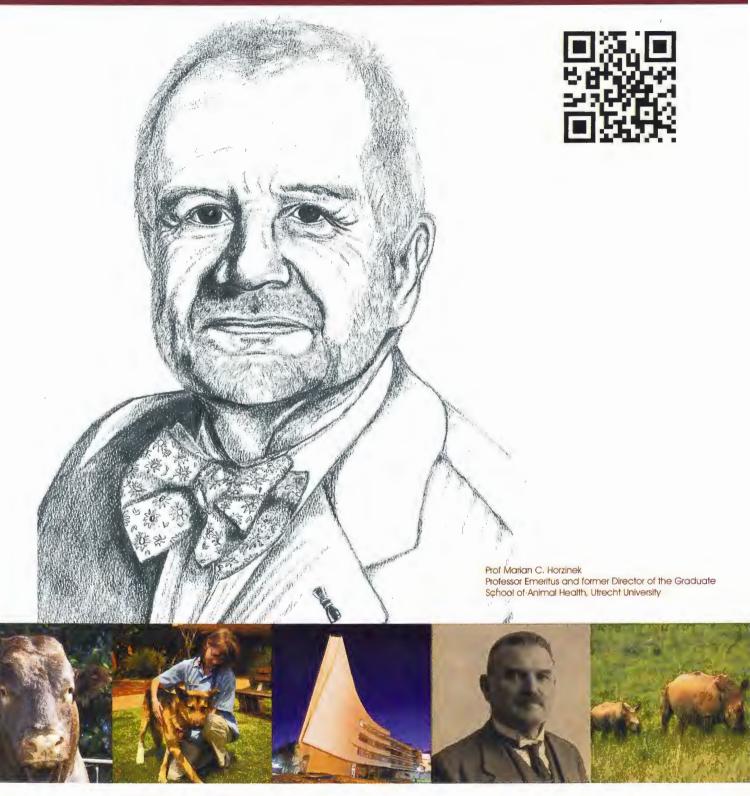
Faculty of Veterinary Science Faculty Day 5 September 2013

Research Overview





Brief history of Faculty Day

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

Since the inception of the Faculty of Veterinary Science at Medunsa in the early 1980s, the staff, and later students, were involved in the activities of the "Academic Day", which was aimed at highlighting the research activities of the University, as well as exposing young researchers to a conference environment. The Faculty of Veterinary Science of the University of Pretoria at Onderstepoort followed this trend shortly thereafter and the first "Faculty Day", which focused on the research activities of the faculty, was held on 5 September 1984, sponsored by the then Dean, Prof JMW le Roux. The combined research skills of the two original institutions are today reflected in the proceedings of the Faculty Day held each year in the spring at the Onderstepoort Campus.

Sponsorships

The Faculty of Veterinary Science wishes to express its sincere thanks to the following sponsors for their very generous contribution in support of the 2013 Faculty Day.



































Faculty Day

Faculty of Veterinary Science University of Pretoria

5 September 2013



Contents/Programme



07:30 - 07:55 Registration and Coffee

Master of Ceremonies: Dr Patrick Page

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SESSION CHAIRPERSON: Prof Estelle Venter

- 09:30 10:25 Sir Arnold Theiler Memorial Lecture: "A Personal Journey Through Coronavirus Evolution" Prof Marian Horzinek

10:30 – 10:55 Faculty Awards 11:00 – 11:40 Tea 11:45 – 13:00 Second Session

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Message from the Dean



Prof Gerry Swan, Dean

The year 2012 marked the end of a very successful year for the Faculty of Veterinary Science in support of strategic direction of the University of Pretoria. This emphasised the importance of increasing research outputs in terms of quantity and quality in support of the University's goal to become a research-intensive institution. This can only be achieved by developing effective postgraduate programmes supervised by world-class staff members without neglecting the basic responsibility of providing the highest quality of undergraduate training.

The basic mandate of Veterinary Science is the protection of animal health, which often also impacts on human health, thereby stimulating economic growth and food security. An efficient research programme therefore must meet the needs of society but remain relevant to a constantly changing environment.

Measuring the growth of the research outputs over the preceding five-year period is useful to evaluate the success of the Faculty. Subsidy earnings, which reflect the number of scientific publications by staff members and students, increased from 65,31 to 89,79, representing a growth of 37,5%. The number of master and PhD qualifications increased by 42% and 113% over the past five years respectively, with the highest number of postgraduate students in the history of the Faculty graduating in 2012. The budget for postgraduate bursaries almost doubled from R386 000 to R610 640, benefitting mostly PhD students. The number of NRFrated staff members, perhaps the most important long-term indicator, increased from 17 in 2008 to 26 in 2012, a growth of 52,9%. A significant achievement is the fact that the per capita publication output per academic staff member is one of the highest in the University in 2012.

