal mixtures against microorganisms related to respiratory ailments in animals. *JK Madisha*
16. Temporal dynamics of *Anaplasma marginale* infection in calves at the wildlife-livestock interface in the Mnisi communal area, Mpumalanga, South Africa. *SM Makgabo*
17. Cattle trade networks in the foot-and-mouth disease (FMD) Protection Zone of Limpopo Province. *KK Malatji*

18. Serotyping and resistance profiling of non-typhoidal *Salmonella* isolates from poultry in South Africa. [E Masitha](#)
19. Abattoir workers' knowledge, perceptions and attitudes regarding zoonotic infections in the Eastern Cape province, South Africa. [KD Mazwi](#)
20. Quantitative detection of *Theileria haneyi* infections in South Africa. [TV Mbaba](#)
21. Reliability of the Enterprise Point-Of-Care blood analyser's calculated arterial oxygen haemoglobin saturation, in immobilized southern white rhinoceros (*Ceratotherium simum simum*). [TK Mtetwa](#)
22. Anti-quorum sensing, anti-biofilm activity, antibacterial activity, total phenolic and flavonoid contents of *Kalanchoe gunniae* and other selected plants with potential for managing mastitis. [EC Ogbuadike](#)
23. Morphological description of the digestive tract of Temminck's ground pangolin (*Smutsia temminckii*). [TJ Saby](#)
24. Development and optimization of a reverse-transcription loop-mediated isothermal amplification (RT-LAMP) assay for the detection and identification of SAT1 serotype foot-and-mouth disease virus. [L Seoke](#)
25. *Ehrlichia ruminantium* and *Rickettsia africae* infection rates in *Amblyomma* species of southern Africa. [A Smit](#)
26. Comparative morphology of the stifle joint of the African lion (*Panthera leo*), African black-footed cat (*Felis nigripes*) and Temminck's ground pangolin (*Smutsia temminckii*). [C Steyn](#)
27. Evaluation of alternative disinfectants to formaldehyde for treating broiler eggs in a commercial hatchery. [AJ van Wijk](#)
28. Development of a multiplex real-time polymerase chain reaction to distinguish between *Mycoplasma* species found in South African poultry. [PP Wambulawaye](#)
29. The effect of developmental genistein exposure on reproductive function in rat offspring. [AM Wulfg](#)



Message from the Dean

After missing two previous in-person Faculty Days due to COVID-19 restrictions and hosting it online, it is a privilege being together again in the same lecture hall where we have presented our Faculty Days for so many years until 2019.

A very warm welcome to staff members, students, sponsors and all our invited guests to this special occasion during which we showcase our research and participate in exchanging scientific knowledge and thought-provoking ideas.

At the moment we are experiencing turbulent times in our country. Besides the ongoing power crisis, slow economic growth and a high unemployment rate all contributing to this, the country is currently facing outbreaks of numerous diseases especially FMD, which has become the worst outbreak since 1957. We are also facing problems with local vaccine production of not only the FMD vaccine but also other vaccines.

While we can stand back and blame State Veterinary Services and other parties for the failures we see, as academics should we be dissociating ourselves from the current situation?

Since the answer is obviously no, we need to add our collective knowledge to the solution and - even more important - look at disease control solutions in different

Prof Vinny Naidoo
Dean: Faculty of Veterinary Science

ways viz. history teaches us that it took an outbreak of Rinderpest to eradicate FMD rather than a successful eradication programme leading to the country obtaining its FMD free status.

This thus brings into question if the current manner of managing a control zone with vaccination is the only way to manage the disease? It has certainly served us well but we need to consider the cost of such a programme and the constitutional fairness of the quarantine zone – just two of the reasons why alternate ways of disease mitigation are needed.

Therefore, another important and exciting new development this year was the UP Senate's approval of our African Centre for Biosecurity and Disease Risk Assessment, which was brought about after lengthy consideration and discussions on how the Faculty could play a more prominent role in the control of high impact veterinary diseases at various levels for research, short courses and degree training.

As we move forward we envision the new Biosecurity Centre playing a bigger role together with industry in tackling issues of importance. Similarly important are our plans to introduce BSL3 laboratories for bacterial and viral research. At the same time, efforts into new vaccine development will also become more common, taking us back to the origins of the Faculty and our history of vaccine development that Theiler brought to the country.

More than just research our new Centre will also form a core part of the new Biosecurity Hub, which was recently launched by the Ministers of Higher Education and Agriculture on 11 October 2022.

The Biosecurity Hub is an initiative of the Department of Science and Innovation's Agricultural Bio-economy Innovation Partnership programme and aims to facilitate collaborative efforts in the national system of innovation to support the prevention, reduction and management of crop and animal disease and other matters related to food safety in South Africa. It will be coordinated by Innovation Africa at the University of Pretoria.

Apart from disease management, there are other crucial issues that we need to consider such as pandemic/epidemic preparedness. More important than being able to control a disease through vaccination and proper biosecurity, would be to be

prepared for the said occurrence by ensuring that we either have sufficient vaccines in storage or being able to place production facilities into operation on short notice.

However, while this may be of value for known agents and serotypes, this won't always be the case. COVID-19 taught us these lessons, and showed the value of having background technology that could be rapidly adapted for mRNA insertion for disease control.

However, since such medical technology is not necessarily translatable to veterinary science, other measures are needed for which new research is required. Concerns about cross reactions between vaccinated and naturally diseased animals, also necessitate consideration towards DIVA or marker vaccines which can induce an immune response while still being differentiated to that of the natural infection.

All in all, this means that there are various opportunities whereby the Faculty can make a major difference in disease management for years to come. The Faculty is perfectly placed to make such contributions and currently we have numerous staff members working on vaccine projects using various vaccine technologies.

Lastly, with up to 75% of new infections originating from animals, the veterinary professions need to play a bigger and more holistic role in a One Health approach. As a result, the Faculty is participating in the current NRF call to establish a Pandemic Preparedness Institute.

While the exact roles of the various University partners is yet to be finalised, the Faculty can already offer support in the diagnoses of zoonotic disease, and as experts of host-pathogen interactions, preclinical development, vaccine development and advanced diagnostics.

In terms of current research, the Faculty has had another excellent research year in 2021. The Faculty produced over 250 publications covering various subject fields and had a publication output of 108.35 units.

In 2021 the Faculty also went through an accreditation visit for the BVSc programme and we received confirmation this year that the BVSc programme has been accredited by the SAVC for a further 7 years. In their accreditation report, the panel acknowledged the significant efforts of the Faculty to meet the University's

goals of being a research intensive University. In terms of ranking as a Faculty, we maintained our position in the top 75 of the Shanghai rankings and top 60 of the QS World University rankings, with numerous staff featuring in the top 2% of top cited researchers in the world.

As a Faculty, I am extremely proud of what we have achieved. We can also be proud that our undergraduate students play a key role in delivering clinical services around our country. We furthermore have over 100 postgraduate students from outside South Africa who are making an impact in their home countries.

Onto the focus of the day. Today we take part in an event that has become a major feature on the Faculty's calendar. Faculty day, as an event, was first held on 5 September 1984, to showcase research undertaken at the Faculty. Over the years the day has evolved and is now dedicated to the presentation of research undertaken by Faculty postgraduate students, and from 2020 also by our final year veterinary students.

The impact of this is evident in that some students have already published their research projects while a few have managed to take their projects forward into MSc degrees.

Another feature that has become part of Faculty Day since its inception is the Arnold Theiler Memorial Lecture. However, this lecture has a much longer history. It was first approved by the University Council in 1962 with the aim of hosting prominent local and international scientists whose experience will contribute to the knowledge of

the veterinary profession in this country. The first lecture was presented in 1963 by Professor C. Remington, F.R.S., Professor of Chemical Pathology, University College Hospital Medical School, London and focused on photosensitization syndromes due to porphyrins in animals.

One comment by Prof Remington in that presentation is still quite relevant today: "It has been said that Theiler put South Africa on the scientific map of the world and the Institute which he founded and directed for many years is indeed revered wherever Veterinary Science is taught and practiced." It is in keeping this tradition, that we start Faculty Day today.

We are therefore also extending a warm welcome to Prof Juergen A. Richt from Kansas State University and Extraordinary Lecturer in the Faculty's Department of Veterinary Tropical Diseases who will be presenting this year's Arnold Theiler Memorial Lecture entitled 'In the footsteps of Arnold Theiler: Vaccines and diagnostics then, now and in the future.'

All of the best to all our presenters today. To our audience, whether you are attending in-person or online, have an enjoyable Faculty Day.

Prof Vinny Naidoo
DEAN



Past deans of the Faculty of Veterinary Science, University of Pretoria

1st



Dean:

Sir Arnold Theiler
1920 – 1927

2nd



Dean:

PJ du Toit
1928 – 1948

3rd



Dean:

G vd W de Kock
1948 – 1950

4th



Dean:

JI Quin
1950 – 1951

5th



Dean:

PJJ Fourie
1951 – 1955

6th



Dean:

H Graf
1956 – 1960

7th



Dean:

RM du Toit
1960 – 1963

8th



Dean:

BC Jansen
1963 – 1969

9th



Dean:

CFB Hofmeyr
1969 – 1981



Dean: Medunsa

N Owen
1980 – 1994

10th



Dean:
JMW le Roux
1982 – 1986

11th



Dean:
RI Coubrough
1987 – 1999



Dean: Medunsa
HM Terblanche
1994 – 1999

12th



Dean:
NPJ Kriek
2000 – 2005

13th



Dean:
GE Swan
2006 – 2014

14th



Dean:
DA Abernethy
2014 – 2018

15th



Dean:
V Naidoo
2018 to present

SOURCES:
Onderstepoort 1908–2008, by courtesy of the Veterinary History Society of South Africa

Universiteit van Pretoria. Ad Destinatum 1910–1960: Gedenkboek van die Universiteit van Pretoria. Johannesburg: Voortrekkerpers Beperk, 1960.

Curriculum Vitae: Juergen A. Richt, DVM, PhD

Dr. Richt came to Kansas State University in 2008 as The Regents Distinguished Professor and Kansas Bioscience Eminent Scholar.

In 2010, he became Director of the Department of Homeland Security (DHS) Center of Excellence for Emerging and Zoonotic Animal Diseases (CEEZAD; www.ceezaad.org) and in 2020 Director of the National Institutes of Health (NIH) Center on Emerging and Zoonotic Infectious Diseases (CEZID; www.k-state.edu/cezid).

He received his Doctorate in Veterinary Medicine (DVM) from the University of Munich and a PhD in Virology and Immunology from the University of Giessen, both in Germany. After coming to the United States in 1989, he completed three years of postdoctoral/residency studies at The Johns Hopkins University and later served for eight years as a Veterinary Medical Officer at the National Animal Disease Center (USDA-ARS) in Ames, Iowa. He has edited several books, obtained several patents, published more than 300 peer-reviewed manuscripts and raised more than \$65 million in grants for veterinary research.

Dr. Richt is a pioneer in veterinary science, most notably in the "One Health" field. His work on high consequence pathogens with zoonotic and transboundary potential led to strategies to identify, control and/or eradicate such agents. His basic and applied research includes studies on animal influenza viruses (swine, bat and avian), animal prion diseases including bovine spongiform encephalopathy (BSE), Rift Valley Fever virus (RVFV), African Swine fever virus (ASFV) and Borna Disease virus (BDV).

Dr. Richt established the first reverse genetics system for swine influenza virus (SIV), and made seminal contributions to the development of a modified live SIV vaccine now sold in the U.S. as "Ingelvac Provenza™" as



Juergen A. Richt, DVM, PhD

Regents and University Distinguished Professor, Department of Diagnostic Medicine/Pathobiology; College of Veterinary Medicine, Kansas State University, Manhattan, KS

well as to understanding the virulence of the reconstructed 1918 “Spanish Flu” virus in livestock. He identified an atypical BSE case with a causative mutation (“genetic BSE”), used gene-editing approaches to develop the first prion protein knock-out cattle which are resistant to prion infection, and provided valuable information on host range of animal prions essential for risk analysis. Dr. Richt’s RVFV work led to the development of novel domestic and wild ruminant models for RVF and a safe, efficacious, and DIVA compatible subunit vaccine which is presently undergoing USDA licensure.

For ASFV, he is developing subunit and modified live virus vaccine candidates as well as Point-of-Need diagnostics (sold as “Pen Check™”) to protect swine from this devastating disease. His recent work focuses on the establishment of preclinical animal models for SARS-CoV-2 to determine the efficacy of COVID-19 vaccines and therapeutics and the development of gene-edited pigs which are resistant to economically important swine diseases. As founding Director of the DHS CEEZAD and the NIH CEZID Centers, he is supporting NIH, DHS and USDA in protecting public health and U.S. agricultural systems from devastating animal and zoonotic diseases.

IN THE FOOTSTEPS OF ARNOLD THEILER: Vaccines and Diagnostics - then, now and in the future

Juergen A. Richt

Department of Diagnostic Medicine/Pathobiology, College Kansas State University, Manhattan, KS, USA

E-mail: jricht@ksu.edu

The *Sir Arnold Theiler Memorial Lecture* will start with introducing the audience to the lecturer’s background and scientific career. Tools and methods used to diagnose infectious diseases and develop vaccines during the *Sir Arnold Theiler* era will then be discussed followed by a summary of the research that Dr. Richt performed in the past 25 years developing vaccines against pathogens such as Borna disease virus (BDV), swine influenza virus (SIV), Rift Valley fever virus (RVFV) and African swine fever virus (ASFV). For BDV, it was demonstrated that passive immunization of rats with BDV-specific T cells before BDV-infection resulted in protection against immuno pathological responses to BDV, where as inoculation of the same cells after infection resulted in rapid onset of clinical BD. For SIV, reverse genetics technology was used to construct an attenuated SIV strain that was used to develop a modified live SIV vaccine commercialized as Ingelvac Provenza™. Dr. Richt’s RVFV work led to the development of a safe, efficacious, and DIVA

compatible sub unit vaccine that is under going USDA licensure. For ASFV, subunit, vector-based and modified live virus vaccine candidates are being developed. In terms of diagnostics, Dr. Richt’s present work addresses point of care diagnostic approaches for various transboundary animal diseases. Mobile molecular platforms (e.g. Pockit™, Biomeme Franklin™) are used and have been validated for ASFV, RVFV and FMD. In comparison with laboratory-based thermocycler. Lateral flow tests are developed for the detection of pathogen-specific antigens (PenCheck™) or antibodies. The lecture will end with a discussion on future technologies and approaches to vaccine and diagnostic development. As an alternative strategy, gene-editing approaches in livestock will be discussed. Dr. Richt will present his work developing prion-protein knock-out (KO) cattle that are resistant to bovine spongiform encephalopathy infection and KO pigs that are less susceptible to SIV infection.

2021 Research Summary

TODAY'S REALITY:

Agriculture and food security are globally areas of key economic and societal importance. With the global population's projected growth, at least 70% more food production is needed by 2050 to feed the world's population.



This is despite the fact that more than 821 million people (10% of the world population) already go to bed hungry every night!

821 million
people go to bed hungry every day



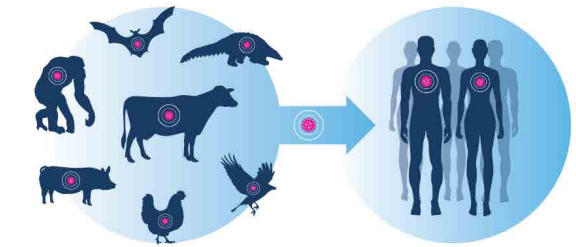
More than **700 MILLION** people worldwide, or 10% of the global population, live below the international poverty line

More than half of the world's extremely poor live in Sub-Saharan Africa, and **42%** of the region's inhabitants live below the international poverty line



More than 75% of the estimated 1.2 billion people living in extreme poverty worldwide (living on less than \$2 per day) depend on subsistence farming and livestock as an essential source of income to survive.

With more than 60% of emerging human infectious diseases being of animal origin, it affects human health and wellbeing directly as common causes of human disease, and indirectly through impacts on livelihoods and food security as a result of livestock production losses.



As such, more than 20% of animal production losses in the world are linked to animal diseases, posing a direct threat to the incomes of these rural communities.

DID YOU KNOW?
More than **20%** of losses in animal production are related to animal diseases
Ensure animal health, ensure food security for all!

Source: World Organisation For Animal Health

Ensuring the health of animals is thus vital to safeguarding the health of people, not only in terms of ensuring the availability of safe, healthy food for all, but also in terms of preventing disease outbreaks or transmission.

OUR COMMITMENT:

The Faculty is, and will continue to be an important contributor to attain optimal health for people, animals and our environment. We continue to aim towards expanding our local relevance and increasing our international visibility. The enhancement of innovative and relevant research, as well as high-quality postgraduate training, therefore remains an integral part of the Faculty's strategic plan. In support of the University's goal of being a top research-intensive institution, the Faculty furthermore requires increased research outputs through effective postgraduate programmes, and making research a primary thrust.

The Faculty's general approach to all teaching and research is orientated towards sustainability. Improving health and productivity of animals are two fundamental areas of focus, which not only improve areas of food security and income generation, but by focusing on animal health, issues such as diseases are minimized, which in turn benefits the environment and promotes sustainable livelihoods.

The Faculty's work also goes beyond the classroom doors; it offers farming programmes that aim to better equip small scale farmers with the knowledge of better farming practices, becoming more responsible environmental stewards while improving their farming outputs. Through our academic programmes and research we are, therefore, committed to promoting sustainability for a healthier environment.

Public and animal health, wildlife conservation, biosecurity and welfare, disease control and animal production are just a few of the focus areas of the Faculty that promote a healthy thriving ecosystem and environment and ensure sustainable livelihoods.

By doing this, we remain committed to support identified priorities of the South African National Development Agenda, Vision 2030, as espoused in our National Development Plan, thereby, supporting Government in its endeavors aimed at sustainable rural development, food security, job creation and nature conservation.



Prof Marinda Oosthuizen

Deputy Dean: Research and Postgraduate Studies

REFLECTIONS 2021:

On the level of postgraduate education and research, 2021 was once again a difficult year with several challenges and many delays experienced due to the COVID-19 pandemic. Although postgraduate students had more access to laboratories and could continue with fieldwork, it remained challenging due to the limited number of students allowed in laboratories, serious delays in the delivery of certain reagents, the increased cost of doing fieldwork (less students per vehicle, and per accommodation unit), just to name a few. Several students struggled (and are still struggling) with financial and emotional difficulties (feeling stressed, overwhelmed, anxious).

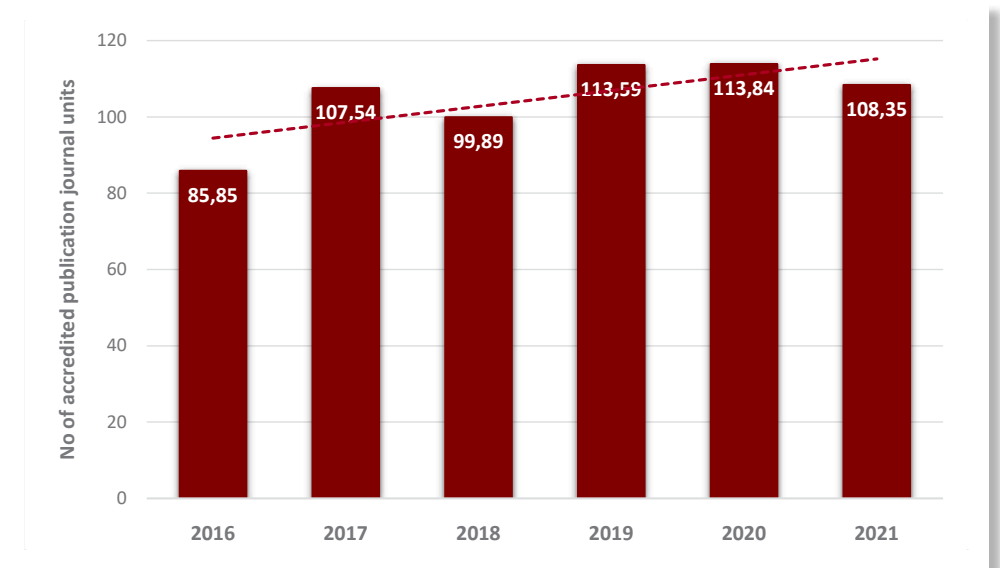
Our Faculty Student Postgraduate Advisor, Dr Monica van Niekerk, did a sterling job in creating several initiatives to support our students, including discussion groups (peer support sessions addressing life/studies/work balance, self-care, conflict styles and resolution), transition programmes for new students, workshops to address common challenges (such as time management, staying motivated, emotional difficulties, bereavement, and relationship difficulties) as well as developing and presenting leadership courses.