An important initiative during 2012 was the implementation by the University of selected Institutional Research Themes (IRTs). These themes were selected on the basis of existing strengths of the university and their potential to stimulate inter-faculty and international collaboration as a method to stimulate research. Five themes were initially approved for

special funding and the Faculty actively collaborate in three of these: Animal and Zoonotic Diseases, Genomics, and Food, Nutrition and Well-being. Six proposals submitted by this Faculty for each of the first two IRTs were approved for funding in 2012/2013.

A second milestone for the Faculty was the allocation of its first Research Chair, for Poultry Health and Production, with the financial support of the poultry industry, and the appointment of Prof Celia Abolnik in this important position.

Another major event was the final signing of a Memorandum of Agreement for the establishment of the Tshwane Animal Health Biocluster between the Technology Innovation Agency (TIA) of the Government and the ARC, CSIR, OBP, NRF and UP. The purpose of the agreement is to stimulate collaboration between these bodies in the development of commercially viable technologies for the control of animal diseases of major social and economic importance for South Africa and the entire Southern African region. In the first round of applications for funding in 2013 the faculty was successful with nine proposals for a total of more than 25 million rand.

Keeping our local and global responsibilities in mind, we will always be faced with new challenges and mindsets that will have to be integrated in our thought processes in order to grow as a leading and renowned veterinary institution. It is thus vital that the Faculty continues to increase its research impact and ensure that this is locally relevant and is keeping pace with research worldwide.

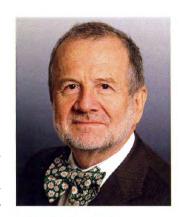
It is a pleasure to welcome you at this year's Faculty Day, which provides an opportunity for our researchers to present the results of their studies and share it with their peers. A total of 18 oral and 13 poster presentations are on this year's programme. The prestigious Sir Arnold Theiler Memorial lecture will be delivered by Prof Marian C. Horzinek, Professor Emeritus of Virology and Viral Diseases of the Faculty of Veterinary Medicine, Utrecht University and former Director of the Graduate School of Animal Health, Utrecht University. The title of the Memorial Lecture will be: A personal journey through coronavirus evolution, a focus on the complex natural history of Coronaviruses, the ideal objects to study evolution because they possess the largest viral RNA genome known to science. Due to their stochastic nature, genetic mistakes are highly probable. Mutations are due to the poor fidelity of the replicases and to the lack of a proofreading mechanism to correct them. Prof Horzinek will be discussing the consequences of point mutations, deletions, recombinations - and the quasi-species concept - to illustrate changes in the occupation of ecological niches by coronaviruses. We are looking forward to share in the insights and personal experience of Prof Horzinek on this topic.

May Faculty Day 2013 be an inspiration to all of us in the Faculty's pursuit for excellence, distinction and innovation in order to grow as a leading and renowned veterinary institution. Congratulations to the Faculty's 2013 teaching and research award winners. I also sincerely thank the Faculty Day Organising Committee for its devotion and hard work in organising this event.

Curriculum Vitae: Prof Marian C. Horzinek

Marian C. Horzinek is Professor Emeritus of Virology and Viral Diseases of the Faculty of Veterinary Medicine, Utrecht University and former Director of the Graduate School of Animal Health, Utrecht University.

Professor Horzinek studied veterinary medicine in Germany at Giessen and Hannover Universities, from 1956 to 1961. A year later he obtained his Doctor of Veterinary Medicine and in 1970 he gained his 'Habilitation' (a PhD equivalent) in Virology. He began his



Prof Marian C. Horzinek

career in virology at the Public Health Laboratory in Hannover, where he worked as a research fellow of the Deutsche Forschungsgemeinschaft. He later helped to establish the Chair of Virology at Hannover Veterinary School, and then spent a year as a research fellow at the Instituto Venezolano de Investigaciones Cientificas in Caracas, Venezuela.

Upon his return, he became Head of the Exotic Diseases Division at the Federal Research Institute for Animal Virus Diseases in Tübingen, Germany. In 1971, he moved to The Netherlands where he was appointed Head of Department and Professor of Virology and Virus Diseases at the Faculty of Veterinary Medicine, Utrecht University. Since 1992, Professor Horzinek was director of Utrecht University's Institute of Veterinary Research, and from 1996 until his retirement in 2001, he directed the Graduate School of Animal Health.

Professor Horzinek has been associate professor at the Veterinary School in Hannover, Germany, courtesy professor at the College of Veterinary Medicine, Cornell University, USA, and clinical professor of virology at the School of Veterinary Medicine, University of California, Davis, USA. During the last ten years, he has established and chaired scientific advisory boards at the universities of Vienna (Austria) and Barcelona (Spain).

During his career, Professor Horzinek has gained prizes and awards from research organizations in Europe, Australia and Asia, and honorary doctorates from the Universities of Ghent (Belgium), Hannover (Germany), Uppsala (Sweden), Vienna (Austria) and Guelph (Canada).