Despite these challenges, the Faculty continued to perform well, and I want to thank each and every one for your contributions.

Research outputs:

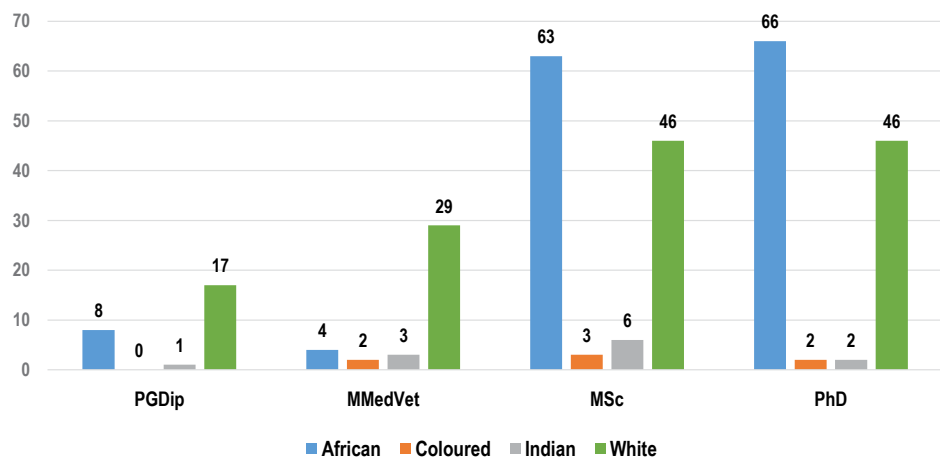
After our record high publication output of 113.84 units in 2020 (exceeding our publication output target of 104 units), we expected to see a similar trend of an increased publication output in 2021. At the time we also expressed concerns that the publication outputs will show a significant drop beyond 2021 due to the delays experienced in field and laboratory research projects. Unfortunately, this drop became a reality already in 2021 with a publication output of 108.35 units.

- The number of publications in the top 10% most CITED publications worldwide (2017-2021) = 187 (11.7%).
- Number of publications in the top 10% JOURNALS worldwide (2017-2021) = 422 (27.1%).
- A total of 63.1% of publications (2017-2021) involved international collaboration, with the top three collaborating institutes (co-authorship) being Utrecht University, Imperial College London and James Cook University.



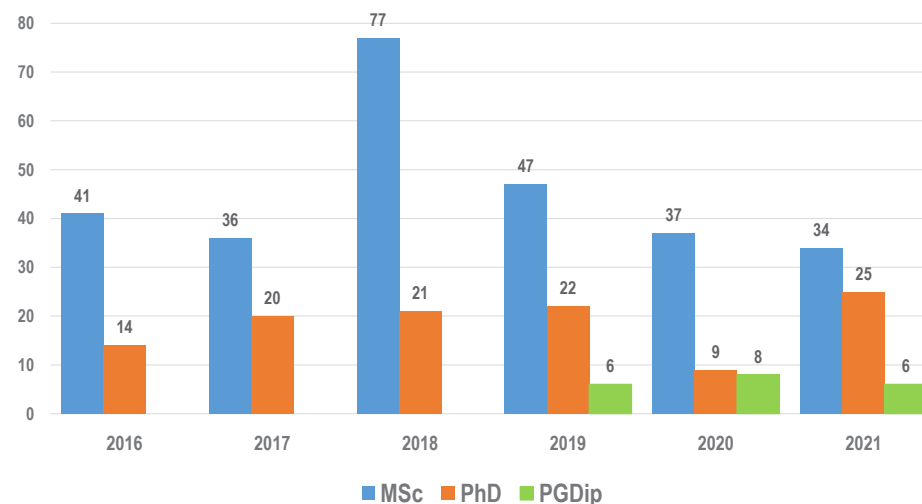
Postgraduate numbers:

For the 2021 registration year, postgraduate students contributed 25% of the total student body. Students from designated groups made up 54% of the total number of postgraduate students, with the group being 57.4% female. The number of postgraduate students and postdoctoral fellows in the faculty in 2021 totaled 294.



Graduations:

In 2021, the faculty awarded a total of 25 PhDs and 34 master degrees, and six postgraduate diplomas at the University's Autumn and Spring graduations.



Research focus areas:

The Faculty is training professionals who will be able to promote animal health, which directly impacts on human health, thereby stimulating economic growth and food security. However, an efficient research programme must remain relevant to the needs of South Africa, but also to a constantly changing international environment. Therefore, a strong research platform is explicitly pursued to continue the growth and development of the Faculty.

Our vision is to create strong internationally recognized research groups in wildlife health, infectious diseases, primary animal health, food production, one health and risk assessment which are already focus areas of the Faculty.

At the same time, it must generate high-impact publications, attract more postgraduate students nationally and internationally, attract competitive and sustainable research funding and escalate the research status of the Faculty.

The Faculty has for the past four years placed much emphasize on four specific research themes which were chosen to establish, achieve and project an African uniqueness and to complement expertise and technology.

New research focus areas:

Although the African continent suffers from one of the highest burdens of infectious diseases of humans and animals in the world, it has the least capacity for their detection, identification and monitoring. In order to strengthen the efficiency of early warning systems, monitoring trends and disease prediction and timely outbreak interventions for the benefit of the national and international community, it is essential that African countries improve their capacity in disease surveillance, recognition (including laboratory competence), monitoring and control.

Ultimately, reducing the risk of disease introduction or spread within animal populations and from animal to human populations, will lead to improved food safety, food security, biodiversity, animal and public health and trade.



African Wildlife Health and Management

- Unique animals of Africa and their management
- Physiology, farming, management, disease management, food safety, disease transmission, innovative surgical management of wildlife conditions/disease



Sustainable Livelihoods and Wellbeing

- Implications of animal diseases on human health and wellbeing
- Bacterial resistance transmission, environmental toxicity, zoonotic diseases, sustainable food production



Pathobiology of Disease

- Study of diseases in animals, including disease epidemiology
- Disease diagnostics, molecular study of disease processes, including descriptions of new pathological agents and/or toxins and the epidemiology of animal disease



Translational Medicine

- The treatment of human and animal diseases
- Development of new chemotherapeutics, vassinology of important veterinary diseases, inflammation and disease

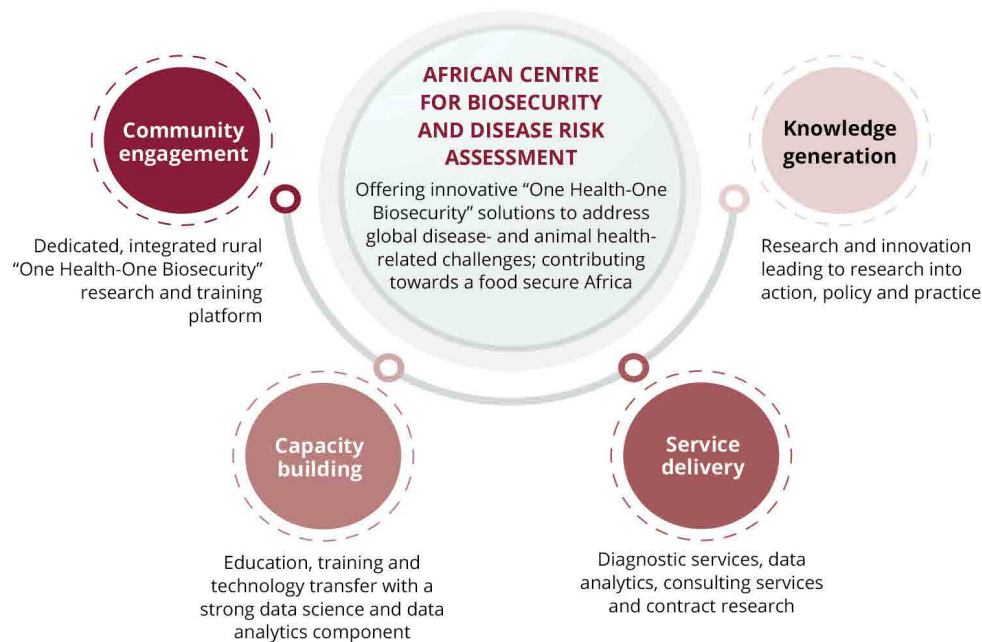
The Faculty has all the relevant epidemiological, clinical, research, and laboratory expertise necessary to assess the risks associated with, and to develop and test surveillance-, diagnostic-, vaccine- and treatment strategies to detect the emergence of animal diseases and animal health-related conditions (including zoonoses, zoonoanthroposis, food-borne diseases, residues and antimicrobial resistance).

This includes assessment on the spread of these diseases and conditions, including the risk of entry into and export from South Africa. We are, therefore, best placed to offer innovative "One Health-One Biosecurity" solutions to the African continent, thereby contributing towards a food secure, healthy and biodiverse Africa.

During 2021, we have worked extensively on conceptualizing the framework of an **African Centre for Biosecurity and Disease Risk Assessment**, which will be a first in Africa. This Centre (which recently received Senate approval) aims to develop Africa's capacity for research and innovation in the field of biosecurity and disease risk assessment by offering innovative and diverse solutions to address global disease- and animal health-related challenges.

Our goal is to advance science, people, and policy; to discover novel approaches for disease intervention and delivery of preventive health care for animals and humans. Our training programmes, research, and transdisciplinary initiatives will ultimately improve the quality of life for animals and humans.

Grounded in four pillars, it cuts across multiple transdisciplinary themes:



Furthermore, the Faculty and the Southern Africa Animal Cancer Association (SAACA) have teamed up to create an **Oncology Centre** for clinical cases and research in animals.

Oncology Unit



- The field of veterinary oncology is a new and exciting field that not only offers new research avenues especially with immunology, but will allow the academic hospital to offer a premier specialist service to our clients.
- And, with the high global burden of cancer in people, the field is multidisciplinary and will allow for better study of cancer pathophysiology from various aspects

Plans for the establishment of an **Aquatic Health Unit** is ongoing – this will ensure improved aquaculture production through optimizing fish health and welfare, capacity development in aquatic and aquaculture, and address local, continental, and globally challenges affecting the aquatic industry.

Aquatic Health Unit



- In recognition of increasing global pressure on food resources, aquaculture is positioned as one of the fastest-growing food production sectors with potential to address issues of food insecurity and economic inequality.
- This unit aims to improve aquaculture production through optimizing fish health and welfare, facilitating capacity development in aquatic and aquaculture sectors, and addressing local, continental, and global health challenges affecting the aquatic industry.

The Faculty furthermore keep on embarking upon several new research areas, such as **Regenerative and Molecular Medicine** and **Smart Diagnostics**.

Increased visibility through media coverage:

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, contributing to an increased visibility of the Faculty. Examples of these can be seen on page 24 - 26 ("Social media HIGHLIGHTS 2021").

IN SUMMARY:

Despite extraordinary challenges we faced due to the COVID-19 pandemic, we are grateful for the continued progress we are making and the successes we are enjoying. It is thus quite appropriate to thank all of you – researchers, postgraduate students and support staff alike – who are making it possible for us to perform at a high level and who are indeed contributing towards the Faculty and the University's goal of being a research-intensive entity.

With my sincere gratitude,

Prof Marinda Oosthuizen
Deputy Dean: Research and Postgraduate Studies







Social media HIGHLIGHTS 2021

In 2021 the Faculty featured extensively in the media and on Faculty and University social media channels. Many of the social media stories were used by mainstream and online external media. Listed below are some of the most important and/or interesting articles and topics that were covered during the course of 2021.

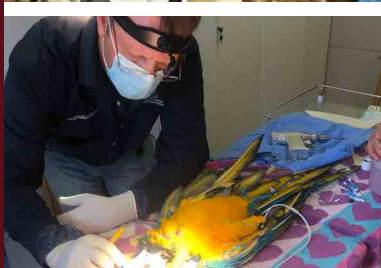
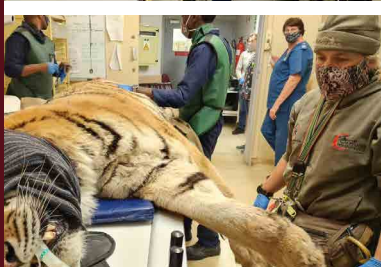
1. In December 2021 the Faculty's Department of Veterinary Tropical Diseases (DVTD) virtually co-hosted the 62nd ITM Colloquium, an annual scientific conference of the Institute of Tropical Medicine in Antwerp, Belgium.
2. On 18 November 2021 the Faculty, in collaboration with the Faculty of Health Sciences and the Faculty of Natural and Agricultural Sciences of the University of Pretoria, hosted a One Health Day Symposium at the Faculty's Onderstepoort Campus.
3. On Thursday, 25 November ten members of the media attended an insightful and exciting networking day at the Faculty.
4. The Faculty celebrated the promotion to full professor of Prof Henriette van

Heerden, researcher in the Department of Veterinary Tropical Diseases on 16 November, when she delivered her inaugural address entitled *'Untangling multi-host, transmission and control dynamics of Anthrax and Brucellosis'*.

5. In October 2021, a team led by Professor Gerhard Steenkamp, veterinary specialist in dentistry and maxillofacial surgery at the Faculty, provided Max, a blue and gold macaw with a fully functional 3D-printed beak.
6. The Faculty and the Care for Wild Rhino Sanctuary celebrated a milestone in veterinary healthcare and diagnostic imaging as well as rhino conservation, when the first ever successful CT scan on a live adult rhino in South Africa was performed in October 2021.
7. Also in October 2021, the Faculty and the Southern Africa Animal Cancer Association (SAACA) teamed up to create an oncology centre for clinical cases and research in animals.
8. In September 2021, Dr Hendrik Swanepoel, a laureate of the joint Faculty/ITM Master of Science in Tropical Animal Health programme won the 2021 Prize for Global Research of the Province of Antwerp for his thesis on viral diseases in African hoofed mammals (ungulates).
9. On 22 September 2021, an important diverse panel discussion entitled *'The future of agriculture and the lessons learnt from COVID-19'* was moderated by Dr Rebone Moerane, HOD: Department of Production Animal Studies in the Faculty. The online discussion was part of the UP Alumni Office's LeadUP: Alumni Thought Leadership virtual series.
10. The Faculty celebrated the promotion to full professor of Prof Lyndy McGaw, Leader of the Phytomedicine Programme in the Faculty's Department of Paraclinical Sciences, when she delivered her inaugural address on 8 September 2021.
11. In August 2021 the Department of Veterinary Tropical Diseases' bovine tuberculosis and brucellosis diagnostic services went live when the Department obtained both SANAS accreditation and Government approval to render a diagnostic service for bovine brucellosis serology (RBT and CFT) and culture for tuberculosis in all animal species.

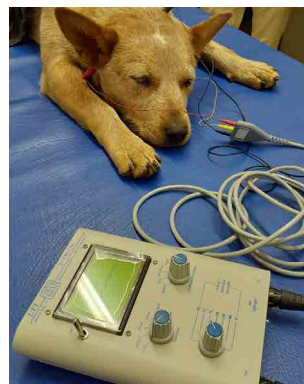
12. Also in August 2021 the Onderstepoort Veterinary Academic Hospital (OVAH) invested in and introduced the latest device to test deafness in dogs, the UFI BAERCOM™ which quickly and painlessly assesses the dog's level of hearing or deafness.
13. In 2021 the OVAH acquired its first magnetic resonance imaging (MRI) scanner and on 26 July 2021 the MRI scanner facility was officially opened by Prof Tawana Kupe, UP's Vice-Chancellor and Principal during an online event.
14. Almost every year the Faculty treats a few tiger patients. One such special patient was Fushan, a nine-year old Siberian tiger from Cheetah Experience in Bela-Bela that was brought to the OVAH in July 2021 to get some dental work done and to assess his hips. Our veterinary experts, among others, Prof Katja Koeppel, Prof Gerhard Steenkamp, Dr Jennie Hewlett and Dr Annette Roug were all on hand to examine and treat the beautiful animal.
15. A team from the Faculty, in collaboration with the Brain Function Research Group (BFRG) at Wits University and the SANParks Veterinary Wildlife Services team, conducted successful research in the Kruger National Park to investigate novel immobilizing drug combinations to facilitate the capture of free-ranging lions for conservation management, monitoring and research purposes. The study formed part of Ashleigh Donaldson's PhD and during the course of three weeks the team - including her supervisors, Prof Leith Meyer, Director of the Centre for Veterinary Wildlife Research, Prof Andrea Fuller, Director of the BFRG and Dr Peter Buss, SANParks Veterinary Senior Manager – assessed the quality of induction, immobilization and recovery of 36 lions immobilized with the various drug combinations.
16. In May 2021 the Faculty's Onderstepoort Veterinary Academic Hospital (OVAH) was selected by Beeld and Netwerk24 readers as their winner in the veterinary category for 2021 and received a Platinum Award.
17. Also in May 2021 Dr Juan Muñoz, then senior lecturer in the Department of Companion Animal Clinical Studies (CACs) at the Faculty, and Dr Felipe Corrêa, equine surgery resident at





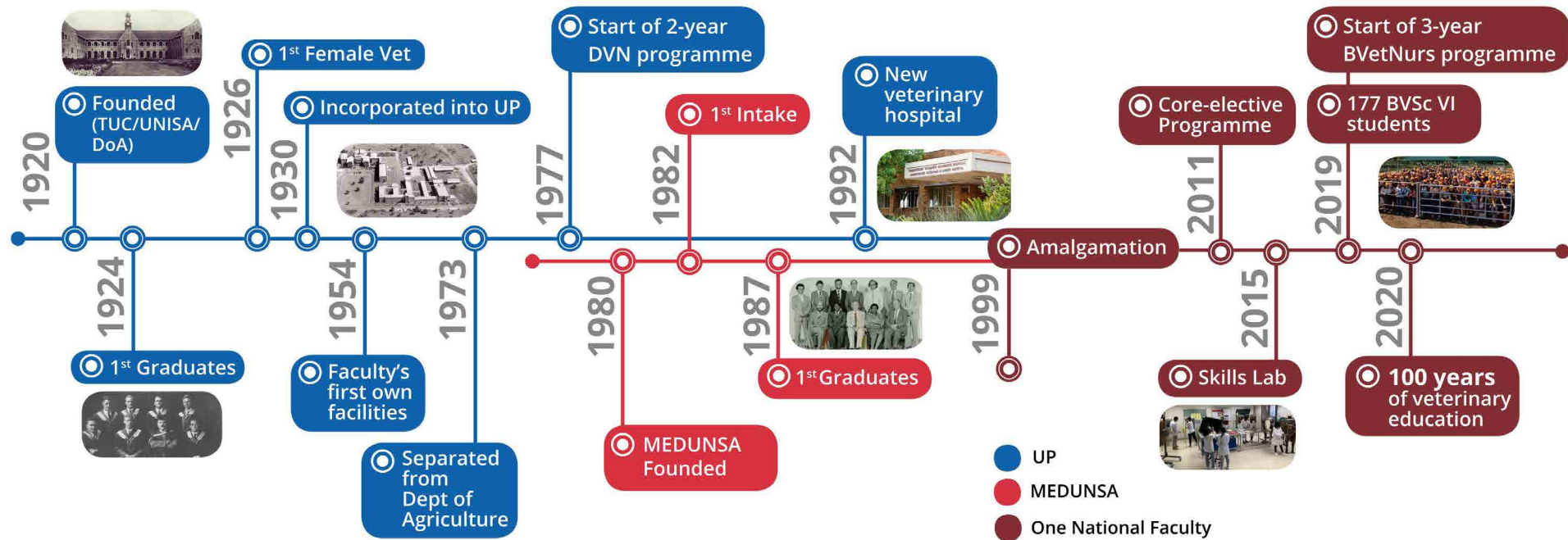
the time performed what was believed to be the first dynamic neuroprosthesis on a horse in South Africa.

18. Staff members and students of the Faculty visited Chimp Eden, the first and only chimpanzee sanctuary in South Africa in May 2021 to perform essential health checks on its 33 chimps housed in night rooms and large semi-wild outside enclosures.
19. In March 2021, Dr Michelle Lewis, veterinarian and lecturer in the Department of Paraclinical Sciences' Pathology Section at the Faculty, used her experience with dolphin post-mortems to produce a video as a learning tool for students and veterinarians alike.
20. A quite popular article about the dangers of lilies to cats by Dr Liesel van der Merwe, senior lecturer and specialist small animal veterinarian in the Faculty's OVAH was published on the Faculty's social media pages in March 2021.
21. Early in March 2021 Dr Elge Bester and Dr Adriaan Kitshoff of the Department of Companion Animal Clinical Studies (CACCS) conducted South Africa's first partial knee replacement on a cat. They used a custom-made artificial groove for the kneecap to glide in, saving the cat from having her right leg amputated.
22. In February 2021, Dr Ned Snelling, Senior Lecturer in the Department of Anatomy and Physiology and Prof Leith Meyer, Director of the Centre for Veterinary Wildlife Research, published a new study on the welfare and survival of Africa's arid-dwelling mammals under the threat of climate change which was utilised on our social media pages.



23. In February 2021, the Association of American Veterinary Medical Colleges (AAVMC) presented their 2021 AAVMC Excellence in Research Award to Dr Juergen A Richt from the Kansas State University College of Veterinary Medicine, and Extraordinary Lecturer in the Faculty's Department of Veterinary Tropical Diseases (DVTD) since 2019. Dr Richt is also presenting the 2022 Arnold Theiler Memorial Lecture.
24. In February 2021, Dr Adriaan Kitshoff and Dr Ross Elliott, specialist veterinary small animal surgeons in the Department of Companion Animal Clinical Studies, performed life-saving heart surgery on two dogs by using a ground-breaking technique, a first in the 100-year history of the Faculty of Veterinary Science.
25. Prof Vinny Naidoo, Dean of the Faculty and specialist in veterinary pharmacology wrote an expert article in January 2021 on the use of the veterinary cattle drug, ivermectin in people against COVID-19 which was widely publicised by social and other media.
26. A widely publicised article written by Dr Rebone Moerane, HOD: Production Animal Studies in January 2021 entitled "*Veterinarians are experienced in dealing with animal pandemics – use our knowledge to fight Covid-19*", argued that veterinary specialists have vital skills gained from dealing with pandemics in the field of animal health, such as rinderpest and foot-and-mouth disease, and could be usefully deployed in the fight against Covid-19.

100 YEARS OF VETERINARY EDUCATION IN SOUTH AFRICA



In 2020, the Faculty of Veterinary Science celebrated a centenary of veterinary education in South Africa. This timeline represents some of the most important developments and highlights in the 100-year history of the Faculty.

In 2021 the OVAH acquired its first magnetic resonance imaging (MRI) scanner.



Faculty Day 2021

Due to the COVID-19 pandemic, a second successful virtual Faculty Day was held on 21 October 2021. A special keynote address entitled *"Science needs to be celebrated – but is that enough?"* was delivered by Prof Tawana Kupe, Vice-Chancellor and Principal of the University of Pretoria while the virtual Arnold Theiler Memorial Lecture was presented by Prof Peter Sutovsky from the University of Missouri in the USA. The title of the lecture was *'Male infertility and semen evaluation: Andrology in the age of precision medicine and agriculture'*. During the course of the day the Dean of the Faculty, Prof Vinny Naidoo also launched a special centenary book to commemorate 100 years of veterinary education in South Africa, celebrated in 2020 but postponed as a result of the COVID-19 pandemic.

Excellence in research performance was recognised at the event with the identification of the Faculty's top 10 researchers as follows:

MSD Animal Health Researcher of the Year

Prof Lyndy McGaw

Nine top researchers in the Faculty

2. Prof Geoffrey Fosgate
3. Prof Vinny Naidoo
4. Prof Anita Michel
5. Prof Leith Meyer
6. Prof Henriette van Heerden
7. Prof Peter Thompson
8. Prof Marinda Oosthuizen
9. Prof Christo Botha
10. Prof Amelia Goddard

OBP Young Researcher of the Year Award

Dr Yolandi Rautenbach

Below are all the other Faculty Day 2021 winners with the sponsor of every prize indicated in brackets:

Undergraduate (VRE) Oral Presenter

Best oral presentation (Sky books)

- Ms Gabriella Naidoo

First time BVSc session chair (Sky books)

- Ms Nina Burger

Postgraduate Oral Presenters

Runner-up: oral presentation (ELANCO)

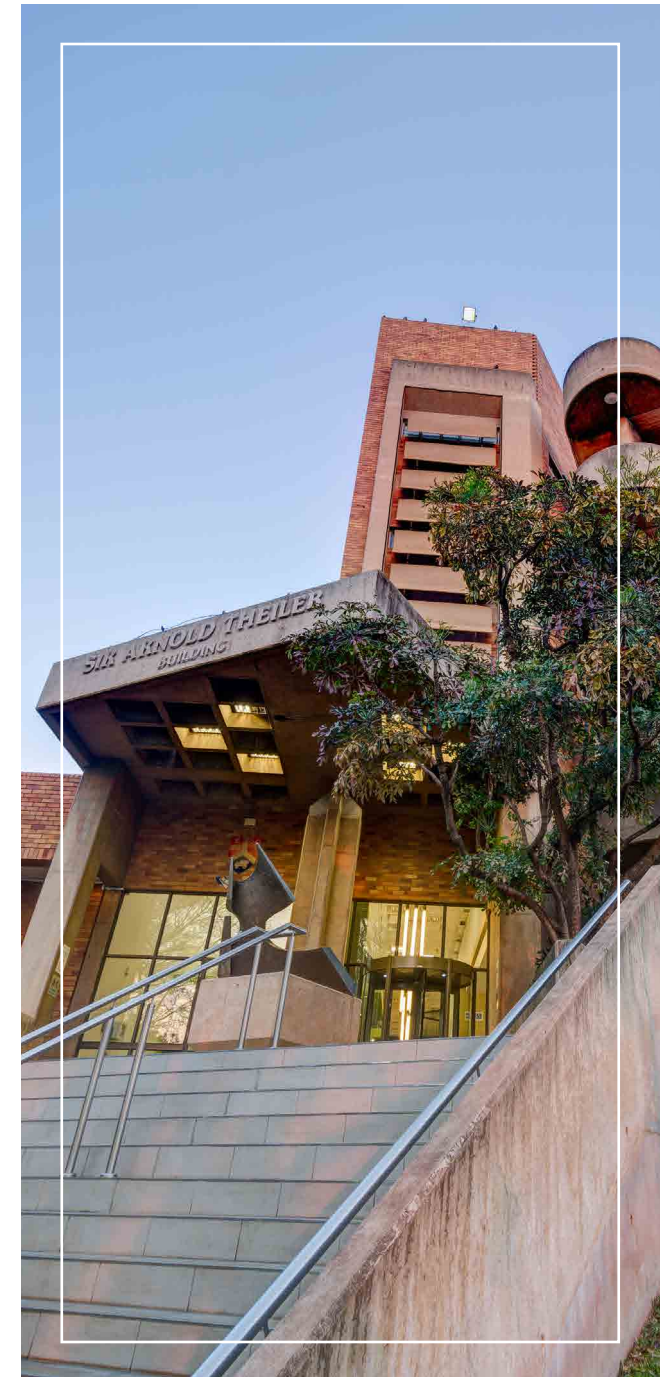
- Dr Chad Berman

Best oral presentation (MSD Animal Health)

- Ms Thembeke Mtetwa

Best speed session presenter (MSD Animal Health)

- Mr Anthony Craig





Session 1:

Postgraduate research



GV Ringani



The effects of nutritional stress and reproduction on stable isotope ratios of ruminants, monogastrics, and hindgut fermenters in South Africa

Pregnancy, lactation, and weaning are energetically demanding periods in the lives of female mammals. Increasing the quantity of food consumed, as well as drawing on body reserves enables lactating females to supply sufficient milk to meet the energetic demands of their young. Stable isotopes of carbon, nitrogen, hydrogen, and oxygen will be used to investigate various aspects of nutrient assimilation and usage of nursing sheep, pigs and rabbits until their offspring are weaned. Carbon isotopes indicate the types of plants (C3 or C4) that formed the diet, while nitrogen is indicative of the protein component of the diet. Hydrogen and oxygen can be used to trace the climatic conditions prevalent when the consumed plants were still growing. The stable isotope composition of animal tissues can be affected by diet quality, fasting, growth rate, and physiological stress. Factors that may cause physiological stress include lactation and the change in the diet of young animals during weaning. The aim of this study is to investigate reproductive, developmental and environmental physiological stresses on monogastrics, ruminants, and hindgut fermenters, through the analysis of naturally occurring stable isotopes in their tissues. The food consumed by nursing ewes, sows and does, as well as the milk produced, will be collected at 14-day intervals. Blood samples will also be collected at 14-day intervals, while fur samples will be collected from the lambs, piglets and kits at 30-day intervals. All the samples will then be processed and a stable isotope mass spectrometer will be used to quantify their isotopic content. The body condition of all the animals, as well as their weights, will be assessed every two weeks.

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P Pazzi



Prospective pathological evaluation of the prevalence of thromboemboli & associated haemostatic dysfunction in canine carcinomas & sarcomas

Haemostatic abnormalities, including disseminated intravascular coagulation (DIC) and thromboembolic complications, are common in people with cancer. Knowledge of the prevalence of thromboemboli and haemostatic status in canine carcinoma and sarcoma might allow earlier intervention and a more favourable prognosis. Prospectively estimate the prevalence of thromboembolic disease in dogs with carcinomas and sarcomas and its association with DIC, and estimate the predictive value of haemostatic variables for thromboembolic disease in dogs. Thirty two dogs with sarcoma, 30 with carcinoma and 20 healthy age-controlled dogs. A haemostasis panel (platelet count, thromboelastography, fibrinogen and D-dimer concentration, factor X, VII and antithrombin activity) was performed in all dogs. Tumour-bearing dogs underwent complete post-mortem and histopathological evaluation for micro- and macrothrombi. Comparisons between healthy dogs and tumour-bearing dogs with and without intracavitary haemorrhage; and tumour-bearing dogs with and without microthrombi were analysed. Thromboembolic disease was identified in 32/62 (52%) tumour-bearing dogs. Microthrombi were identified in 31/62 (50%) dogs, 18/32 (56%) with sarcoma and 13/30 (43%) with carcinoma; 21/31 (68%) had exclusively intra-tumoural microthrombi, 5/31 (16%) had both intra-tumoural and distant microthrombi and 5/31 (16%) had exclusively distant microthrombi. Macrothrombi were identified in three dogs. Compared to healthy dogs, procoagulant activation, inhibitor consumption and fibrinolytic activity consistent with overt and non-overt DIC were identified in tumour-bearing dogs with and without intracavitary haemorrhage, respectively. D-dimer concentrations were significantly higher ($P=0.024$) and platelets significantly lower ($P = 0.028$) in tumour-bearing dogs with microthrombi compared to tumour-bearing dogs without microthrombi. A D-dimer cut-off of 500ng/mL for the prediction of the presence of microthrombi had an estimated sensitivity of 80% and specificity of 41%. Microthrombi are prevalent and positively associated with higher D-dimer concentration in dogs with carcinomas and sarcomas. Non-overt and overt DIC was identified in tumour-bearing dogs and most likely contributes to microthrombi formation.

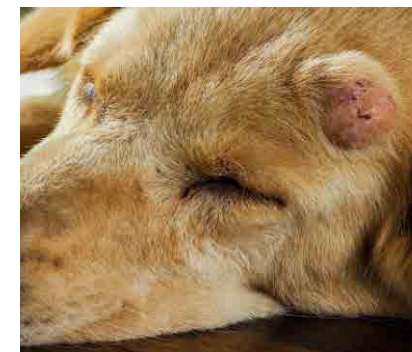
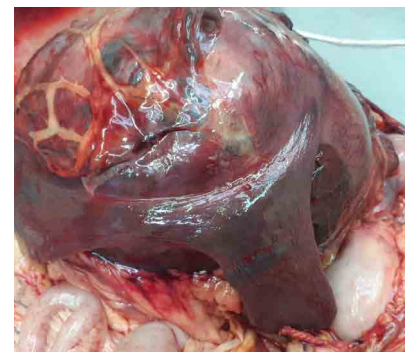
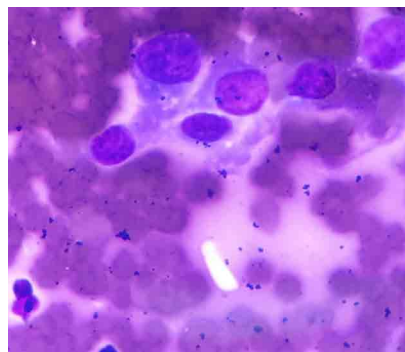
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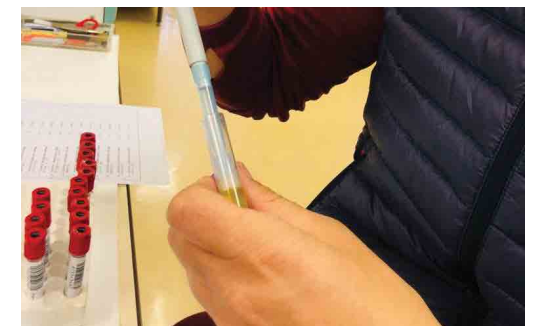
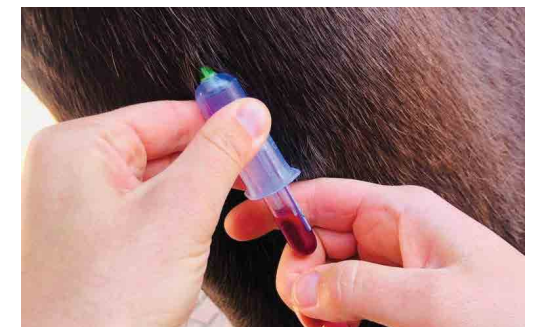
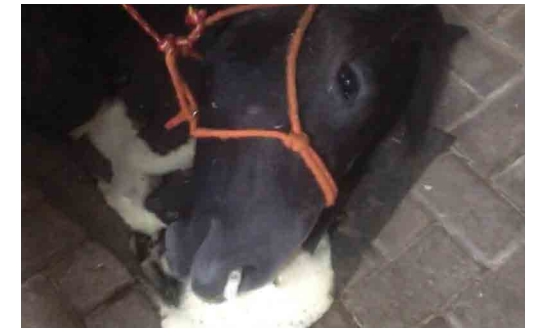


E-C Schliewert



Investigation of the inflammatory response in African Horse Sickness

African Horse Sickness (AHS), a highly infectious disease in equids, is caused by the African Horse Sickness Virus (AHSV). Infection results in high morbidity and mortality in naïve horses. AHSV primarily infects macrophages and endothelial cells and pathogenesis is related to endothelial cell damage with loss of endothelial cell barrier function resulting in oedema, effusion, and haemorrhage. The nature and role of the immune system and inflammatory cascade have only been investigated *in vitro*, in limited studies. The aim of this study was to evaluate the *in vivo* inflammatory response in AHSV infection. Four Boerperd-cross horses negative for AHSV antibodies were infected with AHSV, and blood was obtained every 12 hours throughout the course of the disease until humane euthanasia. Complete white blood cell count (WBC) with manual differentiation, serum amyloid A (SAA) and iron concentration, and cytokines IL-1 α , IL-2, IL-6, IL-8, IL-10, IL-12, IL-17, IFN- γ , TNF- α , and MCP-1 were measured. All horses developed severe clinical signs typical of AHS and were humanely euthanized. Statistically significant changes were observed in WBC; however, these were not deemed clinically relevant. Acute phase reactants SAA and iron also changed significantly but changes were unexpectedly mild and delayed. Substantial changes in cytokine concentration were only observed in IL-10 and TNF- α . IL-10 increased up to 150-fold, TNF- α up to 30-fold towards the final stages of the disease. All other cytokines remained under the detection limit or only displayed minor increases that were inconsistent over time and between horses. Despite severe clinical signs, none of the horses mounted an effective inflammatory response indicated by the lack of an inflammatory leukogram and the subtle changes in acute phase reactants. The lack of a proinflammatory cytokine response is unexpected and these findings suggest viral interference with the host innate immune system resulting in an muted inflammatory response.



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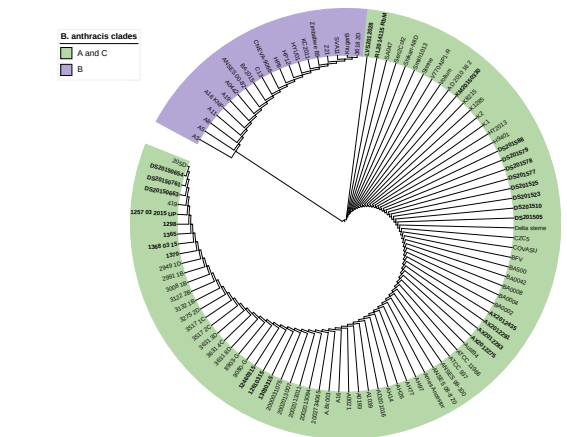
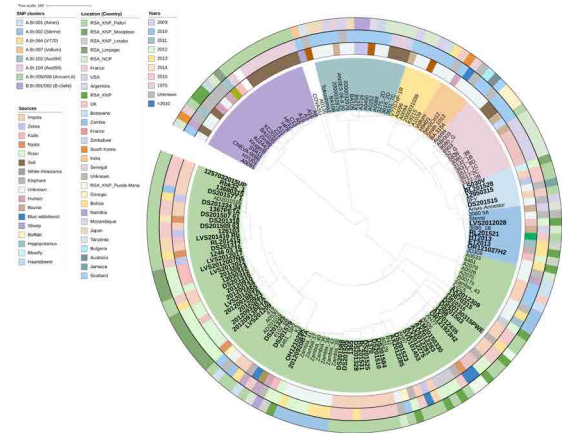
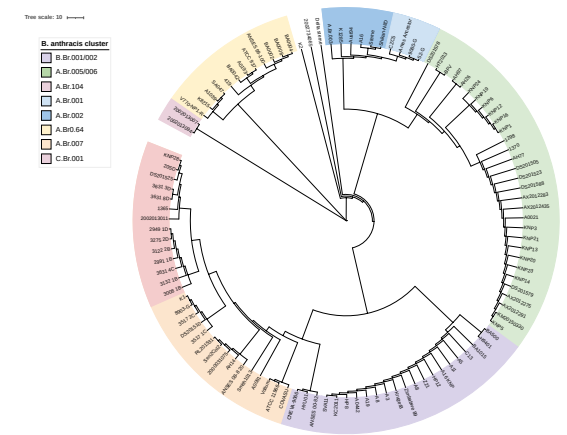


SP Mokgokong



Comparative genomics of *Bacillus anthracis* strains from anthrax outbreaks in Kruger National Park, South Africa

Anthrax is a zoonotic disease caused by a Gram-positive soil bacterium, *Bacillus anthracis*. In South Africa, anthrax is endemic to the Pafuri region of the Kruger National Park (KNP). The genetic structure of *B. anthracis* in KNP consists of distinct A and the rare B clade strains. A whole genome sequencing (WGS) approach was used to survey outbreaks from 2012 to 2015 in KNP. *Bacillus anthracis* isolates (n=80) from animal carcasses and environmental samples were compared to isolates from previous outbreaks and data from GenBank. Whole genome single nucleotide polymorphism (wgSNP) and pan-genomics analysis defined the *B. anthracis* genetic structure and characterized A- and B-clade genomes. Comparative genomics also determined antibiotic resistance profiles amongst the genomes. This study revealed that KNP has diverse strains grouping in the ubiquitous A.Br.005/006 (Ancient A) SNP lineage. The 2012 to 2015 anthrax isolates are dispersed amongst minor sub-clades that present a non-stabilized genetic population, augmented with novel parsimony informative SNPs of the B. anthracis strains across minor sub-clades in the Ancient A clade. Pan-genome analysis identified unique accessory genes that distinguish the A- and B- clade isolates. These included cell wall biosynthesis genes, prophage genes, antibiotic resistance genes and genes involved in the exosporium germination. Genetic differences in the tryptophan operon (*trpA*) determine the survival of the A- and B-clade strains. The study has revealed that comparative genomics using whole genome SNP analysis and pan-genomics are powerful tools for understanding the evolution of *B. anthracis* and for epidemiological surveillance of global anthrax outbreaks.