His publications include in excess of 300 scientific papers and more than 30 books and monographs, handbooks in several languages and CD-ROM articles. He has been an editor or editorial board member for scientific journals published in the Netherlands, Belgium, Great Britain, Germany, Austria, France and Italy. He is the founding president of the European (now: International) Society of Feline Medicine, of the German Gesellschaft für Kynologische Forschung, the Prevention of Equine Diseases (PrEquId) Board, and recently became ambassador of the Black Jaguar Foundation, a non-profit organization with the objective to save the rainforest. One of his most ambitious projects was the establishment of the online veterinary research journal, Veterinary Sciences Tomorrow.

Sir Arnold Theiler Memorial Lecture

A personal journey through coronavirus evolution

Marian C. Horzinek

For companion animal medicine, Feline Infectious Peritonitis (FIP) is an important disease — it is fatal, and prevention is a challenge. FIP is a sporadic viral condition — a contradiction in terms. The explanation: mutants of a coronavirus (CoV) that is endemic in most feline populations, arise in individual cats, change their tropism from enterocyte to macrophage and cause a polyserositis with pyogranulomas. The FIP-causing variants are usually not transmitted, and there is no epidemic spread.

Coronavirology in Utrecht started in the 1970s in a scientific domain that drew little attention; this changed suddenly, and dramatically, when the cause of the Severe Acute Respiratory Syndrome (SARS) was identified: a member of this family had crossed the host species border, from the civet to man. Other conditions followed, like the Middle East Respiratory Syndrome, its causative coronavirus (MERS-CoV) was found related to viruses in bats. Anecdotal exposure histories indicated patients had been in contact with dromedary camels, and neutralising antibodies in camel sera from different locations in Oman suggest widespread infection of camelids. There are more examples to show the complex natural history of these viruses.

Coronaviruses are the ideal objects to study evolution: they possess the largest viral RNA genome known to science, and due to their stochastic nature, genetic mistakes are highly probable. Mutations are due to the poor fidelity of the replicases and to the lack of a proof-reading mechanism to correct them. The consequences of point mutations, deletions, recombinations – and the quasi-species concept – will be discussed to illustrate changes in the occupation of ecological niches by coronaviruses – be it in an organism or in a population.

A short historical note, for a good reason: Sir Arnold Theiler had described acute liver atrophy and parenchymatous hepatitis in horses (1919), referred to as Theiler's Disease ever since. A few months ago, a Flaviviridae relative was identified as its cause (Chandriania et al., 2013). Sir Arnold's son Max was awarded the Nobel Prize for the development of a Yellow Fever vaccine (1951) — flavivirology obviously runs in the family...

Sir Arnold Theiler Memorial Lectures

1984:	T Gutsche	"Theiler – His Personal Significance Today."
1985:	Prof HPA De Boom	"Vlammende Fakkels, Ou Bene, Ivoortorings en Rooi Vlae."
1986:	Prof BC Jansen	"Theiler Gedenklesing."
1987:		Opening of the Sir Arnold Theiler Building - No Lecture
1988:	Dr RD Bigalke	"Important Research Requirements for future Animal Production-Orientated Research with Particular Reference to Veterinary Science"
1989:	Dr R Swanepoel	*
1990:	Dr A Schutte	"The Impact of controlled Breeding on the Cattle Industry in Southern Africa"
1991:	Prof DM Joubert	"Sir Arnold Theilergedenklesing – Theiler en die Fakulteit Veeartsenykunde"
1992:	Dr CM Cameron	"The Environment – Whose Responsibility?"
1993:		Opening of the Onderstepoort Veterinary Academic Hospital – No Lecture
1994:	Dr W Plowright	"Rinderpest and Cell-Culture Revolution"
1995:	Prof WL Jenkins	*
1996:	Prof PV Tobias	"Premature Discoveries in Science."
1997:	Prof DL Block	"Our Universe: Accident of Design?"
1998:	Prof TW Naudé	"A Stroll Through the Wondrous Garden of South African Toxicology"
1999:	*	*
2000:	Dr DW Verwoerd	"The Molecular Revolution in biology and its Influence on Veterinary Science."
2001:	Prof H Huismans	"Molecular Biology and its Impact on the study and Control of Viral Diseases such as Bluetongue and African Horse Sickness."
2002:	Prof I Horak	"The Joy of Research"
2003:	Prof WFO Marasas	"Fumonisins: Historical Perspective and Future Objectives"
2004:	Dr RA Kock	"Wildlife Domestic Animal Disease Interface – Hard or Soft Edge?"
2005:	Prof SS van den Berg:	"The Past, Present and Future of the Clinical Departments in the Faculty of Veterinary Science."
2006:	Dr BD Perry	"The Global Poverty Reduction Agenda: What are the Implications for Animal Health Research and Development?"
2007:	Prof dr AWCA Cornelissen	"What makes an excellent Faculty of Veterinary Medicine?"
2008:	Dr G Brückner	"New challenges for the veterinary profession in global animal disease control and the trade in animals and animal products."
2009:	Prof P Doherty	"Adventures in Infection and Immunity."
2010:	Dr R. Moerane	"The Role of the Veterinary Profession in the Current Developmental Agenda in South Africa."
2011:		World Veterinary Congress in SA – no Faculty Day
2012:	Prof N James MacLachlan	"Emerging viral diseases; the example of bluetongue, from Theiler to climate change"