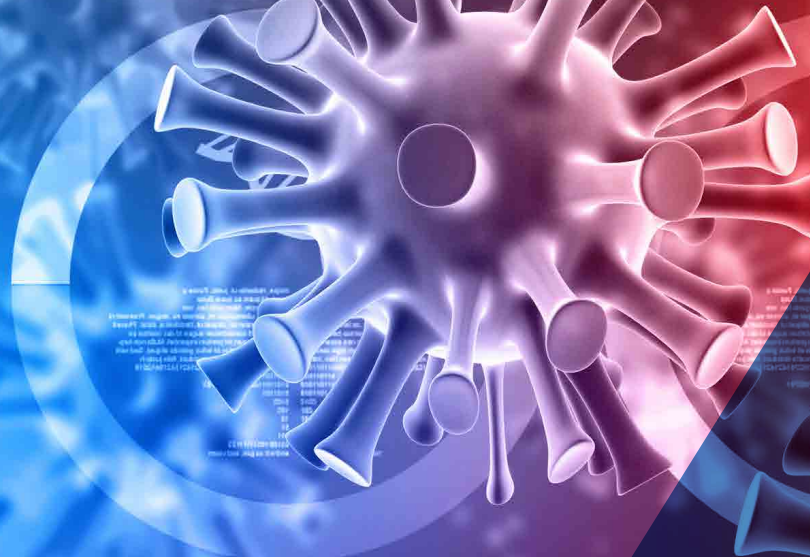
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Session 2: Undergraduate research



M. Duwe

M. Duwe¹, C. Steyn,^{1,2}

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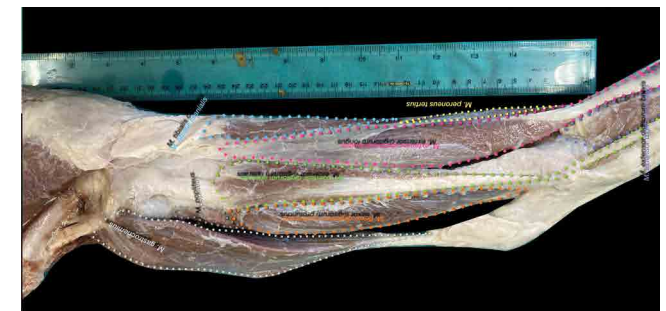
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Pelvic limb myology of the South American tapir (*Tapirus terrestris*)

The South American tapir (*Tapirus terrestris*) is one of four extant tapir species that, together with equine and rhinoceros' species, belongs to the *Perrisodactyla* order. This tapir, also known as the Brazilian tapir, is a herbivore which is well-adapted to swimming and diving. It is the largest indigenous terrestrial mammal in the Amazon and its numbers are in decline due to poaching and deforestation. The pelvic limb myology of tapir species has not been described previously. One Brazilian tapir, which originated from a private game farm, died from a chronic bacterial infection. A full post-mortem was performed and thereafter one pelvic limb was donated for this study. The limb was immersion fixed in 10% neutral-buffered formalin. The pelvic limb muscles were dissected and described. Relevant ethical approval was obtained. The tapir possesses three digits, similar to the rhinoceros. Very thick skin and fascia are present over the entire pelvic limb. Proximally, the muscles are very fibrous and similar in arrangement to equine species. Distally, the muscles are fleshier. The axial and abaxial digits (II and IV) are extended by tendon slips from the *M. extensor digitorum longus* while the middle digit (III), which bears the majority of the animal's weight, is only extended by the *M. extensor digitorum brevis*. The thick skin and fascia are thought to play a role in the weight-bearing capacity of the limb. An understanding of pelvic limb myology may assist wildlife practitioners in treating injured tapirs.





N Tar



Diagnostic accuracy of cytological examination for the identification of neoplasia in the OVAH Clinical Pathology laboratory

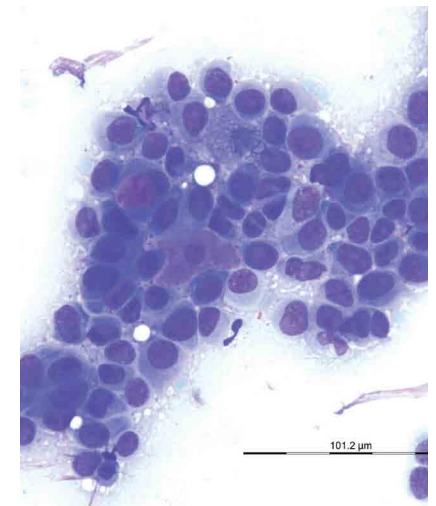
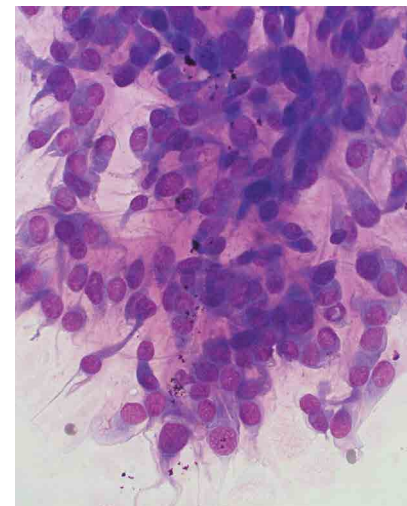
Cytology is a valuable and rapid tool used to aid the diagnosis of neoplastic, non-neoplastic and inflammatory lesions. Agreement between cytology and histopathology (considered the gold standard) differs between organ systems and laboratories and measures of diagnostic accuracy of cytology are important quality assurance indicators for cytologists and clinicians alike. Quality goals for the cytologic diagnosis of neoplasia in human medicine are: absolute sensitivity >60%, complete sensitivity >80% and specificity >55%. The aim of this study was to determine the diagnostic accuracy of cytology for the identification of neoplasia in dogs in the Clinical Pathology Laboratory of the OVAH. This retrospective study used 150 corresponding cytology and histopathology reports from OVAH canine patients, from 2018-2021. Results for each modality were categorized as non-diagnostic (C1), benign (C2), atypical (C3), neoplastic with tissue type unknown (C4) or neoplastic with tissue type known (C5 epithelial, mesenchymal or round cell). C1 samples were excluded from further analysis. Measures of diagnostic accuracy (absolute and complete sensitivity, specificity, predictive values (PV), accuracy) were calculated using 2x2 tables and standard formulae. 6.9% of samples were non-diagnostic. Overall agreement was 69.3%. For the diagnosis of neoplasia (C4+C5), complete sensitivity of cytology was 84.5%, specificity 84.3%, positive PV 89.5%, negative PV 76.8% and accuracy 84.1%. The absolute sensitivity of cytology for C5 epithelial was 74.4%, for C5 mesenchymal was 70.8%, and for C5 round cell was 81.8%. Diagnostic categories were the same in 79.7% of cases from cutaneous and subcutaneous lesions while the highest percentage of discordant cases were from spleen (56.2%) and lung (75%). Measures of diagnostic accuracy exceeded quality goals, and predictive values were high. These results are similar to those reported in other veterinary cytology studies. Cytological analysis performed in the OVAH clinical pathology laboratory is useful and accurate for diagnosing neoplasia in canine patients.

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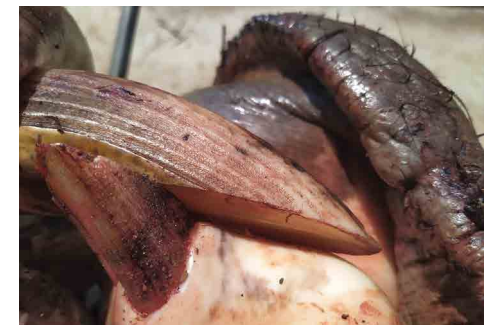


M Engelbrecht



Characterisation of bacterial isolates located in the oral cavity of free-ranging hippopotami (*Hippopotamus amphibius*)

This study aimed to identify the normal flora in the oral cavity of *Hippopotamus amphibius*. It created a foundation for an upcoming study that involves antibiogram sensitivity and resistance testing. The follow-up study will identify suitable antibiotics for hippopotamus bite wounds in human patients. Oral swabs were collected from 34 hippopotami that were culled in the southern Kruger National Park and Timbavati Private Nature Reserve, which is part of the Greater Kruger National Park Complex. Conventional bacteriology, including primary and secondary bacterial identification, was performed in the Department of Veterinary Tropical Diseases Bacteriology Laboratory at Onderstepoort, Pretoria. The samples were analysed to identify all the bacterial species present in the swab samples. The main aerobic bacterial species that were isolated include *Aeromonas hydrophila*, *Shewanella putrefaciens*, *Aeromonas sobria*, and *Vibrio parahaemolyticus*. There were only two types of anaerobic bacteria isolated, namely *Prevotella melaninogenica* and *Clostridium* spp. Most of the aerobic bacteria that were isolated are gram-negative bacteria that inhabit the aquatic environment. It has been found previously that both *Aeromonas hydrophila* and *Shewanella putrefaciens* are highly pathogenic and can cause severe wound infections and complications. These two bacterial species may also cause bacteraemia. Most of the isolated bacteria, including the anaerobes, have previously been identified in dog and cat bite wounds. Therefore, existing treatment protocols for dog and cat bite wounds, including wound management and antibiotic treatment, may be adapted for the treatment of hippopotamus bite wounds.



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S Baxter



The use of client-created video clips in testing the effectiveness of an over-the-counter product in the management of thunderstorm phobia in dogs

Noise phobia is a common behavioural condition in dogs and is especially prevalent in areas such as Gauteng where there are many thunderstorms. Thunderstorm phobia in dogs can lead to a decrease in the animal's well-being as well as an increase in owners' anxiety. There are many different methods and products to pharmacologically manage thunderstorms and other noise phobias. Many different methods have been utilized to test the effectiveness of treatments for noise phobias ranging from double-blind placebo-controlled studies to client questionnaires. Gahwiler et al. (2020) utilized citizen science in the form of client-taken video clips and concluded that this method allowed one to objectively assess situations in real-time. This is advantageous as thunderstorms are difficult to recreate for testing purposes as there are many elements associated with thunderstorms such as thunder, lightning, and changes in atmospheric pressure. This pilot study utilises citizen science in the form of owner-taken video clips and questionnaires to test the effectiveness of over-the-counter medication in alleviating the expression of fear and anxiety in dogs with thunderstorm phobia. The study aims to test this novel methodology of using owner-made video clips to assess behavioural medications in the dog's own environment and secondarily aims to test the effectiveness of the medication. Five dogs partook in the study, and all showed at least mild improvement in their fear and anxiety scores when scored both by the owners and researcher. It was also noted that clients more readily filled in multiple choice answers compared to open-ended questions, leading to a lack of qualitative data about the individual storms. The methodology used was user-friendly, however, the format of conveying the data to the researcher would need to be improved to make it more cohesive for larger studies.



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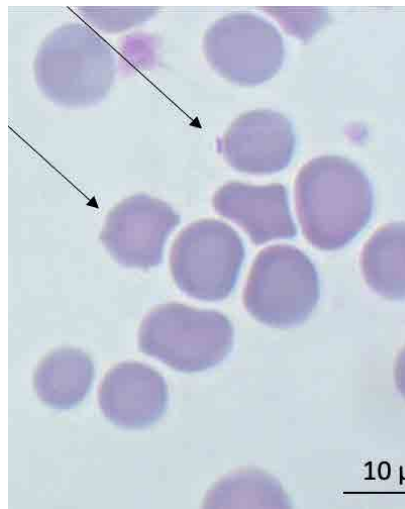
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Mycoplasmosis in black-footed cats (*Felis nigripes*): diagnosis, treatment, and prevention

Mycoplasmosis has been associated with severe regenerative anaemia in felids. This is a case study of a black-footed cat (*Felis nigripes*) named Hercules, who was presented to the Onderstepoort Veterinary Academic Hospital (OVAH) between January and March 2021 and includes information about another *F. nigripes* kitten that died of mycoplasma infection at the OVAH in 2019. The aim was to provide an overview of the disease course, diagnosis, treatment, and prevention. Hercules was diagnosed with renal disease on 14/01/2021 and was given supportive therapy. Though regenerative anaemia was noticed on 29/01/2021, it was only on 09/02/2021 that haemoplasma species were identified and antibiotic treatment was started. Diagnosis by visual identification of mycoplasma organisms is definitive but has very low sensitivity. The gold standard is a polymerase chain reaction assay. Other diagnostic techniques include haematological parameters and DnaK protein assays. The treatment for mycoplasmosis, in this case, was somewhat successful, as none was detected on PCR after prolonged doxycycline treatment. The recommended treatment plan is doxycycline at 5 mg/kg BID orally for 28 days followed by marbofloxacin at 2 mg/kg OID orally for 14 days. From the literature, predisposing factors include ectoparasite infestation, warmer climates, outdoor access, genetics, and immunocompromise through disease or immunosuppressive drug administration. Possible routes of infection are aggressive interactions, haematophagous arthropods, blood transfusions, and rodent blood and faeces. Despite small improvements, Hercules degenerated and was euthanised on 05/03/2021. The cause of death determined on post-mortem was renal failure due to severe renal amyloidosis, common in captive black-footed cats. Amyloidosis may have predisposed to mycoplasmosis, which in turn caused severe regenerative anaemia.



SIR ARNOLD THEILER
BUILDING



Session 3:

Arnold Theiler Memorial Lecture

Lectori Salutem

Prof Juergen A Richt

delivered the
Sir Arnold Theiler Memorial Lecture
 on the occasion of
Faculty Day 2022
 of the Faculty of Veterinary Science,
 University of Pretoria, Onderstepoort

We honour an esteemed colleague, scientist and
 researcher for his outstanding contribution to the
 promotion of science

20 October 2022

Date

J. Richt
 Dean



Faculty of
 Veterinary Science

Fakulteit Veeartsenykunde
 Lefapha la Disaense tša Bongakadiruiwa

100
YEARS
 OF VETERINARY EDUCATION



Session 4:

Postgraduate research



A Olawole



Prevalence and characterization of Shiga toxin-producing *Escherichia coli* in dairy cattle in South Africa

Shiga toxin-producing *Escherichia coli* (STEC) is a foodborne pathogen that has been associated with human disease characterized by watery diarrhoea, bloody diarrhoea and complications including hemorrhagic colitis and the hemolytic uremic syndrome (HUS). STEC is defined by the possession of two main Shiga toxin genes. Furthermore, some STEC strains may carry intimin and hemolysin which are responsible for the attachment of STEC on the intestinal epithelium and causing bloody diarrhoea. The aim of this study was to determine the occurrence of STEC in dairy cattle, in South Africa. A total of 771 faecal samples from dairy cattle were screened for STEC using microbiological culture and polymerase chain reaction (PCR). Furthermore, 355 STEC isolates were characterized by serotype and major virulence genes (*stx1*, *stx2*, *eaeA* and *hlyA*). STEC was found in 42.2% (325/771) of dairy cattle faecal samples. The distribution of major virulence genes among the 355 STEC isolates was as follows: 14.6% (52) isolates carried *stx1* only, 28.2% (100) isolates carried *stx2* only while both *stx1* and *stx2* were found in 57.2% (203) of isolates. Furthermore, *eae* and *hlyA* genes were detected in 12.9% (46) and 68.2% (236) of the isolates respectively. The serotyping of the STEC isolates yielded 51 distinct serotypes which comprise 35 O-serogroups and 19 H (flagellar) types. Serotype O82:H8 (27.3%) was the most prevalent in the dairy cattle investigated in this study. Serotypes from the major STEC serogroups (O157, O26 and O103) which are commonly associated with human disease accounted for 9.6% of the isolates. STEC O8:H2 and O8:H19 which have been associated with HUS in Europe were also detected in this study. The findings showed dairy cattle in South Africa are an important reservoir and may be a source of virulent strains of STEC that are pathogenic to humans. These data underscore the need for further molecular investigation of STEC isolates from dairy cattle to assess their full virulence potential and capacity to cause disease in humans. This will further enhance the strategy to minimize the risk of food contamination with these pathogens and their threat to public health.

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KM van Boom



The effect of glycine supplementation on the identified urinary metabolites in captive cheetahs (*Acinonyx jubatus*)

Cheetahs are a vulnerable species and their conservation is of global importance. In captivity, cheetahs are prone to a wide range of gastrointestinal diseases that are believed to be linked to the captive diet. In the wild, cheetahs eat whole carcass diets rich in collagen. This is not always feasible in captive settings resulting in most facilities feeding their cheetahs only raw meat. Glycine has many physiological functions in mammals, most notably its role in collagen synthesis, and anecdotal evidence showed that diet supplementation may improve gastritis. Thus, this study aimed to determine the effect of glycine supplementation on the metabolism of the cheetah. Ten healthy male and female captive cheetahs were fed either a meat-only or glycine-supplemented diet for four weeks, followed by a 4 weeks cross-over. Urine samples were collected at baseline, four weeks (diet 1) and eight weeks (diet 2). Untargeted H^1 nuclear magnetic resonance analysis was performed followed by the appropriate spectra, data and statistical analyses. A total of 151 metabolites were identified in the urine with 10 metabolites highlighted as important contributors to the diet. Specifically, the relative concentration of dimethyl sulfone and trimethylamine, both linked to the gut microbiome, were lower in the glycine diet compared to the meat-only diet. This decrease may be linked to the intermediary role of glycine in methionine and choline metabolism, reducing pressure on these pathways, and leading to a decrease in detrimental decomposition products of gut bacteria. The identified metabolites play various potential metabolic roles in the cheetah. Glycine supplementation may be a simple, inexpensive method to improve gastrointestinal health in captive cheetahs.

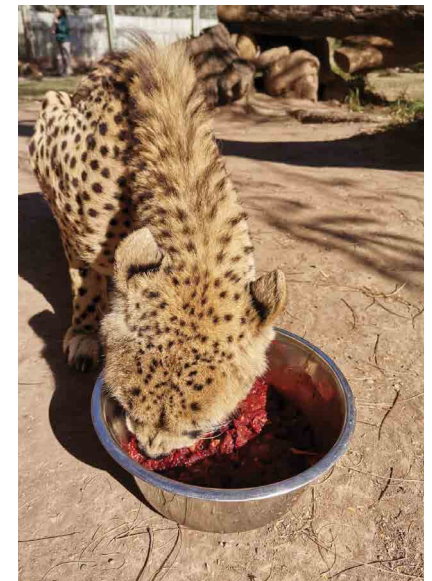
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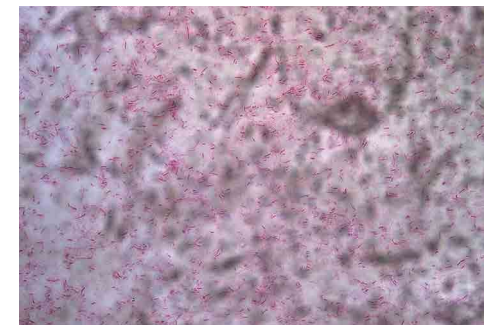
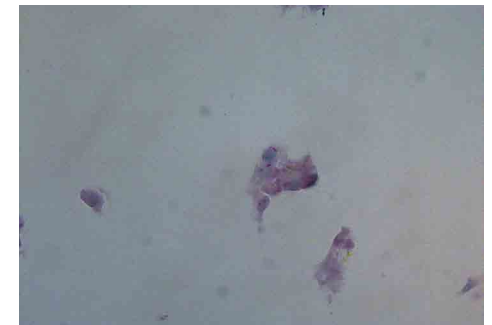
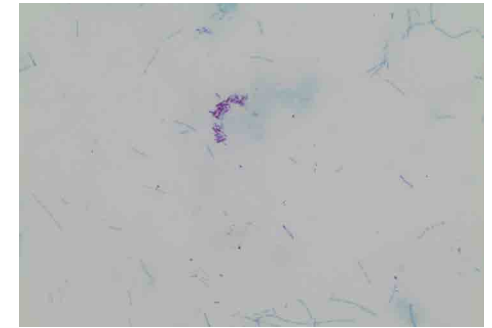


A Jonker



Bacterial and fungal causes of domestic ruminant abortion in South Africa

Abortions of domestic ruminants (cattle, sheep, goats) represent important economic losses in the agricultural industry. Determining the cause of abortions is important for control efforts, but it can be challenging. The aim of this study was to investigate detection methods for and the significance of bacteria and fungi as agents of abortion in domestic ruminants in South Africa. Retrospective data was collected by a keyword search of clinical cases (2006-2016) in pathology and bacteriology laboratory registers at the Faculty of Veterinary Science, Onderstepoort, and electronic data from Vetdiagnostix laboratory. Samples from 135 abortion cases (2017-2019) were analysed during a prospective study by a combination of conventional bacteriology methods: an examination of wet preparations and impression smears; and aerobic, *Brucella*, anaerobic, microaerophilic, *Leptospira*, *Mycoplasma* and fungal culture. Primers and probes for detection of the Chlamydiales, *Chlamydia abortus*, *Chlamydia pecorum*, *Parachlamydia acanthamoeba* and *Waddliachondrophila* were selected from literature and combined to create quantitative real-time PCR (qPCR) assays. These assays were optimized and employed to analyse samples from 25 cases, with placentitis and/or pneumonia lesions, selected from the 135 cases above. The retrospective study reported 288 cases from six provinces. The diagnostic rate was 35.1%. *Brucella* species were reported in 7.3% of cases. From 2017 to 2019, 135 cases from six provinces were analysed. The diagnostic rate was 42.2%. *Brucella* species were isolated from 7.4% of cases. The qPCR assays detected Chlamydiales in 60% of cases with placentitis and/or pneumonia. *Waddlia chondrophila* was detected in bovine and ovine, and *C. pecorum* in ovine cases. Increased submission of placenta, and additional conventional and molecular diagnostic methods can improve diagnostic rate. The significance of *Brucella abortus* as a bovine pathogen in South Africa was highlighted. Detection of *C. pecorum* and *W. chondrophila* indicate that these bacteria play a previously unknown role in abortions.