^{**} We do apologise that the above list is not complete. It will be appreciated if anyone who has access to some of the missing information, contacts either Dr Paul van Dam (paul.vandam@up.ac.za or 012 529 8203) or Mr Chris van Blerk (chris.vanblerk@up.ac.za or 012 529 8436)

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This can only be achieved by developing effective postgraduate programmes supervised by world-class staff members without neglecting the basic responsibility of providing the highest quality of undergraduate training. The basic mandate of Veterinary Science is the protection of animal health, which often also impacts on human health, thereby stimulating economic growth and food security. An efficient research programme therefore must meet the needs of society but remain relevant to a constantly changing environment. To meet these requirements the Faculty decided on the following focus areas:

Molecular studies on infectious and parasitic diseases of

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

Phytomedicine and ethno-veterinary medicine

An established multidisciplinary and collaborative research programme focusing on the development of extracts from plants with antimicrobial or anti-parasitic activity for use in animal production

Wildlife and Environmental Health

This is an inclusive research focus with contributions from all five departments of the faculty, including studies on tuberculosis in buffalo, immune-contraception in elephants, theileriosis in roan and sable, toxicity of non-steroidal antiinflammatories in vultures and endocrine disruptors in the environment

Veterinary aspects of food safety and food security

This is an established research focus of the faculty which includes, *inter alia*, programmes in veterinary public health, community development, epidemiology and risk assessment and poultry health

Equine and companion animal health and welfare

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

Research output and growth

Measuring the growth of the research outputs over the preceding five-year period is useful to evaluate the success of the Faculty. Subsidy earnings, which reflect the number of scientific publications by staff members and students, increased from 65,31 to 89,79, representing a growth of 37,5%. The number of master and PhD qualifications increased by 42% and 113% over the past five years, respectively, with the highest number of postgraduate students in the history of the Faculty graduating in 2012. The budget for postgraduate bursaries almost doubled from R386 000 to R610 640, benefitting mostly PhD students,





and the number of NRF-rated staff members, perhaps the most important long-term indicator, increased from 17 in 2008 to 26 in 2012, a growth of 52,9%. A significant achievement is the fact that the *per capita* research output per academic staff member is one of the highest in the university in 2012.

Highlights of 2012

An important initiative during 2012 was the implementation by the University of selected Institutional Research Themes (IRTs). These themes were selected on the basis of existing strengths of the university and their potential to stimulate inter-faculty and international collaboration as a method to stimulate research. Five themes were initially approved for special funding and the Faculty actively collaborate in three of these: Animal and Zoonotic Diseases, Genomics, and Food, Nutrition and Well-being. Six proposals submitted by this Faculty for each of the first two IRTs were approved for funding in 2012/2013.

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During 2012 a total of 82 research protocols were approved by Rescom. The themes involved illustrate how research is influenced by needs and opportunities. Five projects support the protection of Rhinos, ranging from the genetic identification of individual animals, anatomical features, susceptibility to various diseases and the treatment and prognosis of animals injured during poaching. Pollution of the environment, especially of river systems by mine effluents, was addressed in studies on its effect on the reproduction of domestic and wild animals, the pansteatitis problem in crocodiles and on using catfish cultures for the measurement of pollution. Six new projects were initiated in the Mnisi area involving the training of emerging small scale farmers and research at the animal/human/wildlife interface, including diseases such as TB, brucellosis, foot and mouth disease, rabies, tick-borne diseases and the development of acaricide resistance by ticks. Finally, studies on vaccine development and improvement included work on Rift Valley fever, anthrax and African horse sickness vaccines.

The annual Faculty Day in 2012 again provided an opportunity for our researchers to showcase the research activities in the Faculty to colleagues and peers, and was well attended by staff members, visitors and sponsor companies alike. The prestigious Sir Arnold Theiler Memorial lecture was delivered by Prof NJ MacLachlan, Distinguished Professor and internationally renowned expert on orbiviruses from the University of California, and Extraordinary Professor in the Faculty's own Department of Veterinary Tropical Diseases. The title was 'Emerging viral diseases: the example of bluetongue, from Theiler to climate change'. Highly relevant in terms of the recent spread of bluetongue to Europe, his contribution also fittingly illustrated the dedication of our faculty to international collaboration with experts all over the world.

Research Summary: 2012 (continued)



A similar successful event in 2012, highlighting the importance of research and cooperation, was the second Postgraduate Student Symposium hosted in September in collaboration with the Institute of Tropical Medicine, Antwerp, Belgium. The symposium was attended by 92 delegates from 16 countries. Topics related to "One Health" were addressed and postgraduate students from the SADC region presented their research to their peers, supervisors and invited scientists with international standing.