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KM Sepotokele



The production of a plant-expressed virus-like particle vaccine against infectious bronchitis coronavirus and efficacy in chickens

Infectious Bronchitis Coronavirus (IBV) is a gamma-coronavirus causing severe respiratory disease in chickens and other poultry species. IBV has a high mutation ability which leads to the continuous emergence of new variants that make it difficult to control using normal vaccine programs. Most commercial vaccine types do not offer cross-protection against heterologous challenge with variant IBV strains. Inactivated autogenous vaccine types such as virus-like particles (VLPs) which are based on variant strains of IBV offer broad-spectrum protection against multiple strains of the virus. For this research, the IBV spike protein was modified to substitute its original transmembrane domain and cytosolic tail with that of the Fusion protein of Newcastle Disease Virus (LaSota strain). Two consecutive proline mutations were substituted into the spike protein's S2 subunit to stabilise the prefusion state in order to assist with protein expression and improved immunogenicity. The modified spike protein was co-expressed with the matrix protein of NDV in *Nicotiana benthamiana* plants in order to assemble IBV VLPs using a plant expression system. The size and morphology of the resultant VLPs were similar to native IBV particles. A single dose immunogenicity study revealed that the plant-produced IBV VLP vaccine was able to elicit high titres of spike-protein specific antibodies in SPF chickens ranging between 9.1 log₂ and 10.5 log₂ after just two weeks. In a challenge study against the live virus, the VLP vaccine elicited immune responses comparable to those elicited by a combined IBV Ma5 and IBV 4/91 live vaccine cocktail. It was also able to effectively protect 100% of the chickens against damage to the tracheal cilia and reduce both cloacal and oropharyngeal viral shedding. The vaccine caused no observable adverse side effects in the chickens. This vaccine has major potential for the global poultry industry as it has the potential to offer broad-spectrum protection against emerging IBV variants.

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Session 5:

Postgraduate research



JD Chetty



Effects of developmental genistein exposure on anxiety and depressive-like behaviour in rat offspring

Endocrine-disrupting chemicals (EDCs) possess similarities, both molecularly and structurally, to endocrine chemicals (hormones). Phytoestrogens, a group of EDCs, can bind to oestrogen nuclear receptors alpha and beta and the cell surface oestrogen receptor, G-protein coupled oestrogen receptor (GPER), leading to either physiologically beneficial or adverse effects, depending on the receptor type and cellular context. Developmental exposure to EDCs has been implicated in many negative effects including lower birth weights, reproductive defects, as well as neurological implications, such as aggression. The phytoestrogen genistein is well-known for its beneficial effects in cancer treatment due to its anti-proliferative action. However, when exposed developmentally, it may have negative consequences on the developing foetal brain and may interrupt neural networks. Therefore, the aim of this study is to investigate the effects of developmental genistein exposure on cognitive function by evaluating anxiety and depressive-like behaviour in rat offspring. Pregnant Sprague-Dawley rats will be exposed to genistein (10mg/kg; 50mg/kg; 100mg/kg) at two timelines: developmental exposure during gestation and lactation (GND7 - PND21), and lifetime exposure (GND7 - PND85). Control groups with no exposure to genistein will also be included. Behavioural tests will be conducted on the offspring at adulthood (PND 86-89). The Sucrose Preference and the Elevated Plus Maze tests will be utilized in the present study. By measuring the preference for a sucrose solution, the Sucrose Preference Test assesses anhedonia which is a common symptom of depression. The elevated plus maze test evaluates a preference for enclosed spaces, which is indicative of anxiety-like behaviour. Upon sacrifice, blood and brain tissue will be collected for neurochemical analysis. The stress hormone, corticosterone, will be measured, and western blots will be performed to assess the expression of the proteins neuropeptide Y (NPY) and brain-derived neurotrophic factor (BDNF), both of which have been implicated in anxiety and depression.

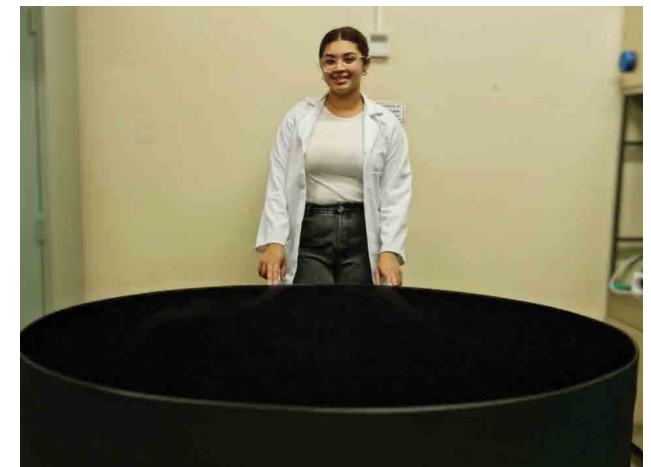
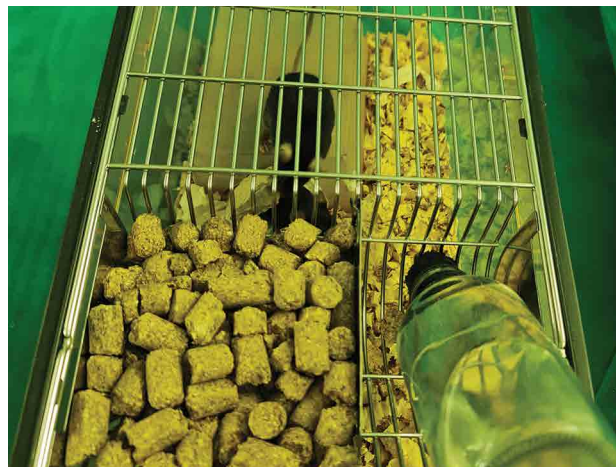
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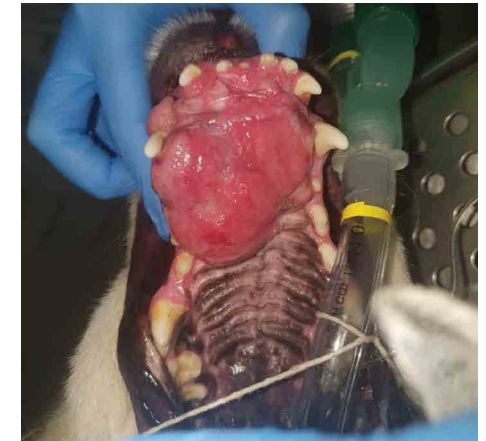
AJ Rixon



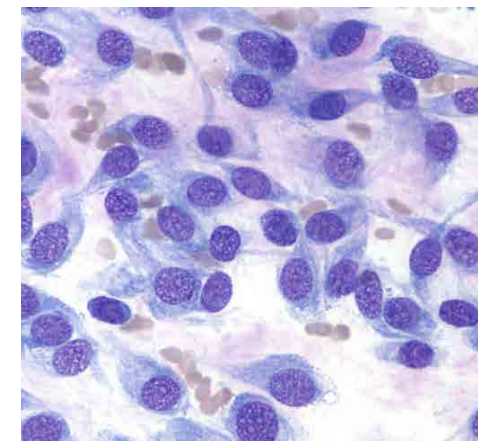
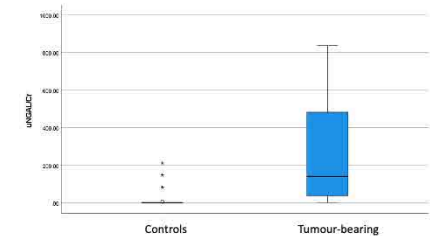
Neutrophil gelatinase associated lipocalin and symmetrical dimethylarginine in dogs with carcinoma or sarcoma

The specificity of elevations in the renal biomarkers neutrophil gelatinase-associated lipocalin (NGAL) and symmetric dimethylarginine (SDMA) is questioned in humans and dogs with tumours. Studies evaluating biomarker concentrations in dogs with carcinoma, sarcoma and metastatic disease in light of renal histopathology are lacking. Determine the influence of tumour presence, tumour type and metastatic disease on sSDMA, sNGAL, uNGAL and uNGAL/Cr concentrations in dogs with carcinoma and sarcoma. Twenty three dogs with carcinoma, 25 dogs with sarcoma and 20 healthy age-controlled dogs.

Methods: Serum SDMA, sNGAL, uNGAL and uNGAL/Cr concentrations were prospectively determined on banked serum and urine samples and interpreted in a review of patient medical and renal histopathology records. Dogs with renal azotaemia or evidence of moderate to severe histopathological evidence of renal disease were classified as having a significant renal disease. Biomarker concentrations were compared between tumour-bearing dogs, without significant renal disease, and healthy age-controlled dogs. Additionally, tumour-bearing dogs without significant renal disease with carcinoma were compared to those with sarcoma, and dogs with metastasis to those without metastasis. Tumour-bearing dogs without significant renal disease had significantly elevated uNGAL ($P < .001$) and uNGAL/Cr ($P < .001$) concentrations as compared to healthy age-controlled dogs, while sNGAL and sSDMA were not significantly different between the two groups. Biomarker concentrations did not differ between tumour-bearing dogs with carcinoma or sarcoma or between dogs with and without metastasis. uNGAL/Cr concentrations, but not sNGAL or sSDMA concentrations, are influenced by tumour presence in dogs with carcinoma and sarcoma. Metastasis and tumour type do not influence biomarker concentrations.



uNGAL/Cr in tumour-bearing dogs vs controls



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SM Makgabo



Identification of *Anaplasma* species in wild animal species in the Kruger National Park and surrounding game reserves using a bacterial microbiome approach

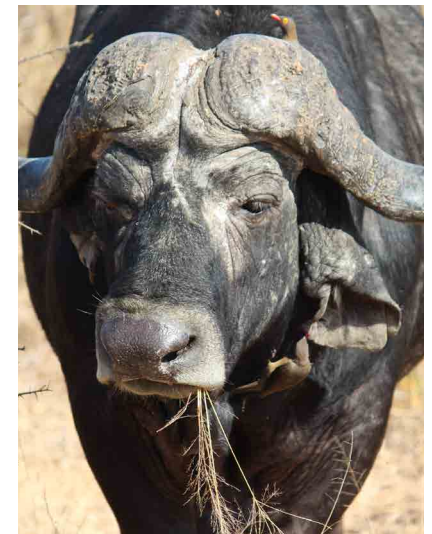
The rapid advancement of next-generation sequencing technologies has led to the discovery of many novel sequences ascribed to the genus *Anaplasma*, with nearly 20 new species being proposed since the last formal organization of the genus. Most of the 16S rRNA gene surveys for *Anaplasma* were conducted on cattle and to a lesser extent on rodents, dogs, and ticks. Little is known about the occurrence, diversity, or impact of *Anaplasma* species circulating in wildlife species. Therefore, we conducted a 16S rRNA gene survey to identify *Anaplasma* species in a variety of wildlife species in the Kruger National Park and neighbouring game reserves, using an unbiased 16S rRNA gene microbiome approach. An *Anaplasma* genus-specific qPCR assay revealed the presence of *Anaplasma* species in 70.0% (21/30) of African buffalo, 86.7% (26/30) of impala, 36.7% (11/30) of greater kudu, 3.2% (1/31) of African wild dog, 40.6% (13/32) of Burchell's zebra, 43.3% (13/30) of warthog, 22.6% (7/31) of spotted hyena, 40.0% (12/30) of leopard, 17.6% (6/34) of lion, 16.7% (5/30) of African elephant and 8.6% (3/35) of white rhinoceros samples. Microbiome sequencing data from the *Anaplasma*-positive samples revealed genotypes that phylogenetically group with known and previously published *Anaplasma* sequences, as well as novel *Anaplasma* genotypes. Our preliminary results reveal a greater genetic diversity of *Anaplasma* species circulating in wildlife species than currently classified within the genus *Anaplasma* and suggest the potential for transmission to livestock or companion animals. Our findings highlight the need for genetic and genome sequencing of putative species for correct classification and further assessing occurrence in livestock and companion animals.

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ET Khunoana



Anti-cancer, anti-inflammatory and antioxidant activity extracts, fractions and compounds from *Ptaeroxylon obliquum* leaves

Ptaeroxylon obliquum (Thunb.) Radlk. (Rutaceae) is traditionally used to treat many infections in South Africa, including inflammatory-related diseases. Approximately 20% of cancers are induced by chronic inflammation or other infections. In this study, *in vitro* anti-cancer, anti-inflammatory and antioxidant activity of *P. obliquum* acetone and aqueous leaf extracts, as well as fractions and compounds were determined. Cytotoxicity was evaluated against normal Vero cells, human breast cancer (MCF-7), hepatocarcinoma (HepG2), lung adenocarcinoma (A549) and human cervical cancer cells (Hela) using a colorimetric (MTT) assay. Soybean 15-lipoxygenase (15-LOX) inhibitory assays were used to evaluate the anti-inflammatory activity. Radical scavenging activity was tested using 2, 2-diphenyl-1-picrylhydrazyl (DPPH) and 2, 2'-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS) assays. Obliquumol and a mixture of lupeol β -amyirin and O-methylalloptaeroxylin were isolated from the chloroform fraction using silica gel open column chromatography. Acetone extracts were toxic to HepG2 carcinoma cells with LC₅₀ values ranging from 10 to 14 μ g/ml and less toxic to other tested cell lines including Vero cells with selectivity index values ranging from 5 to 8. Aqueous extracts and fractions were non-toxic at the concentrations tested against all the cell lines with LC₅₀ > 100 μ g/ml. Morphological analysis of HepG2 and Hela cells using light microscopy showed that acetone extracts were cytotoxic and changed the morphology of the cells. Water extracts scavenged ABTS and DPPH radicals with IC₅₀ values as low as 29.06 μ g/ml and 43.4 μ g/ml. Isolated compounds and acetone extracts had good activity against 15-LOX with IC₅₀ values of 3-14 μ g/ml and 6-10 μ g/ml respectively. Acetone extracts had anti-inflammatory activity and selective anti-cancer activity.

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L Marais



Monitoring and controlling aspiration fluid temperature during ovum pick-up in southern white rhinoceros (*Ceratotherium simum simum*)

Artificial reproductive technologies need to be developed to assist in the battle against the extinction of endangered and threatened species. Temperature regulation during oocyte recovery and transportation is essential for ensuring *in vitro* embryo development. In this study, we examined the temperature pathway of oocyte aspiration fluid during ovum pick-up (OPU) in the southern white rhino (*Ceratotherium simum simum*) in South Africa, and its role in IVF success. The large aspiration instrument used was made from PVC plastic. As a poor heat conductor, it needed to be pre-heated for several hours before OPU commenced to avoid cooling of the oocytes. Fewer temperature fluctuations were recorded when oocytes were transported in a portable transport heating/cooling incubator than in an Equitainer. A greater number of oocytes were harvested per aspirated follicle during the suggested predominant breeding season (November – April), after adjusting for the duration of the OPU procedure. Oocytes harvested from rhino cows given a warm water enema before the OPU procedure were 2.31 times more likely to mature *in vitro* than those harvested from cows receiving a cold-water enema, after adjusting for the negative effect of follicular wave stimulation. This study indicates that to ensure the successful maturation of oocytes in southern white rhinos, oocyte hypothermia during aspiration and transportation is a major concern. The results and conclusions of this study may inform future research with respect to the temperature regulation of oocytes during recovery and transportation for other endangered wildlife species.



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Session 6:
3-minute thesis
presentations and 1-minute
dissertation presentations

NA 1.25



The seroprevalence of African horse sickness in dogs living in Tshwane, South Africa (2014-2019)

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Dogs are the only non-equid species to develop the fatal form of African horse sickness (AHS). Before 2013, all cases of AHS were believed to have been contracted through ingestion of contaminated horsemeat. *Culicoides* midges, the AHS virus (AHSV) vector for horses, have an aversion to dog blood meals and dogs are believed to be dead-end or incidental hosts. More recently, dog mortalities have occurred in the absence of horsemeat consumption and vector transmission has been suspected. The current study is a retrospective serological survey of AHS in dogs from an endemic area. Sera (n=385) collected from dogs living in the city of Tshwane, Gauteng Province, South Africa, were randomly selected from a biobank at the Onderstepoort Veterinary Academic Hospital, corresponding to the years 2014-2019. The study utilised an in-house recombinant VP7 antigen-based indirect antibody enzyme-linked immunosorbent assay (ELISA) for seroprevalence determination and viral neutralization test (VNT) for serotype determination. The seroprevalence was 6% (22/366). Incidence was estimated for dogs with multiple serological results with seroconversion occurring at a rate of 2.3 seroconversions per 10 dog years at risk (95% CI = 0.6-6.2). A subsection of the study sera was tested with AHS VNT (n=42) for serotype determination. AHS serotype 6 was most prevalent (90%) in VNT seropositive dogs (n=20). Seroprevalence descriptively varied by year with dogs sampled in 2017 accounting for 59% of seropositive dogs and positive results appeared linked to rainfall. Identified risk factors were consistent with midge-borne transmission of AHSV to dogs. The relatively high seroprevalence and seroconversion rates suggest frequent exposure of dogs to AHSV indicating a need to investigate the role dogs play in the epidemiology of AHS.



Interrelationships of warthogs, *Ornithodoros* ticks and African swine fever virus in South Africa.

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African swine fever virus (ASFV) causes a contagious and lethal disease in domestic pigs. In the savannah areas of eastern and southern Africa, the virus is maintained in a sylvatic cycle involving warthogs (*Phacochoerus africanus*) that develop benign viraemic infection and eyeless argasid ticks of the *Ornithodoros moubata* complex, commonly found in warthog burrows. However, where biosafety measures are not applied, the infection can be introduced and maintained in pig populations in the absence of wild hosts or vectors. In South Africa, the disease was first seen in the north of the country where domestic pigs were in contact with warthogs, ostensibly through consumption of infected warthog tissues in swill or transmission through the intermediary of ticks. Outbreaks elsewhere in the country were initiated by the movement of infected pigs or pork products from the north. Consequently, a controlled area was declared in 1935, including the known distribution range of warthogs at the time. The movement of pigs and pork products from this area was regulated. These measures successfully limited the spread of ASF until 2016, when a series of outbreaks south of the controlled area occurred that could not be linked to the movement of infection from the north. Meanwhile, there has been a marked increase in informal/small-scale pig farming throughout the country as well as widespread translocation of warthogs south of the controlled area associated with the growth of an extensive wildlife industry. The extralimital warthogs flourished to become an invasive species. We tested warthog sera acquired opportunistically for the presence of antibodies to the virus, and *Ornithodoros* ticks for the virus at selected locations, and confirmed geographic extension of the sylvatic circulation of the virus beyond the controlled area in South Africa. However, the mechanisms involved, and the extent of the spread requires further investigation.



Plant extracts influences surface hydrophobicity, cell adhesion, quorum sensing and extracellular matrix production involved in biofilm formation of multidrug resistant *Escherichia coli* O157:H7

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Biofilm-mediated infectious diseases are generally difficult to treat because of their potential for the acquisition of multi-drug resistance. Hence, there is a need to find novel anti-biofilm agents to combat the resistance of diarrhoeagenic pathogens to antibiotics. The study aimed to determine the effect of 32 plant extracts from 16 medicinal plants on bacterial cell aggregation, surface hydrophobicity, cell attachment, quorum sensing and production of extracellular polymer substances (EPS), thereby inhibiting biofilm formation. The sub-minimum inhibitory concentration (subMIC) of acetone and methanol plant extracts were determined (both 0.156 mg/mL) against *E. coli* ATCC O157:H7. Thereafter, cell surface hydrophobicity was assessed by two protocols, namely Microbial Adhesion to Hydrocarbon (MATH) and Salt Aggregation Test (SAT). The degree of flagella-directed swarming and pillus-directed twitching motility was detected by measuring the diameter (mm) of migration. Subsequently, the capacity of plant extracts to prevent bacterial cell attachment to a polystyrene surface of microtitre plates using 0.1% crystal violet was investigated. Quorum sensing ability was explored using the biosensor strain *Chromobacterium violaceum* ATCC 12472. Lastly, the EPS was quantified from a 24h pre-formed biofilm biomass. This study established that surface hydrophobicity of *E. coli* ATCC O157:H7 changed after exposure to subMIC of 0.156 mg/ml. Sixteen extracts aggregated with >2M SAT (highly aggregative or hydrophilic). In contrast, 30 extracts examined by the MATH test showed hydrophobic behaviour $\geq 50\%$. Maximum prevention of cell attachment percentage was observed in the methanol extracts of *Senegalia galpinii* (90.37 \pm 1.49), *Dichrostachys cinerea* (91.43 \pm 0.04) and *Vachellia sieberiana* (84.48 \pm 1.77). Furthermore, the lowest concentration to inhibit 50% (IC₅₀ 0.001 mg/mL) violacein production was measured in the methanol extracts of *Searsia lancea*, *Grewia monticola* and *V. gerrardii*. Regarding anti-motility activity, *V. gerrardii*, *V. nilotica* and *V. tortilis* plant extracts caused significant ($p < 0.001$) differences in twitching and swarming motility after 72h compared to untreated bacteria. Finally, the plant extracts reduced a substantial amount of biofilm extracellular polymer substances. This study provides evidence that botanical resources may be excellent sources of therapeutic agents in combating biofilm-mediated infections.



Molecular epidemiological investigation of contagious equine metritis in South Africa

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Contagious equine metritis (CEM) is a non-systemic, venereally-transmitted disease of horses caused by the bacterium *Taylorella equigenitalis*. The second member of the genus, *Taylorella asinigenitalis*, is found in donkeys and is considered non-pathogenic. Contagious equine metritis is a World Organisation for Animal Health (WOAH) notifiable disease and is regarded internationally as one of the most highly regulated equine diseases of global importance to equine health and international trade. South Africa was considered free of *T. equigenitalis* until May 2011 when an outbreak was confirmed. Initially, the outbreak was thought to be confined to one property, affecting the index case, a stallion and a mare bred to him via fresh semen artificial insemination. However, institution of a national stallion screening programme and further epidemiological traceback of in-contact animals during the period July 2011 to May 2020, revealed a total of 42 horses on 15 properties that were affected. All affected animals were traced back to the index property. In this thesis, the risk factors contributing to outbreaks of equine infectious diseases were considered and the pertinent literature on both *T. equigenitalis* and *T. asinigenitalis* were reviewed. The materials and molecular diagnostic methods used, and results obtained during the South African *T. equigenitalis* outbreak investigation and subsequent epidemiological surveillance using real-time PCR were described. These included the progressive development of an adapted treatment protocol for the South African context. Finally, we describe how the use of newer molecular technologies such as multi-locus sequence-type (MLST), whole genome sequencing and the application of comparative phylogenetic analysis, definitely traced the origin of this outbreak. Experience gained during the investigation and control of these outbreaks informed subsequent legislation and knowledge regarding the diagnosis and treatment of both *T. equigenitalis* and *T. asinigenitalis*.



Acute stress response in southern white rhinoceros (*Ceratotherium simum simum*) immobilized with four different drug protocols

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Efforts made to ensure the conservation of the southern white rhinoceros (*Ceratotherium simum simum*) include dehorning and translocation, which necessitate chemical immobilization. Rhinoceros experience high levels of stress during capture, and numerous immobilization drug combinations have been proposed, with each claimed to be less stressful and safer than others. The purpose of this study was to evaluate the effects of four commonly-used drug protocols on the acute stress response in white rhinoceros. Eight sub-adult males were captured in the southern Kruger National Park area and housed in individual bomas. The rhinoceros were immobilized with four etorphine-based drug protocols every two weeks for 10 weeks, using a cross-over design. The protocols used were etorphine + butorphanol (control), etorphine + azaperone + butorphanol, etorphine + medetomidine + butorphanol, and etorphine + midazolam + butorphanol. Venous blood samples were collected at three time points during immobilization (0, 20 and 40 min). The following were measured: noradrenaline, adrenaline and their metabolites, dopamine, serotonin, glucocorticoids, glucose, neutrophil:lymphocyte ratio, haematocrit and leukocyte coping capacity. Differences in biomarkers between drug protocols were analysed using a linear-mixed model. All rhinoceros mounted an acute stress response, as shown by increases in noradrenaline and cortisol. With the medetomidine protocol, glucose was higher compared to etorphine alone ($p = 0.003$), cortisol was lower compared to the azaperone protocol ($p=0.027$), neutrophil:lymphocyte ratio was higher than with azaperone and midazolam protocols ($p < 0.001$) and haematocrit was lower compared to all protocols ($p < 0.001$). Noradrenaline was lower ($p = 0.008$) with the midazolam protocol compared to the control and medetomidine protocols. Although the medetomidine protocol appeared to blunt the increase in cortisol, the decrease in haematocrit, which is a result of alpha-2 agonist-mediated splenic dilation with erythrocyte sequestration, may adversely affect tissue oxygenation. Midazolam may curtail the acute stress response during capture.



Investigation of *Bacillus anthracis* spore survival in soils from Kruger National Park in South Africa and Etosha National Park in Namibia

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Bacillus anthracis is a soil-borne pathogen which causes anthrax. The endospores may persist in suitable soils, and this results in endemic regions such as Kruger (KNP) and Etosha (ENP) National Parks. In these parks, Pafuri (KNP) and Okaukuejo (ENP) are high anthrax incidence regions, while Skukuza (KNP) and Otjovasandu (ENP) are low incidence regions. *Bacillus anthracis* strains mainly from the A-subclade occur in ENP, whilst there are strain types from the A- and B-subclades in KNP. The main aim of this study was to investigate how the soils from these high- and low-incidence areas affect the survival of different *B. anthracis* strains in a laboratory experiment. A fully insulated terra-simulator with sensors which measured the soil moisture, ambient temperature, humidity and light was designed to mimic Pafuri's weather conditions. The four selected strain types (one KNP A-, one B-, and two ENP A-clade strains) and the Sterne strain were inoculated into the soils and incubated in the terra-simulator. Spore counts were done at monthly intervals and reported as CFU/g. Soil from Pafuri had the best spore survival rates for all strains except for Sterne, followed by Otjovasandu, Okaukuejo and Skukuza soils. The Sterne and the Kruger B-clade strain showed significant environmental lability. The overall spore survival in KNP differed rather noticeably when compared to ENP. This suggests that spore survival is supported equally well in both areas of ENP, especially for the endemic strains while this was not the case in KNP. At six months, spore survival rates had stabilised, except for Sterne. Strain type, time, relative humidity and temperature are significant determinants of spore survival across all soil types ($p < 0.0001$). As these factors are constantly changing over time due to a culmination of climatic events, this study highlights the importance of consistent disease surveillance for this pathogen and improved predictive modelling systems.



Prevalence and factors associated with *Salmonella* species in mechanically recovered poultry meat imported through a port of entry into South Africa.

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Salmonella species have been reported in foodborne illnesses and persons with underlying clinical conditions are at a higher risk of infection. Although studies have reported *Salmonella* species in poultry and poultry products, there is limited information on the prevalence of *Salmonella* species in mechanically recovered poultry meat imported into South Africa. This study uses secondary data of mechanically recovered poultry meat consignments imported and tested for *Salmonella* species between 2016 and 2017. The proportion of *Salmonella* positive consignments and their 95% confidence interval based on country of origin, month, season, and importer were calculated. A logistic regression model was fit to data to assess associations between the predictor variables and the outcome of *Salmonella* status. In total 8127 consignments were imported, of these, 5733 (70.54%) were tested for *Salmonella* species and 985 (17.18%, 95% CI: 16.23-18.18) tested positive for *Salmonella* species. The majority of *Salmonella* positive consignments came from Country-B (26.95%) followed by Country-A (16.89%). Importer-VI (26.10%) had the highest proportion of consignments testing positive, followed by importer-IV (18.80%). The year 2016 (21.12%) had more consignments positive for *Salmonella* species compared to the year 2017 (14.45%). The proportions of consignment testing positive for *Salmonella* also differed depending on the season. Based on the univariable logistic regression model, country ($p < 0.0001$), importer ($p = 0.0001$), season ($p < 0.0001$), and year ($p = 0.0001$) were potential predictors of a consignment testing positive for *Salmonella* species at a liberal $\alpha \leq 0.20$. Therefore, they were included in the multivariable model. In the final model, the odds of testing positive for *Salmonella* spp. was significantly higher among consignments from country-B (OR: 3.958, $p < 0.0001$) compared to "All others". Consignments imported in 2016 were 1.563 times ($p < 0.0001$) as likely to test positive for *Salmonella* when compared to consignments imported in 2017. Seasonally, the odds of testing positive for *Salmonella* were higher in consignments imported in autumn (OR: 1.488, $p < 0.0001$) but lower in summer (OR: 0.843, $p < 0.0001$) and spring (OR: 0.767, $p = 0.0004$) when compared to the winter season. Importer-V (OR: 0.238, < 0.0001) had lower odds of importing a consignment positive for *Salmonella* species compared to "All others". While the odds of importing a *Salmonella* positive consignment were higher among those imported by importer-VI (OR: 0.461, < 0.0001) compared to "All others". *Salmonella* species that were isolated in mechanically recovered poultry meat consignments in this study suggest that the risk of importing contaminated consignments exists. In addition, the odds of importing a *Salmonella* positive consignment differed significantly based on the country of origin, the type of importer and the time of importation. Therefore, efforts to mitigate the risk including monitoring and controlling must be continued.



A population model to investigate the drivers that cause the replacement of *Rhipicephalus decoloratus* by *Rhipicephalus microplus* on cattle

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Cattle ticks cause considerable economic losses worldwide, especially the one-host *Rhipicephalus* ticks (sub-genus *Boophilus*). In southern Africa, *R. microplus* (the Asiatic Blue tick) is expanding its range at the cost of *R. decoloratus*. Different theories have been put forth to explain the relative success of *R. microplus* but the order of importance of these theories remains unknown. Slight differences in the life cycle lengths and fecundity; different rates of maturation and sterile matings; and different responses to micro-climate conditions have all been proposed. A population dynamics model was developed to compare the influence of the various theories under the same modelling assumptions. Each life stage of the ticks was represented in a compartmental model for which the entry and exit dynamics were modelled using stochastic functions and daily time steps. The location of the ticks was either on a cattle host (for parasitic life stages) or in a grazing camp (for the off-host life stages). The movement of cattle through the camps on a rotational grazing system determined where engorged females were dropped and contributed to the success of larvae questing for hosts. Climate data including temperature and relative humidity influenced many of the functions of the life-stage dynamics including mortalities. The model allowed for an assessment of the relative importance of the differences in the life cycles of *R. decoloratus* and *R. microplus* while controlling for climatic conditions.



Comparative antimicrobial efficacy of four surgical hand preparation procedures prior to application of an alcohol-based hand rub in veterinary students

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The objective of this clinical, prospective study was to determine the influence of skin preparations before the application of an alcohol-based hand rub (ABHR) on bacterial counts before and after elective surgery. Final year veterinary students (n=113) performing ovariohysterectomies on 140 dogs were assigned randomly to perform one initial surgical hand preparation just before surgery: A - hand preparation with medicated solution (4% w/v chlorhexidine bigluconate followed by an ABHR; B - application of a medication solution (benzalkonium chloride 0.1-1% and polymeric biguanide hydrochloride 0.01-0.1%) followed by an ABHR; C - non-medicated pH neutral soap (pHN) hand wash followed by ABHR and D - direct application of an ABHR. Samples were taken by pressing the distal fingertips to an agar plate before the hand preparation, after the hand preparation (n=3), after ABHR application and 120 minutes later. Colony forming units (CFU) for samples were determined. Total log CFU and CFU log₁₀ reduction were calculated and used for comparison with $p < 0.05$. Two hours after surgery commenced, the participants of groups that performed a hand preparation had lower total CFU than those that did not perform a hand preparation ($p = 0.001$). In particular, the number of CFUs was lower when ABHR was performed after the application of pHN compared to direct ABHR ($p = 0.001$). In this population, performing a hand preparation with pHN before applying an ABHR had a better antimicrobial effect for the duration of surgery than not performing a hand preparation. The results suggest that veterinary surgeons should wash their hands before the application of ABHR before performing surgery, even when their hands appear clean.



POSTER PRESENTATIONS



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

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Many emerging communicable diseases amongst humans can be ascribed to zoonotic pathogens arising from animals. *Rhipicephalus sanguineus* ticks are ideal vectors of zoonotic pathogens, and although capable of parasitizing most vertebrates they prefer dogs, and are thus prevalent on dogs, particularly in rural, resource-poor communities. The Mnisi community in Mpumalanga, South Africa is one such community located at a wildlife-livestock-human interface where humans are at risk of infection with various tick-borne zoonotic pathogens. The aim of this study was to characterize the bacterial microbiome of *R. sanguineus* ticks collected from dogs in the Mnisi community. To achieve this, we analysed the bacterial microbiome of ticks sampled from community dogs over three non-consecutive years. After collection, ticks were kept in a humidity and temperature-controlled chamber for two days to digest their blood meal. A total of 10 dogs were sampled in 2016, 7 dogs in 2017 and 6 dogs in 2019. From each dog, ten male *R. sanguineus* ticks were collected, surface sterilized and dissected. Separate pools were made from midguts (MG) and salivary glands (SG) from all 10 ticks. We processed 9 MG pools and 9 SG pools from 2016, 7 MG and 7 SG pools from 2017 plus 5 MG and 5 SG pools from 2019. Genomic DNA was extracted and amplified using universal 16S rDNA barcoded primers. Samples were then sequenced using Pacific Bioscience's circular consensus sequencing strategy. Our study detected *Anaplasma platys* from two samples in 2017 (2.53%) and one sample in 2019 (1.05%). *Anaplasma centrale* was detected in all 2019 samples (17.80%). *Coxiella*-like endosymbionts were detected in all samples from 2016 (74.54%), 2017 (84.29%) and 2019 (42.78%). Our study highlights the need for further research into the role that *R. sanguineus* ticks play as a vector of bacterial pathogens.



Efficacy of South African plants against drug-resistant staphylococci isolated from clinical cases of bovine mastitis

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Drug-resistant strains of *Staphylococcus aureus* and non-aureus staphylococci (NAS) threaten the success of antibiotic treatment of bovine mastitis in the dairy industry. This study aimed to investigate the antibacterial and anti-biofilm activities of selected South African plants against drug-resistant staphylococcal bacteria isolated from milk of clinical cases of bovine mastitis. Plant species were selected based on a history of known antimicrobial activity against *S. aureus*. The minimum inhibitory concentration (MIC) values of acetone and ethanol extracts of *Pleurostyliya capensis*, *Antidesma venosum*, *Searsia leptodictya*, *S. lancea*, *Indigofera frutescens*, *Erythrina caffra*, *Elaeodendron croceum*, *Ziziphus mucronata* and *Trichilia emetica* against 12 clinical isolates of staphylococcal bacteria and an ATCC strain of *S. aureus*, were determined using a serial microdilution assay. Cytotoxicity was evaluated against Vero cells and anti-biofilm activities were also determined using the crystal violet assay. All plant extracts (acetone and ethanol) had antimicrobial activity against each of the clinical isolates tested with MIC = 0.01 to 1.41 mg/ml. The acetone extract of *S. lancea* was the most active against all clinical isolates with MIC values between 0.01 and 0.05 mg/ml. The tested extracts were relatively non-cytotoxic with most LC50 values greater than 0.1 mg/ml. The acetone extract of *S. lancea* inhibited biofilm formation completely and disrupted 48 hours pre-formed biofilm by 81.57%, which is highly promising. The ethanol extracts of *E. caffra*, *A. venosum*, and *S. lancea* also had good antibiofilm activity. These extracts demonstrated antibacterial activity against the tested isolates combined with low cytotoxicity and promising antibiofilm activity. Further investigation is warranted regarding the potential development of complementary treatments from these plants to alleviate the burden of mastitis.



***In silico* identification and comparative analysis of *Theileria parva* vaccine candidates identified using reverse vaccinology methods**

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Theileria parva causes a fatal lymphoproliferative disease in cattle throughout East, central and southern Africa. Although various prevention and treatment methods exist, the genetic and antigenic diversity occurring in field isolates, especially buffalo-derived *T. parva*, undermines these efforts. A subunit vaccine that will provide broad protection is needed. Reverse vaccinology has been instrumental in identifying numerous potential vaccine candidates, however, there are discrepancies in candidates identified in different studies from the same parasite proteome. Thus, we report on the *in silico* identification of *T. parva* vaccine candidates and comparison with candidates reported by two other recent studies. Candidates were identified from the 4051 *T. parva* proteome by screening for classically and non-classically secreted proteins with GPI anchors, one or no transmembrane domain, antigenic properties and MHC class-I binding affinity. At least three tools were used for each analysis step. Only proteins with a hit in 50% or more tools were selected for subsequent analysis. This analysis resulted in the identification of 24 potential vaccine candidates, compared to a total of 26 candidates reported in the comparative studies. From identified candidates, only two were also reported in these studies, with other candidates from comparative studies excluded at various stages of our analysis, especially classically secreted (n=2), non-classically secreted (n=13) and GPI anchors (n=9) analyses. Differential expression analysis against the sporoblast, reported in the comparative studies, revealed that 21 of our vaccine candidates are expressed in the schizont developmental stage of *T. parva*, with 20 upregulated in this stage. Twelve of these were also upregulated in the sporozoite, with five candidates at comparable levels to the schizont. The sporozoite and the schizont are important in the initiation and establishment of infection in the mammalian host, thus candidates that are highly expressed in these stages could be good targets for vaccine development.



Mandibular fractures in cats: a comparison of signalment, distribution, conformation and association with teeth between referral practices in South Africa and Canada

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Studies describing feline mandibular fracture location and distribution are abundant, in contrast, there is a paucity in the literature describing mandibular fracture morphology and their association with teeth. The aims of this study included: 1) Comparing the aetiology and signalment of feline mandibular fracture cases in two populations of cats, from a referral centre in South Africa (OVAH) and Canada (WCVDS), and 2) Describe mandibular fracture conformation and association with teeth. Materials and Methods: The study was conducted as a retrospective descriptive case series evaluating mandibular fractures on preoperative radiographic and/or computed tomographic studies. Fifteen patients from the OVAH and 31 patients from the WCVDS were included. The most common aetiology of mandibular fractures was unknown. The symphysis and mandibular horizontal ramus were affected in 64.5% and 41.9% of WCVDS cases compared to 40% and 60% of OVAH cases respectively. However, when considering the overall number of fractures in a region the mandibular horizontal ramus represented the most fractured region in both groups (48.6% OVAH, 42.8% WCVDS). The caudal mandible and symphyseal region were least affected in the WCVDS group and OVAH group respectively. The vast majority of mandibular fractures were displaced and affected dentulous regions. Overall, caudo-ventral oblique and caudo-lingual oblique fractures were most common in the horizontal ramus in OVAH and WCVDS groups respectively. Within the horizontal ramus, most fractures were centred around the canine tooth in both groups with caudo-ventral oblique fractures most common in the OVAH group. The canine tooth represented 90.9% (OVAH) and 56.2% (WCVDS) of teeth in a fracture line. Type A fractures were mostly associated with teeth in a fracture line (72.7% OVAH, 56.2% WCVDS). Mandibular fracture distribution and morphology are relatively unpredictable when comparing different populations of cats.



Farmer's knowledge, perceptions and attitudes of FMD in the control zone of Limpopo Province.

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Cattle in the FMD protection zone are not simply means of social and cultural relations among farmers, but they are also marketable commodities that can play a critical role in poverty reduction. However, farmers in this zone are typically smallholder, communal farmers who do not have access to more lucrative markets within the FMD-free zone. The study area was Vhembe and Mopane districts in Limpopo Province and a total of 12 dip-tanks were randomly selected and a cross-sectional study was performed to assess the level of knowledge, attitudes, and perception of FMD among small-scale, communal cattle producers with vaccination using a structured questionnaire and a two-stage interview process. A total of 275 interviews were conducted at diptanks and 110 at homesteads. Most (75%) interviewed farmers were male and the majority (67%) were 60 years of age or older. Forty-five percent of interviewed farmers were involved in livestock farming as the main activity and 38% did not have a formal education. Sixty percent of interviewed farmers knew the name of FMD in the local language and 35% reported that the cause of the disease was buffalo. The majority described the clinical signs of FMD in their animals including lameness (34%), salivation (24%), weight loss (8%), and death of calves (2%). According to interviewed farmers, co-grazing with infected cattle (32%), contact with buffalo (12%) and transporting cattle (1%) were among the main causes of FMD spread. Thirty-one percent of farmers believed that the injection of an antibiotic such as oxytetracycline is an effective treatment for FMD in cattle. Also, forty-six percent of farmers strongly agreed that the FMD vaccine is effective in preventing outbreaks in cattle. Collecting information from farmers can help identify practices at the local farm/household level that potentially cause FMD spread and is an important step towards FMD control.



Evaluation of the efficacy and safety of ketamine-medetomidine and ketamine-butorphanol-medetomidine for the immobilization of free-living African lions (*Panthera leo*).