Excellence in research performance was again recognised by the identification of the Faculty's Top 10 researchers and the allocation of the following research awards:

Researcher of the Year

 Prof Moritz van Vuuren (Department of Veterinary Tropical Diseases)

Young Researcher of the Year

 Ms Lizette du Plessis (Department of Anatomy and Physiology)

Following nine (top 10 names are published and qualify for Dean's lunch)

- Prof Vinny Naidoo
- Prof Anita Michel
- Prof Montague Saulez
- Prof Estelle Venter
- Prof Christo Botha
- Prof Eran Dvir
- Prof Banie Penzhorn
- Prof Peter Thompson
- Dr Marinda Oosthuizen

In June 2012, Prof Kobus Eloff, Head of the Phytomedicine Programme in the Faculty was awarded the National Science and Technology Forum (NSTF)-BHP Billiton Award, sponsored by Eskom. This award is presented annually to a researcher for outstanding contributions to science, engineering, technology and innovation through research capacity development over the last five to ten years.

Research Programme: Oral Presentations

A health and demographic surveillance system in dogs (HDSS-Dogs) as a platform for research into rabies epidemiology and control

A Conan¹, A Meyer¹, O Akerele¹, G Simpson², J van Rooyen¹, DL Knobel¹

¹Department of Veterinary Tropical Diseases & ²Department of Production Animal Studies, Faculty of Veterinary Science, University of Pretoria, Onderstepoort, 0110; darryn.knobel@up.ac.za



A health and demographic surveillance system (HDSS) is defined as "a set of field and computing operations to handle the longitudinal follow-up of well-defined entities or primary subjects (individuals, households, and residential units) and all related demographic and health outcomes within a clearly circumscribed geographic area" (1). This geographic area is known as a demographic surveillance area (DSA). Health and demographic surveillance systems are widely used in human public health in low- and middle-income countries, to generate high quality, population-based, longitudinal health and demographic data. One of the key characteristics of an HDSS is the longitudinal measurement of demographic and health variables, achieved through repeated visits at regular intervals to all residential units within DSA. Unlike a cohort study, an HDSS follows up the entire population of a given geographic area. HDSS sites not only collect vital baseline information on population health and demographics, but also provide a platform for the design and evaluation of a wide range of health, social, economic and behavioural interventions and research studies. The overall objective of these HDSS sites is to establish a reliable information base to help policy-makers set health priorities and allocate resources more efficiently (2).

Despite similar needs for accurate, reliable population-level data on which to base policy decisions, HDSS's are not widely used in animal health research. Understanding the size and demographics of dog populations (in particular, rates of birth, death, in- and out-migration) is critical for planning and implementing effective dog rabies control campaigns (3,4). However, little is known about the dynamics of dog populations in countries in sub-Saharan Africa where

dog rabies is endemic. Here, we describe an HDSS in a population of owned, free-roaming dogs in Bushbuckridge Local Municipality, Mpumalanga Province, South Africa. The DSA for this HDSS-Dogs covers 10.4 km², incorporating over 2,500 residential units ('stands') and a population of around 1,000 owned dogs. All stands are permanently and uniquely identified, and are visited bi-annually by the HDSS-Dogs field team. All owned dogs are recorded at each visit. as are demographic events including births, deaths, and migration in and out of the property. All 'residence episodes' of individual dogs in stands are tracked and used to calculate birth, death and migration rates. Residence episodes begin with birth or in-migration events, and terminate with death or out-migration events. All dogs in the population are permanently and uniquely identified by a subcutaneouslyimplanted RFID microchip. Properties such as age, sex and rabies vaccination status are recorded for the enrolled dogs. Data are stored in a relational database that is updated biannually. Dogs are owned by 15.4% of households, with an average of 2.7 dogs per dog-owning household. The ratio of dogs to humans in the DSA is 1:7. Of the dogs enrolled into the HDSS at the start of the study in July 2011, only 15.8% had been vaccinated against rabies within the preceding 3 years. Of the residence episodes that terminated during the course of the study, 64.3% were due to death of the dog, and 28.7 % due to the dog being given away. Half of all deaths were reportedly due to disease. The potential of the HDSS-Dogs as a platform for specific studies into rabies epidemiology and control is discussed.

- INDEPTH, 2002, Population and Health in Developing Countries Volume 1: Population, Health and Survival at IN-DEPTH Sites, International Development Research Centre, Ottawa.
- Sankoh, O. & Byass, P., 2012, 'The INDEPTH Network: filling in vital gaps in global epidemiology', *International Journal of Epidemiology* 41, 579-588
- Lembo, T., on behalf of the Partners for Rabies Prevention, 2012, 'The blueprint for rabies prevention and control: a novel operational toolkit for rabies elimination', PLoS Neglected Tropical Diseases 6(2), e1388.
- Hampson, K., Dushoff, J., Cleaveland, S., Haydon, D.T., Kaare, M., et al, 2009, 'Transmission dynamics and prospects for the elimination of canine rabies', *PLoS Biology* 7(3), e1000053.