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We compared ketamine-medetomidine (KM) and ketamine-butorphanol-medetomidine (KBM) to tiletamine-zolazepam-medetomidine (ZM) as potential alternative immobilizing drugs for free-living African lions by immobilizing 12 lions with each drug combination in the Kruger National Park. Duration and quality of induction, immobilization and recovery for each study animal were measured and assessed using established subjective criteria. Respiratory and cardiovascular variables, rectal temperature, and arterial blood gases were monitored at 10-minute intervals for 30 minutes. Heart rate and respiratory rate were manually measured. An intra-arterial blood pressure monitor was used to measure blood pressure and an exercise physiology system was used to determine expired minute ventilation, oxygen consumption, carbon dioxide production and respiratory exchange ratio. Arterial blood samples were analysed for blood gases. Induction times and level of ataxia in lions did not differ between drug combinations. Animals immobilized with KM had a shallower depth of immobilization than those immobilized with ZM or KBM. Animals immobilized with all three drug combinations exhibited tachycardia and hypertension. Arterial blood pressure and heart rate were highest in animals immobilized with KM but decreased with time for all drug combinations. Mean PaO₂ was below 80 mmHg at T₀, but increased with time for all drug combinations, and was lower in animals immobilized with KBM than in those immobilized with ZM. Mean PaCO₂ did not differ between drug combinations or over time and was within the expected range for an awake lion. Mean recovery time was greatest in animals immobilized with ZM and lowest in animals immobilized with KBM. The level of ataxia observed during recovery did not differ between drug combinations. Two KM animals and one KBM animal spontaneously recovered before the end of the procedure. In free-living lions, KBM allowed for quicker recoveries compared to ZM and produced stable anaesthesia with fewer negative cardiorespiratory side effects compared to KM.



***In vitro* biological activities of *Combretum molle* R.Br. exG.Don (Combretaceae) against mastitis-causing organisms**

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Bovine mastitis, caused mostly by *Staphylococcus* spp., is a recurring disease resulting in huge financial losses. Antibiotic resistance of organisms may be exacerbated by their ability to form biofilms. This study aimed to explore anti-biofilm and quorum quenching activities of extracts of *Combretum molle* against six clinical strains of *Staphylococcus aureus* (SA1-6) and two ATCC strains (*S. aureus* ATCC 29213, *S. epidermidis* ATCC 35984) as well as their antioxidant, anti-inflammatory and cytotoxic effects. The antibacterial and anti-biofilm potential of the extracts was determined via serial microdilution and crystal violet assays. Quorum quenching activity was ascertained via inhibition of violacein production in *Chromobacterium violaceum* ATCC 12472. Antioxidant activity was determined using in vitro chemical assays. The 15-lipoxygenase enzyme inhibition assay was utilized to ascertain the anti-inflammatory activity of the extracts. The tetrazolium-based colorimetric (MTT) reduction assay was used to determine the cytotoxicity of the extracts against Vero cells. Antibacterial activity of the extracts was moderate to good, with MIC values ranging from 0.02-0.63 mg/ml. The hot-water extract (AQh) had the best anti-biofilm (ABF) activity against SA5 and *S. epidermidis* at time zero (T0) and after 24-hour incubation (T24), while at T48, the ethanol extract had the best ABF activity against SA4. All extracts had moderate to excellent quorum quenching activity with minimum quorum sensing inhibitory concentration (MQSIC) ranging from 0.04 to 0.08 mg/ml. The cold-water extract had the best anti-quorum sensing activity (96.12 % violacein inhibition) at 1.25 mg/ml with MQSIC of 0.08mg/ml. All extracts had lipoxygenase inhibitory activity. Polar extracts had the best antioxidant efficacy and all extracts were non-toxic to Vero cells. Thus, *C. molle* may be an alternative source of antibacterial, antibiofilm, anti-quorum sensing, antioxidant and anti-inflammatory constituents useful in mastitis prevention and treatment.



Development and evaluation of an RT-qPCR assay for the detection of *Rift Valley fever phlebovirus* formalin-fixed, paraffin-embedded tissues in natural cases of sheep

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Formalin-fixed, paraffin-embedded (FFPE) tissues are important specimens for diagnosing fatal Rift Valley fever (RVF) cases in livestock, mainly when fresh convalescence- or acute-phase clinical specimens are unavailable. Although immunohistochemistry (IHC) is well established for FFPE tissues and has excellent precision for *Rift Valley fever phlebovirus* (RVFV) detection, a rapid supplementary molecular test is valuable to obtain a definite diagnosis when IHC results are inconclusive. However, FFPE-derived RNA is in most cases degraded and cross-linked in peptide bonds, making it less applicable for downstream molecular analysis. A novel semi-automated method for deparaffinisation and RNA extraction from FFPE that uses a combination of commercialised reagents was applied to archived FFPE samples of sheep (n= 98) necropsied during the 2010 South African RVF epidemic. A TaqMan hydrolysis probe reverse transcription quantitative PCR (RT-qPCR) targeting the RVFV M segment was also developed for testing FFPE-derived RNA. The RT-qPCR assay was efficient and linear over a broader dynamic range ($R^2= 0.9898$). The assay was highly specific because it did not detect common non-RVFV pathogenic and toxicogenic aetiological agents causing ruminant abortions. Relative to IHC, the sensitivity and specificity of the new assay were determined to be 83.0% (95% CI: 69.2-92.4%) and 86.3% (95% CI: 0.737-0.943%), respectively. In addition, the assay had a moderate Cohen's Kappa coefficient ($k= 0.578$) and a statistically significant ($p < 0.05$) agreement with fresh tissue RT-qPCR done at the World Organisation for Animal Health's South African RVF reference laboratory. The RT-qPCR assay for RVFV detection in FFPE samples is a biologically safe, specific, and sensitive supplementary test to IHC. The strategy for deparaffinising FFPE samples and RNA extraction can be adapted to discover novel molecular biomarkers of rare fatal livestock diseases for which prospective sample collection is unfeasible, yet for which there are archived FFPE samples.



The seroprevalence of African horse sickness in dogs living in Tshwane, South Africa (2014-2019)

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Dogs are the only non-equid species to develop the fatal form of African horse sickness (AHS). Before 2013, all cases of AHS were believed to have been contracted through ingestion of contaminated horsemeat. *Culicoides* midges, the AHS virus (AHSV) vector for horses, have an aversion to dog blood meals and dogs are believed to be dead-end or incidental hosts. More recently, dog mortalities have occurred in the absence of horsemeat consumption and vector transmission has been suspected. The current study is a retrospective serological survey of AHS in dogs from an endemic area. Sera (n=385) collected from dogs living in the city of Tshwane, Gauteng Province, South Africa, were randomly selected from a biobank at the Onderstepoort Veterinary Academic Hospital, corresponding to the years 2014-2019. The study utilised an in-house recombinant VP7 antigen-based indirect antibody enzyme-linked immunosorbent assay (ELISA) for seroprevalence determination and a viral neutralization test (VNT) for serotype determination. The seroprevalence was 6% (22/366). Incidence was estimated for dogs with multiple serological results with seroconversion occurring at a rate of 2.3 seroconversions per 10 dog years at risk (95% CI = 0.6-6.2). A subsection of the study sera was tested with AHS VNT (n=42) for serotype determination. AHS serotype 6 was most prevalent (90%) in VNT seropositive dogs (n=20). Seroprevalence descriptively varied by year with dogs sampled in 2017 accounting for 59% of seropositive dogs and positive results appeared linked to rainfall. Identified risk factors were consistent with midge-borne transmission of AHSV to dogs. The relatively high seroprevalence and seroconversion rates suggest frequent exposure of dogs to AHSV indicating a need to investigate the role dogs play in the epidemiology of AHS.



An Epidemiological Study of Canine Lymphoma in South Africa

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Breed, age, and sex predispositions for canine lymphoma (cL) have been reported for various locations. However, epidemiological information concerning cL in South Africa is scarce. To describe the epidemiologic features of cL and the frequency of World Health Organisation (WHO) subtypes in South Africa. Material and Methods: A retrospective case-controlled study was performed that included 342 cases with a histopathological diagnosis of cL matched with non-cL control cases. Associations between cL and breed, sex and age were assessed using univariate and multivariable conditional logistic regression. Associations were reported as odds ratios (OR) and significance was set as $P < 0.05$. Breed, in general, was significantly associated with cL, but not age, sex, or neutering status. The median population age was 8 years, with a male-to-female ratio of 1.2:1. The Boerboel had an increased (OR = 3.25, $P = 0.004$) and the Yorkshire Terrier a decreased risk (OR = 0.17, $P = 0.004$) of developing cL. The Boerboel cL group ($n=27$) had a younger median age of 6 years and a higher male-to-female ratio of 1.5:1. Immunophenotyping was performed on 119 (35%) cases, of which 82 (69%) were B-cell, 34 (29%) T-cell and 3 (2%) neither. World Health Organisation subtype was available for 88 cases; of these 66 (75%) were diffuse large B-cell lymphoma (DLBCL) with the remaining subtypes each $\leq 7\%$. All Boerboel cases ($n=4$) that were WHO subtyped were DLBCL. This study confirmed a breed predisposition for cL in the Boerboel, a South African mastiff-type dog, as previously reported. However, the lack of association between age, sex, and other breeds, and cL, was unexpected. The frequency of immunophenotypes and WHO subtypes were similar to findings in other locations. This study might indicate a predilection for B-cell lymphoma in the Boerboel.



Molecular epidemiology of pigeon paramyxovirus (PPMV) in South Africa

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Pigeons are susceptible to a variety of diseases, the most common of which are viral infections. Newcastle disease (ND), caused by Newcastle disease virus (NDV) or Pigeon paramyxovirus (PPMV), a pigeon-specific genotype that causes pigeon epidemics regularly, is one of the most serious infectious illnesses. Humans keep pigeons for a variety of reasons, including racing, meat production in relevant countries and as pets. Pigeons, doves, and other birds play an important role in the occurrence, epidemiology, and dispersion of ND viruses, stressing the need of tracking these birds. Since this disease is known to inflict serious economic losses, quick detection and confirmation are critical. The molecular epidemiology of PPMVs circulating in South Africa (SA) since 2012 was determined, from which eight new samples submitted to the Poultry Section and 18 existing samples located in the University's repository were analysed by real-time reverse transcriptase-polymerase chain reaction (RT-PCR). Different tissue samples from each isolate were examined, with the kidney tissue sample being found to have the highest viral load overall. The tissue sample from each isolate with the highest viral load was screened using conventional RT-PCR for ribonucleotide acid (RNA) extracts, followed by Sanger deoxyribonucleotide acid (DNA) sequencing on four RT-PCR amplicons. Ion Torrent Next Generation Sequencing (NGS) was used to sequence the whole genomes of five representative samples, the genetic data collected was combined and is now being phylogenetically analysed. The evolution and epidemiology of PPMV are always changing due to persistent genetic variety that arises around distinct strains of PPMV, which is constantly updated through a uniform phylogenetic classification system and revised nomenclature. Given a result, it's critical to update our understanding of the various strains of PPMV circulating in SA, as previous research on PPMV molecular epidemiology in SA was undertaken between 2001 and 2006.



Detection of bacterial tick-borne pathogens in two provinces of South Africa using a microbiome sequencing approach

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Ticks are obligate ectoparasites recognised worldwide as major vectors of several disease-causing pathogens and are good indicators of disease distribution and epidemiology. However, global warming has affected climate change and consequently expanded tick distribution. As a result, there is a growing concern about emerging and re-emerging of tick-borne pathogens. Therefore, this study seeks to identify ticks and detect bacterial tick-borne pathogens using a 16S rRNA next-generation sequencing (NGS) approach in three neighbouring towns: Harrismith, Phuthaditjhaba and Bergville. A total of 50 blood samples were collected from cattle in each study site and 418 ticks were collected from these cattle, comprising 126 ticks from Harrismith, 160 from Phuthaditjhaba and 132 from Bergville. Ticks were identified morphologically. *Rhipicephalus evertsi evertsi* was the most dominant tick species in the three study sites, whereas *Hyalomma truncatum*, *R. decoloratus* and *R. microplus* tick species were only present in Harrismith. The 16S rRNA NGS was used to explore the bacterial pathogens transmitted by the ticks in cattle. A total of 7,687,581 reads were obtained. The downstream analysis will be conducted using R studio (Alpha diversity, Beta diversity and Principal Component analysis). The results from this study will update the current tick distribution and the pathogens they transmit in the three study sites. Moreover, the study will help in improving the strategies for the prevention and control of tick-borne diseases.



The effects of developmental genistein exposure on learning and memory function in rat offspring

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Endocrine-disrupting chemicals (EDCs) are environmental contaminants that may result in disruption in processes involved in the endocrine system. These in turn may cause various dysfunctions and diseases associated with oestrogen, thyroid hormones, and androgens. Phytoestrogens are plant-derived EDCs found in various food sources that include vegetables, nuts and most commonly in soy products. These compounds possess multiple health benefits but may also exert adverse effects on the body when exposed developmentally or by chronic exposure. Investigating this becomes a priority, especially for countries where soy food products are a staple in the diet. Genistein is a phytoestrogen that may affect cognitive function by altering the oestrogen-sensitive neural pathway development. Therefore, the aim of this study is to assess whether developmental genistein exposure affects learning and memory function in rat offspring. Pregnant Sprague Dawley rats will be exposed to genistein (10mg/kg; 50mg/kg; 100mg/kg) in two timeframes: developmental (GND7 – PND21) and lifetime (GND7 – PND85). Following treatment with genistein, two behavioural tests (Morris Water Maze test and Novel Object Recognition test) will be performed on the rat offspring. The Morris Water Maze test assesses spatial learning and memory, while the Novel Object Recognition test evaluates recognition memory. Upon sacrifice, hippocampal brain tissue will be collected for neurochemical analysis. Hippocampal synapses will be analysed using transmission electron microscopy to assess for any ultrastructural changes. Western blots will be performed to measure the expression of the proteins cAMP response element-binding (CREB) protein and synaptophysin, both of which are involved in learning and memory. The neurotransmitters, glutamate and gamma aminobutyric acid (GABA) will also be measured using high-performance liquid chromatography (HPLC).





A survey on medicinal plants used by indigenous people of Limpopo for treatment of Covid-19 and respiratory symptoms in humans and animals

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The COVID-19 pandemic has been termed the most consequential global crisis since the World Wars. The first line of defence against the COVID-19 spread comprises non-pharmaceutical measures like social distancing and personal hygiene which only reduce the spread. Coronavirus disease 2019 (COVID-19) is a pandemic disease that spreads primarily through droplets of saliva or discharge from the nose. There are no specific antiviral drugs or vaccines available for the treatment of this disease. Prevention measures recommended by the World Health Organization (WHO) can only delay the spread of the virus. This calls for scientific research to be carried out to develop novel drugs and vaccines that may be used to combat and minimize the spread of the coronavirus. Medicinal plants used to treat viral infections with a long history of use in traditional medicine are commonly assumed to be safe. In this study, an ethnobotanical survey was conducted to document the indigenous knowledge of medicinal plants used to treat Covid-19 and related ailments. Using semi-structured interviews and questionnaires, ethnobotanical data were collected from five farmers and 30 traditional healers in the Sekhukhune region, Limpopo. Plant leaves were commonly used, crushed in water, and administered orally or topically. During the survey, 47 plant species were recorded to be traditionally used by indigenous people to treat various human and veterinary respiratory diseases such as pneumonia, flu, bronchitis, tonsillitis, influenza, tuberculosis and conditions such as chronic obstructive pulmonary disease. These plants may play a role in limiting the impact of respiratory ailments and comprise an important component of primary health care in South Africa.



Antimycobacterial activity of traditional herbal mixtures against microorganisms related to respiratory ailments in animals

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Tuberculosis (TB) is a life-threatening disease for both humans and animals and is caused by various *Mycobacterium* species. It is a leading cause of human mortality in the developing world. The high incidence of infection and the increased occurrence of multi-drug resistant and extensively-drug resistant strains of the organism further complicate the problem of TB control with the Covid-19 pandemic worsening the matter. In this study, the *in vitro* activity of leaves of *Eucalyptus camaldulensis* and *Euphorbia tirucalli*, *Aloe marlothii*, *Schotia brachypetala* (bark and roots) and *Elephantorrhiza elephantina* was evaluated against *Mycobacterium tuberculosis*, *M. smegmatis* and *M. bovis*. Toxicity to African green monkey kidney (Vero) cells was evaluated using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay. The antibacterial efficacies of the different combinations of *E. elephantina*, *A. marlothii*, *E. camaldulensis*, *E. tirucalli* and *S. brachypetala* plants varied. All the tested plant extracts had antimycobacterial activity with minimum inhibitory concentration (MIC) values ranging from 0.02 to 2.50 mg/ml. The extracts had IC_{50} values in the cytotoxicity assay above 0.1 mg/ml implying that they have low cellular toxicity. The extracts were also able to inhibit bacterial biofilms. The results support the indigenous use of these plants in the treatment of TB and it is suggested that these plants may have the potential for development as preventative or therapeutic treatments. Synergistic interactions observed between the extracts indicate that combinational therapy may improve biological activity.



Temporal dynamics of *Anaplasma marginale* infection in calves at the wildlife-livestock interface in the Mnisi communal area, Mpumalanga, South Africa

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Bovine anaplasmosis, caused by *Anaplasma marginale*, is one of the most important tick-borne diseases of cattle in South Africa. Data collected in the study area of the Mnisi Community in the Mpumalanga Province indicate the presence of *A. marginale*, with occasional bovine anaplasmosis cases reported at villages close to the wildlife-livestock interface. The infection dynamics, and *A. marginale* strain diversity, during 12 months were examined in ten calves in a peri-urban area and a wildlife-livestock interface within the Mnisi community. Blood samples collected monthly from five calves in each area were screened using an *A. marginale/A. centrale* duplex real-time PCR, *A. marginale* strain diversity was determined by *msp1a* genotype analysis and 16S rRNA microbiome analysis was conducted on blood samples taken at the last time point. The real-time PCR assay confirmed the presence of *A. marginale* in all five calves in the peri-urban area from the first month, but in only two calves at the wildlife-livestock interface and only after six months. Microbiome analysis detected *A. marginale* 16S rRNA sequences in the same calves, but eight of the ten calves also contained other *Anaplasma* sequences. *Msp1a* genotype analysis revealed 42 *A. marginale* genotypes in calves in the peri-urban area and 10 genotypes from the wildlife-livestock interface. Methods of cattle management, acaricide treatment and cattle density could explain differences in exposure to *A. marginale* in the two areas. Most calves were superinfected by distinct *A. marginale* strains within the 12-month study period, indicating continuous challenge with multiple strains that should lead to robust immunity in infected calves and endemic stability. However, the occasional bovine anaplasmosis cases observed at the wildlife-livestock interface might be attributed to a lack of endemic stability in that area, since calves at the wildlife-livestock interface are not all continually infected with *A. marginale* in their first year when natural immunity is highest. Cattle might benefit from cross-protection afforded by infection with other *Anaplasma* species.



Cattle trade networks in the foot-and-mouth disease (FMD) Protection Zone of Limpopo Province

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Foot-and-mouth disease is an economically important disease of livestock and a global threat to national and international trade. Foot-and-mouth disease virus is a positive-sense, single-stranded and non-enveloped virus belonging to the genus *Aphthovirus* in the family *Picornaviridae*. The virus naturally infects cloven-hoofed species and camelids and causes an acute illness characterised by fever and lesions in the oral cavity, coronary band, interdigital space and teats. The objective of this study was to describe the trade network of livestock farmers in the FMD Protection Zone of Limpopo Province, which will be useful for the development of a strategic surveillance system within the livestock movement network. All 55 diptanks within the FMD Protection Zone with vaccination of Limpopo Province were selected for the study. Farmers were interviewed at diptanks concerning the management and movement of their livestock. The questionnaire included four sections: owner demographics, livestock marketing, enterprise economics and the impact of FMD. One hundred and ninety questionnaires were administered, 79% males and 21% females were sampled. Sixteen percent of participants had no formal education, 38% had an education level between grades 1-7 and 45% between grades 8-12. Of those interviewed, 93% were more than 40 years of age and only 7% were less than 39 years. Twenty-one diptanks have been sampled to date with respondents reporting that 87% of livestock movements are within the FMD Protection Zone. Most livestock movements are due to trade; funerals play a major role in these movements. The remaining 13% are movements outside the FMD Protection Zone mostly representing trade to obtain better pricing. Livestock movements outside of the FMD Protection Zone put the country at risk of continuing FMD outbreaks. It is therefore recommended that more marketing opportunities with better prices be created within the FMD Protection Zone to limit these movements.