First report of Avian Gyrovirus Type 2 in South African poultry

C Abolnik¹, DBR Wandrag¹ et al

1Poultry Section, Department of Production Animal Studies, Faculty of Veterinary Science, University of Pretoria, Onderstepoort, 0110; celia.abolnik@up.ac.za

In late March 2013, a small-scale broiler operation near Johannesburg submitted live and dead 35-day old chickens for post mortem. The farmer reported mortalities of 500 out of 1400 birds over a seven day period, and the remainder of the flock were also displaying symptoms that included nervous signs (paresis, paralysis and torticollis). On post mortem

80% showed various degrees of rhinitis, tracheitis, air sacculitis and peritonitis. Cultured air sac swabs tested positive for *Escherichia coli*. Newcastle disease and avian influenza as causes of the nervous signs were initially excluded by PCR conducted at ARC-OVI.

To identify the pathogen causing the nervous symptoms, random amplification deep sequencing of the transcriptome extracted from pooled tissue samples was performed. Following de novo assembly of 928 000 paired sequence reads, a near-complete genome of Avian Gyrovirus Type 2 (AGV2) was identified, as well as the presence of Mycoplasma

gallisepticum. Genomes of other pathogens (infectious bronchitis virus, influenza A virus, West Nile virus, infectious bursal disease) were not detected, but an avirulent genotype I strain of Newcastle disease virus (NDV) was isolated from pooled tissues. A Taqman real-time PCR assay was designed and applied to extracts from separate tissue pools, indicating that the NDV and AGV2 viruses were only present in the brain. The strain of NDV isolated is not known to cause neurological symptoms.

Avian Gyrovirus Type 2 (AGV2) is a newly-discovered circovirus (single stranded DNA virus) that has only been

reported in Southern Brazil and the Netherlands before now (1, 2). It shares only 40% genomic nucleotide sequence similarity with Chicken Anaemia Virus (CAV), but up to 92% sequence identity with Human Gyrovirus, raising concerns that this may be a zoonotic infection or one that is transmissible through the consumption of chicken meat (3).



Attempts to isolate the South African AGV2 strain are currently underway, following which experimental infections of specific pathogen-free chickens and surveys to determine the prevalence of the infection of the virus in South Africa are planned.

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Transpla

Transplacental infection in goats experimentally infected with a European strain of bluetongue virus serotype 8

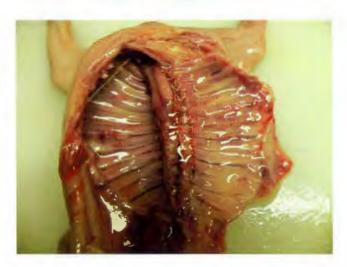
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Bluetongue (BT) is a globally important, non-contagious arthropod transmitted viral disease of domestic and wild ruminants that is caused by the bluetongue virus (BTV) (genus Orbivirus, family Reoviridae). Twenty six serotypes of the virus have been identified that are transmitted primarily through the bites of haematophagous midges (Culicoides spp. - Diptera: Ceratopogonidae), the biological vectors of the virus. During a recent outbreak of bluetongue serotype 8 (BTV-8) in northwestern Europe (2006-2008), it was found that the virus demonstrated the capability to cross the placenta of sheep and cattle, resulting in some cases in the birth of lambs and calves that demonstrated either a transient infectious viraemia and/or severe teratological defects. The ability of the virus to cross the placenta was unusual as transplacental infection had previously only been associated with the vaccination of pregnant sheep and cattle with egg/cell culture adapted modified-live virus vaccines produced by Onderstepoort Biological Products (OBP) in South Africa. The BTV-8 strain was in addition able to persist in Europe during winter, when the host-vector cycle is interrupted due to the death of adult midges as a result of inclement climatic conditions. Transplacental infection may be one of several mechanisms by which the virus may have been able to overwinter. Sero-prevalence rates in goats in north-western Europe were high during the outbreak of BTV-8; however the capability of the virus to infect goats through the transplacental route has not been established.

The objectives of this study were to determine whether (a) the European strain of BTV-8 had the capability to infect goat foetuses through the transplacental route, (b) to document congenital defects and/or gross lesions that may be associated with transplacental infection in goats and (c) to evaluate the importance of transplacental infection in goats in regards to overwintering of the virus in Europe. Four Saanen goats pregnant at 62 days of gestation were inoculated with the European strain of BTV-8. Adult goats were sequentially euthanised at roughly 2 week intervals post infection and the foetuses recovered. Foetal serum, blood and tissue were tested by using serological and molecular methods in order to investigate the presence of the virus.

Infection of adult goats resulted in mild clinical signs (limited to behavioral changes, a transient pyrexia and petechial haemorrhaging in the conjunctivae); however gross lesions observed post mortem were more severe (hyperaemia, haemorrhaging and oedema in several tissues). The virus crossed the placenta in all four goats. Viral RNA was demonstrated by real-time RT-PCR in blood and tissue



samples from three foetuses harvested from two goats at 43 days post infection. Conventional PCR and sequencing confirmed infection of two of these foetuses with BTV-8. Viral RNA was also detected in placental tissue and fluid from the remaining two goats at 13-25 days post infection. In total, five of six foetuses demonstrated lesions (haemorrhaging/oedema in the lungs, heart and liver) that may have been associated with transplacental infection. No group or serotype specific antibodies were observed in the serum of any of the infected foetuses. Furthermore no neurological lesions could be demonstrated. Infected goat fetuses demonstrated low viral RNA concentrations in blood and tissue and would likely not have been born viraemic.