Serotyping and resistance profiling of non-typhoidal *Salmonella* isolates from poultry in South Africa

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Nontyphoidal *Salmonella* serovars account for 93.8 million cases of human gastroenteritis of which 80.3 million cases are foodborne and 155000 deaths globally. Poultry birds are considered an important reservoir and source of foodborne Salmonellosis for humans. In this study total of 705 *salmonella* isolates which were collected from organs and farm environments of diseased birds on 30 poultry farms throughout South Africa were characterised by serotype using traditional serotyping and PCR. Furthermore, 200 *Salmonella* isolates were tested for antimicrobial resistance against a panel of 16 antimicrobials by the Kirby Bauer-disc diffusion method. Serotyping revealed that the top 5 *Salmonella* serotypes were *S. serovar Muenchen* 24.1% (170/705), *S. serovar Senftenberg* 19.0% (134/705), *S. serovar Isangi* 4.4% (31/705), *S. serovar Schwarzengrund* 4.1% (29/705) and *S. serovar Heidelberg* 2.6% (18/705). Antimicrobial resistance profiling of 200 isolates showed that 51% (102/200) of isolates were resistant to tetracycline, 13% (26/200) ampicillin, 12.5% (25/200) amoxicillin, 8.5% (17/200) kanamycin, 4.5% (9/200) chloramphenicol, 2% (4/200) sulfamethazine/trimethoprim, 1.5% (3/200) gentamycin and 0.5% (1/200) nalidixic acid. All *S. serovar Muenchen* were resistant to at least one antimicrobial. Multi-resistance was observed in 13% (26/200) of isolates. Multiresistance was more frequent among serotypes *Muenchen* 6.5% (13/200), *Heidelberg* 6% (12/200), and *Schwarzengrund* 0.5% (1/200). Of particular interest, was one *S. serovar Heidelberg* strain 367 which was resistant to six antimicrobials including tetracyclines, ampicillin, amoxicillin, chloramphenicol, kanamycin, and sulfamethazine. This study shows that poultry birds in South Africa carry antimicrobial-resistant *Salmonella* serotypes that have been associated with Salmonellosis in poultry and humans, worldwide. The presence of antimicrobial-resistant *Salmonella* serotypes in poultry is a public health concern as *Salmonella* can be transferred to humans through poultry products such as meat and eggs causing diseases that are difficult or impossible to treat. Further surveillance of *Salmonella* on poultry farms and along the food chain and detailed characterisation of *Salmonella* isolates for antimicrobial resistance and virulence genes will be needed to understand the epidemiology of this important zoonotic foodborne pathogen in South Africa.



Abattoir workers' knowledge, perceptions and attitudes regarding zoonotic infections in the Eastern Cape province, South Africa.

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Exposure to infectious pathogens from apparently healthy animals is a growing concern that warrants attention and awareness campaigns. The lack of proper use of Personal Protective Equipment (PPE) and knowledge regarding zoonotic infections among abattoir workers can result in outbreaks. The objective of this study was to evaluate the knowledge, perceptions and attitudes regarding zoonotic infections among abattoir workers. Workers (n=116) from five abattoirs were purposely selected for the study. Of these, 40 respondents participated in the initial study on pathogens, while 76 participated in the risk factors study. An awareness session was held to demonstrate the presence of microorganisms on work surfaces, the human body and clothes. Semi-structured questionnaires were administered to the abattoir workers regarding infectious diseases and causative microorganisms. Of the 40 workers, 87.5% knew about germs while 22.9% believed that germs can be seen with the naked eye. Of the 76 respondents, 18.4%, 19.2% and 47.4% of workers knew Q-fever, Toxoplasmosis and Brucellosis, respectively, whereas 15% did not know all the diseases. Consumption of undercooked meat was reported in 11.8% of the workers, while 31.6% of the workers drank unpasteurized milk. Most abattoir workers (84%) wore PPE. Abattoir workers (75.3%) also reported hand-cut injuries which occurred at work. This study showed that there is a gap in the knowledge and understanding of microorganisms and the use of PPE among workers in the studied abattoirs. The lack of knowledge regarding microorganisms, as well as the paucity of understanding of the use of PPE in the abattoirs, raises a concern. Transmission routes of zoonotic infections include inhalation of contaminated aerosols and direct contact with infected carcasses/secretions. The presence of infectious diseases in apparently healthy animals is of concern and we recommend regular awareness campaigns and educational activities, which we perceive are essential to mitigate human infections.



Quantitative detection of *Theileria haneyi* infections in South Africa

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Genetically diverse *Theileria equi* and *Babesia caballi* parasite genotypes have compromised the molecular and serological detection of equine piroplasmiasis globally, and this has been further complicated by the recent description of *Theileria haneyi* in the USA. *Theileria haneyi* was recently reported to occur in South African horses infected with *T. equi* genotype C. Differentiation between *T. equi* and *T. haneyi* is accomplished either by nested PCR assays targeting the *ema-1* gene of *T. equi* and a *T. haneyi*-specific gene, or a *ThEMA-11* indirect ELISA based on a protein exclusive to *T. haneyi*. We developed a quantitative real-time PCR assay targeting the *ema-11* gene for the detection of *T. haneyi* infections in horses from South Africa. Field samples were screened for the presence of *T. haneyi* using established molecular genotyping and nested PCR assays. The reference *T. haneyi* Eagles Pass *ema-11* gene sequence was used to design primers to amplify and sequence the *ema-11* gene from *T. haneyi*-positive field samples. An alignment of the *ema-11* gene sequences obtained in this study was used to design a *T. haneyi*-specific TaqMan minor groove binder (MGB™) real-time PCR assay. The diagnostic efficacy of the assay was evaluated by screening field samples. The assay was able to detect *T. haneyi* and, in conjunction with a *T. equi*-specific real-time PCR, to distinguish between *T. haneyi* and *T. equi* infections. The *T. haneyi*-specific real-time PCR assay could be used as a complementary diagnostic assay, together with the indirect *ThEMA11* ELISA for importing and exporting horses.





Reliability of the Enterprise Point-Of-Care blood analyser's calculated arterial oxygen haemoglobin saturation, in immobilized southern white rhinoceros (*Ceratotherium simum simum*)

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Enterprise Point-Of-Care (EPOC) blood analysis is routinely used in wildlife veterinary practice to monitor blood oxygenation but the reliability of the EPOC's calculated arterial oxygen haemoglobin saturation (cSaO₂) has never been validated in southern white rhinoceros (*Ceratotherium simum simum*), despite their susceptibility to hypoxaemia during chemical immobilization and anaesthesia. The study aimed to evaluate the reliability of EPOC's cSaO₂ by comparing it against SaO₂ measured by anAVOXimeter 4000 co-oximeter (reference method) in immobilized white rhinoceros. Eight male white rhinoceros (Study 1) were immobilized by darting with etorphine and received either butorphanol or an equivalent volume of saline intravenously after 30 minutes of immobilization. An additional eight rhinoceros (Study 2) were immobilized four times using different drug combinations (etorphine + saline, etorphine + azaperone, etorphine + midazolam, etorphine + medetomidine) and were given butorphanol (intravenously) after 12 minutes. Animals in both studies also received oxygen via intra-nasal insufflation after 60 minutes. Blood samples were drawn, at predetermined time points, from a catheter inserted into the auricular artery and analysed using the EPOC and AVOXimeter 4000 co-oximeter. Bland-Altman (bias and precision) and area root mean squares (ARMS) were used to determine the reliability of the EPOC's cSaO₂ when compared with simultaneous AVOXimeter co-oximeter's SaO₂ readings. The EPOC's cSaO₂ readings were unreliable (inaccurate and imprecise) when compared across the entire SaO₂ range (bias = - 6, precision = 5, and ARMS = 8). The EPOC's cSaO₂ is unreliable and should not be used to monitor blood oxygenation in immobilized white rhinoceros.



Anti-quorum sensing, anti-biofilm activity, antibacterial activity, total phenolic and flavonoid contents of *Kalanchoe gunniae* and other selected plants with potential for managing mastitis

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Infectious mastitis in dairy cattle, an inflammation of the udder parenchyma has significant financial consequences. The increasing occurrence of antibiotic resistance to bacterial agents such as *Staphylococcus aureus* (*S. aureus*) has led to challenges in the prevention and treatment of bovine mastitis. The synergistic activity of extracts and fractions of medicinal plants can possibly be a novel strategy to overcome the development of resistance toward single antimicrobial compounds. In view of this, acetone and ethanol extracts of *Maurocena frangula*, *Maytenus undata*, *Kalanchoe gunniae*, and *Bryophyllum pinnatum* were investigated for their anti-quorum sensing activity. Extracts with good anti-quorum sensing activity and lack of *in vitro* cellular toxicity were further investigated for antibiofilm activity. These plant extracts were active against *S. aureus* reference strain and isolates from clinical cases of mastitis in earlier research and among them, *K. gunniae* extracts had the best antibacterial activity. Thus, fractionation was done on 80%-methanol extract of *K. gunniae*. Antibacterial activity, total flavonoid and phenolic contents of fractions were also investigated. *Kalanchoe gunniae* extracts had the best quorum sensing activity with minimum quorum sensing inhibition concentration (MQSIC) and minimum inhibition concentration (MIC) values of 0.04 mg/ml and 0.63 mg/ml respectively on the quorum sensing model organism *Chromobacterium violaceum* ATCC 12472. Significant biofilm inhibition against *S. aureus* mastitis isolates was observed at time zero (0 h). MIC values of fractions of *K. gunniae* against *S. aureus* isolates from clinical cases of mastitis were as low as 0.02 mg/ml. Flavonoids and markedly high content of phenolics were present in the extract and active fractions of *K. gunniae*. To overcome antibiotic resistance, extracts and fractions of *K. gunniae* can potentially be used to develop herbal therapeutic spray formulations to manage mastitis. Work is ongoing to identify compounds in the active fractions of *K. gunniae* through chromatographic analyses.



Morphological description of the digestive tract of Temminck's ground pangolin (*Smutsia temminckii*)

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Temminck's ground pangolin, of the order *Pholidota*, is one of eight extant pangolin species and the only pangolin species present in southern Africa. These myrmecophagous mammals are posited as the most trafficked mammal species globally and their numbers are drastically decreasing due to various anthropogenic threats. Existing research on pangolins focuses mostly on ecology, husbandry of captive pangolins and the increase in trafficking. The anatomy of pangolins has received little attention with most focus on the digestive tract of Asian pangolins and the reproductive tract of the Chinese pangolin, although recently the thoracic limb of Temminck's ground pangolin has been described. This study aims to provide a gross morphological description of the digestive tract and associated organs of Temminck's ground pangolin. Pending the approval of research and animal ethics, all specimens used in the study will be obtained from animals that either died in the wild or that succumbed to illness despite treatment. Two whole pangolin carcasses will be dissected to describe the abdominal topography as well as the digestive tract organs *in situ*. In addition, the digestive tracts and associated organs from five pangolins will be removed, described and digitally recorded. A detailed anatomical study of the digestive tract will contribute baseline data which may be used in future studies to understand digestive function. This may aid in the treatment and successful recovery of pangolins in captivity and allow for a better understanding of their dietary choices and restrictions.



Development and optimization of a reverse-transcription loop-mediated isothermal amplification (RT-LAMP) assay for the detection and identification of SAT1 serotype foot-and-mouth disease virus

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Foot-and-mouth disease (FMD), caused by infection with foot-and-mouth disease virus (FMDV), is an infectious disease of cloven-hoofed domestic and wild animals that impacts national and international trade of livestock and their products. Southern African territories (SAT) serotypes of FMDV are endemic to southern Africa where SAT1 is the second most detected serotype during outbreaks in cattle. Rapid detection of FMD is crucial to its control. Shipping samples to reference laboratories for testing can be time-consuming, expensive and labour-intensive, delaying effective decision-making. Early detection could be accomplished by the development of pen-side tests that can be used at the site of outbreaks. This study developed and preliminarily validated a reverse-transcription loop-mediated isothermal amplification (RT-LAMP) assay for the detection of SAT1 viruses circulating in southern Africa. Twenty-seven P1 nucleotide sequences of viruses belonging to SAT1 topotype II FMDV, which is found in South Africa, southern Mozambique and Zimbabwe, were selected. A consensus sequence created from the alignment was used to design five sets of primers using the PrimerExplorer software. The primer sets were tested across a range of temperatures and reaction times while keeping other parameters constant. From these, one primer set demonstrated satisfactory performance and was chosen for further analysis. The primer set amplified a region of the FMDV VP2 gene at a constant temperature of 68°C for 50 minutes. Thirty SAT1, 20 SAT2, and 10 SAT3 viruses were tested in triplicate as part of the validation. Positive results were defined by sigmoid-shaped fluorescent signal curves and negative results by a linear fluorescent signal. During preliminary validation, 69% of the SAT 1 topotype II viruses were successfully detected. Of the non-SAT1 viruses selected 73% were negative, as expected, while the remaining produced discordant amplification signals across replicates. Additional work is underway to investigate the reasons for these discrepant results.



***Ehrlichia ruminantium* and *Rickettsia africae* infection rates in *Amblyomma* species of southern Africa**

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Ticks of the genus *Amblyomma* are known for their aggressive hunting and biting behaviours and are well documented as vectors of a variety of disease-causing pathogens of socio- and economic concern. *Ehrlichia ruminantium* and *Rickettsia africae* are well-established pathogens transmitted by the *Amblyomma* genus which occur predominantly in sub-Saharan Africa. *Ehrlichia ruminantium* causes heartwater in ruminants and *R. africae* causes African tick bite fever (ATBF) in humans. The focus of the study was to determine the *E. ruminantium* and *R. africae* infection rates of southern African *Amblyomma* ticks. *Amblyomma* ticks were collected from livestock in South Africa, Zambia, Zimbabwe, Angola and Mozambique, identified, and screened using molecular methods for the aforementioned pathogens. In total, 7784 ticks were collected and identified as *Amblyommaeburneum*, *Amblyommahebraeum*, *Amblyommapomposum*, and *Amblyommavariegatum*. The overall infection rates for *E. ruminantium* (n = 6041) were 11.49% with the highest infection rates in South Africa. This could be attributed to the fact that ticks were collected from areas with a high prevalence of heartwater. For *R. africae* (n = 1874) the infection rates were much higher, ranging from 35.23% to 77.36%. The high positivity rates of these pathogens in *Amblyomma* ticks imply that heartwater and ATBF remain a problem to livestock rearing and human health, respectively, in southern Africa. Continuous surveillance of these pathogens and the tick vectors are needed to establish relevant control and disease prevention measures, especially in communities where these diseases affect the livelihood of the people that live there.



Comparative morphology of the stifle joint of the African lion (*Panthera leo*), African black-footed cat (*Felis nigripes*) and Temminck's ground pangolin (*Smutsia temminckii*)

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The morphology of felids is known to be highly conserved amongst the different species. The African lion, an apex predator and cooperative hunter is the largest cat species in Africa. In comparison, the black-footed cat is the smallest cat species in Africa and is a nocturnal, solitary hunter. Temminck's ground pangolin belongs to the order Pholidota, which is postulated to be closely related to the order Felidae. The conservation status of all three of these species is listed as vulnerable. The stifle joint is one of the most complex joints in the body and its pathology can severely affect locomotion in both humans and animals. Although the morphology of the stifle joint has been well documented in domestic cats, little is known about the anatomy of this joint in wild felids. An *Os meniscus* is present in the domestic cat and is an apparently normal finding. Similarly, a meniscal bone has been noted in some wild felids, although it is unclear whether this bone is associated with pathology. In contrast, meniscal ossification in humans is a pathological finding. This study aims to compare the stifle joint morphology of the African lion, black-footed cat and Temminck's ground pangolin and will entail gross and microscopic descriptions, diagnostic imaging, scaling of muscle geometry and application to biomechanics. Pending approval by research and animal ethics, the specimens used for the study will be from live animals (imaging only), animals that died in the wild, succumbed despite treatment or that were culled. A detailed morphological description of the stifle joint may provide information of veterinary significance for the treatment of musculoskeletal disorders in the species under study. The comparative aspect of the data will be used to elucidate the functions of structures in the stifle joint across related animals with varying locomotor functions and body sizes.



Evaluation of alternative disinfectants to formaldehyde for treating broiler eggs in a commercial hatchery

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Formaldehyde is a commonly used disinfectant in poultry hatcheries to fumigate eggs and chicks during incubation. While formaldehyde is a very effective disinfectant, it's a potential human carcinogen and it has been proven to cause tracheitis and negatively affect the tracheal cilia of the newly-hatched chicks. Previous research predominantly focused on alternatives to formaldehyde that were applied prior to the incubation of eggs. On the contrary, this research intended to specifically evaluate alternatives to formaldehyde during the final stage of incubation in a large commercial broiler hatchery, when the eggs hatch and a large number of bacteria and fungi are released in the incubators with the potential to infect other eggs and chicks. Formaldehyde fumigation served as control and was applied to 5.76 million eggs, while Virocid (glutaraldehyde and quaternary ammonium compound) from Cidlines and Imazigard (imazilil and polyhexamethylene biguanide) from Technblend served as treatments, with another 5.76 million eggs being exposed to each treatment over an 18-week trial period at Rainbow Chickens' Broiler Hatchery in Worcester. Bacteriological (Total Viable Counts, *E. coli* and *Pseudomonas*) and mycological (yeasts and moulds) counts on hatcher fluff from the three groups as well as seven-day chick mortalities were analyzed and compared. A cost comparison was also done between the three disinfectants to establish the cost per egg disinfected. The microbiological results have proven that for all metrics evaluated, both Virocid and Imazigard managed to perform similar or better than formaldehyde. For 7-day chick mortalities, there was no statistically significant difference between the control and treatment groups. Contrary to what is commonly assumed, the cost per egg was less for the alternative products when compared to formalin as applied during the trial. The research has proven that good alternatives exist to effectively, safely and cost-effectively disinfect poultry eggs in hatcheries.



Development of a multiplex real-time polymerase chain reaction to distinguish between *Mycoplasma* species found in South African poultry

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Avian mycoplasmosis is a serious and chronic bacterial disease caused by *Mycoplasma* species, and the most significant pathogens to poultry are *M. gallisepticum* and *M. synoviae*. Previously, six mycoplasma species were identified from South African poultry flocks, viz. *M. gallisepticum*, *M. synoviae*, *M. gallinarum*, *M. pullorum*, *M. iners* and *M. gallinaceum*, and minimum inhibitory concentration tests performed demonstrating evidence of multidrug resistance in some of the non-pathogenic mycoplasma species. If the transfer of antibiotic-resistant traits between species were to occur, there could be major implications for the control and management of avian mycoplasmosis in the country. Thus, the development of a molecular test to detect all the mycoplasmas circulating in the national flock would bring a better understanding of the persistence of mycoplasma infections. The aim of this project was therefore to develop, validate and test a multiplex real-time PCR. Using the currently available whole *Mycoplasma* genomes, a pan-genome analysis was done to identify genes in conserved regions for primer and probe design and synthesis; and a literature review was conducted to compare published primer and probe sequences for mycoplasma detection and differentiation. Five primer pairs for each species were designed together with two group-specific 16S RNA primer sets. Following primer testing for specificity and sensitivity using conventional PCR, one group-specific (16SRNA GC-341&R543) and one primer set for each species (mgc2 - *M. gallisepticum*, vlhA - *M. synoviae*, oligo *M. gallinaceum*& RpiR - *M. pullorum*) were selected for probe design. A plasmid containing genes for use as a laboratory positive control was designed and synthesised using the forward, probe, and reverse sequences of the selected primer/probe pairs. The multiplex PCR was validated and finally tested against two hundred and four field samples.



The effect of developmental genistein exposure on reproductive function in rat offspring

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There is growing interest in the adverse reproductive health effects posed by exposure to endocrine-disrupting chemicals (EDCs). Several EDCs including pesticides, phthalates, and plasticizers have been shown to negatively affect reproductive health. Phytoestrogens are plant-derived EDCs which have been reported to cause long-term disruption in the male reproductive system, by mimicking endogenous estrogen or acting as an estrogen antagonist. Developmental exposures to EDCs are particularly harmful as they may pass the placental barrier and affect the developing foetus during sensitive male-programming windows. Therefore, the aim of this study is to investigate the effect of genistein exposure, during development on the reproductive function of rat offspring. Pregnant Sprague Dawley rats will be exposed to genistein (10mg/kg; 50mg/kg; 100mg/kg) during two treatment timeframes: developmental exposure (GND 7 - PND 21) and lifetime exposure (GND 7 - PND 85). On PND 90, the male offspring will be euthanized, and blood, semen, as well as testicular and prostate gland tissue samples will be collected for analysis. Testosterone levels will be measured in whole blood while the testes and prostate gland will be processed for histological and histomorphometry analysis. Computer-assisted sperm analysis (CASA) will be used to analyze semen samples for sperm parameters, such as sperm morphology, motility, sperm concentration, and acrosome reactions. The outcome of this research will provide resourceful data that will contribute towards a better understanding of reproductive health related to genistein consumption.



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