The potential consequences for goat fetuses and overwintering of the virus are difficult to determine from the results of this study due to the limited number of pregnant animals that were included. Future studies should include more pregnant animals and bring fetuses to term, in order to determine: (1) whether transplacentally infected kids can be borne viraemic; (2) the serological status of transplacentally infected kids at birth, and (3) to document possible BTV-8 associated teratogenicity.

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Investigating the possible presence of Theileria parva carrier cattle in Mnisi area

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Corridor disease (buffalo-derived Theileria parva) caused by Theileria parva lawrencei is the most important Theileria sp. posing a threat to the cattle farming industry in South Africa. The African buffalo (Syncerus caffer) is the reservoir host for this protozoan parasite which is transmitted by the threehost ticks Rhipicephalus appendiculatus and Rhipicephalus zambeziensis. Although it is considered a self-limiting disease because most cattle die before the parasites reach the tickinfective stage. Recent experimental studies have shown that a carrier state can be attained in infected cattle that survive the disease. Field and laboratory studies in Northern KwaZulu-Natal indicated that some infected and recovered cattle were potential T. parva carriers as they remained parasitaemic (polymerase chain reaction [PCR]-positive) for periods ranging from 30 to 50 days. Attempts to infect susceptible cattle from these cattle using Rhipicephalus appendiculatus however, failed. A study to identify T. parva carrier cattle in Mnisi, a wildlife/livestock interface area, was started in 2012. Records from Hluvukani Animal Health Centre and Bushbuckridge State Veterinary office were scrutinized and 670 blood samples in plain tubes were collected from herds that recorded Corridor disease cases in the past three years. Herds that grazed in the same areas with buffaloes when they broke from the Kruger National Park or private game reserves in the past three years were also sampled. The indirect fluorescent antibody test was used to check for T. parva antibodies. Deoxyribonucleic acid (DNA) was extracted from ethylene-diamine-tetra-acetic-acid (EDTA) blood samples collected from sero-positive herds and screened for the presence of piroplasm parasite DNA using a T. parva-specific quantitative real-time PCR (qPCR). The full-length parasite 18S ribosomal ribonucleic acid (rRNA) gene of selected positive samples will be amplified, cloned and sequenced. The sequences will be compared with those found in clinical Corridor disease cases in Mnisi area as well as with isolates from buffalo.

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Radiographic changes in Thoroughbred racehorse yearlings in South Africa: Prevalence at the time of the yearling sales (2008-2010)

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Radiographic examination of Thoroughbred racehorses at the time of the yearling sales is common practice in South Africa. Although it is generally accepted that most yearlings will have some radiographic changes, there has currently only been one study done to estimate the prevalence of these changes in Thoroughbred racehorse yearlings during 2008 at the time of the yearling sales in South Africa (1).

The objective was to describe the prevalence and distribution of radiographic changes in the metacarpophalangeal joint, metatarsophalangeal joint, carpi, tarsi, stifle and fore digits of racing Thoroughbred yearlings in South Africa when examined as part of pre-purchase examination during the Annual National Yearling Sales extending from 2008 - 2010.

Thoroughbred racehorse yearlings were subjected to radiographic evaluation included the digit (n=566), metacarpophalangeal joint (n=566), metatarsophalangeal joint (n=566), carpi (n=566), tarsi (n=566) and stifle (n=566). The radiographic changes were categorised by location and type of change present for each series.

In South Africa Thoroughbred racehorse yearlings radiographic changes most commonly involved the metacarpo- and metatarsophalangeal joints, carpi and tarsi. The current study showed an increase in prevalence of supracondylar lysis, dorsomedial carpal disease, carpal osteochondral fragmentation and degenerative joint disease of tarsi when compared to similar studies performed.

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Delayed gadolinium enhanced magnetic resonance imaging and T2 mapping of cartilage of the cadaver distal metacarpus3/metatarsus3 of the normal Thoroughbred horse

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Introduction: Delayed gadolinium enhanced imaging of cartilage (dGEMRIC) and T2 cartilage mapping in humans can indicate osteoarthritis early in the disease process. T2 mapping characterizes hyaline articular cartilage and repair tissue. In dGEMRIC, the negatively charged Gd-DTPA²⁻, injected either intra-articularly or intravenously, penetrates hyaline cartilage inversely proportional to the cartilage proteoglycan concentration. In osteoarthritic proteoglycan-depleted cartilage, penetration of Gd-DTPA²⁻ is increased.

Objectives: The feasibility of dGEMRIC and T2 mapping of the distal metacarpus3/metatarsus3 (Mc3/Mt3).

Methods: Twelve racing Thoroughbred cadaver midcondylar distal Mc3s/Mt3s underwent six precontrast short *tau* inversion recovery (STIR) sequence scans for dGEMRIC T1 relaxation time calculation, as well as T2 scans using a 1.5T machine. Gd-DTPA²⁻ was injected intra-articularly and the STIR sequences repeated at 30, 60, 120, and 180 minutes postinjection. T1 and T2 maps of the distal Mc3/Mt3 cartilage were created and mean values of regions of interest calculated. (Approved: Animal Ethics Committee, University of Pretoria.)

Results: T1 relaxation time (dGEMRIC) decreased from 1027 ms (mean) to its lowest at 642 ms, 120 minutes post intra-articular injection. Mean T2 relaxation time was 85 ms. At the distal Mc3/Mt3 - proximal phalanx cartilage interface, the opposing cartilages could not be differentiated.

Discussion: T2 and dGEMRIC mapping in the fetlock is feasible, with T1 relaxation time decreasing to its lowest 60-120 minutes post Gd-DTPA²⁻ injection. Limitations include poor spatial resolution of the thin cartilage, overlap of the distal Mc3/Mt3 - proximal phalanx cartilage, and low number of limbs. Future studies envisioned include evaluation of intravenous administration of Gd-DTPA²⁻, and cartilage mapping in live exercised *vs* non-exercised horses. dGEMRIC



and T2 mapping of horse fetlocks with osteoarthritis should be performed in future to determine whether early cartilage injury can be ascertained.

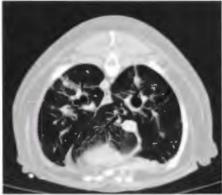
Conclusions: Reference values and technique of dGEMRIC and T2 mapping in the cadaver distal Mc3s/Mt3s of normal Thoroughbred horses were determined.

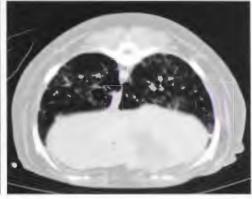
Cryptogenic organising pneumonitis in two dogs

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The aim of this case report was to highlight that cryptogenic organising pneumonitis (COP) should be considered in dogs that present with persistent pyrexia, coughing, malaise with radiological features consistent with bronchopneumonia. Typically dogs with this condition will consistently fail to respond to the standard therapy of antibiotics, but it carries an excellent prognosis and is reversible with corticosteroid treatment. Two dogs presented with pyrexia, tachypnoea, coughing and adventitious lung sounds. Both dogs had a 3 to 4 week history of persistent pyrexia, reduced exercise tolerance, inappetence, coughing and lymphadenopathy that failed to respond to repeated courses of antibiotics. Haematology in both dogs revealed an inflammatory leukogram. Radiological abnormalities revealed a patchy, mixed alveolar-interstitial lung pattern. Thoracic computed tomography of the one dog showed diffuse ground glass attenuation with a multifocal subpleural alveolar pattern. Broncho-alveolar lavage (BAL) submitted for cytology, aerobic and fungal culture in both dogs confirmed a sterile suppurative pneumonia. Prednisilone was started (2 mg/kg, q 12 hours) and within 48 hours of initiating therapy clinical and radiological abnormalities had resolved. Both dogs were weaned off prednisolone (2 mg/kg q 24 hr for 7 days, 1 mg/kg, q 24 hr for 3 weeks, 0.5 mg/kg, q 24 hr for 3 weeks then 0.5 mg/kg, q 48 hr for 3 weeks). One of the dogs relapsed with clinical and radiological changes 3 weeks later but resolved completely after initiating therapy again. Cryptogenic organising pneumonitis, is an extremely rare idiopathic interstitial lung disease (ILD) that is characterised by alveolitis and intraluminal 'lung buds', interstitial fibrosis but the lack of bronchial scaring and is reversible (Cordier 2006). There are three reports of organising pneumonitis in the small animal veterinary literature (Phillips et al. 2000; Norris et al. 2002; Li et al. 2006). Differentials of idiopathic ILDs in dogs include idiopathic pulmonary fibrosis, eosinophilic bronchopneumopathy, histiocytosis X, sarcoidosis and hypersensitivity pneumonitis, which can be differentiated from COP based on radiological and BAL findings (Norris et al. 2002). Lung biopsy and histopathology is definitive but is often not performed in frail or respiratory compromised patients as was the case in these dogs due to the associated risk of death (Norris et al. 2002). There was rapid resolution of clinical and radiological signs with corticosteroids and relapse in the one dog after discontinuation of steroid therapy. Neither dog had microbial infection detected and both had vaccinations that were current There was a lack of exposure to inhaled toxins or exposure to drugs prior to the onset of clinical signs and rapid resolution of clinical and radiological findings to corticosteroids, supporting the diagnosis of this rare condition.

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Clinical experience with the use of non-cemented total hip replacement systems in dogs

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Introduction:

BioMedtrix cemented total hip replacement (THR) implants that have been used in previous studies revealed latent infections and implant loosening in some dogs. These complications prompted veterinary surgeons over the past decade to divert to non-cemented implant systems. Advantages of non-cemented over cemented implants include longer implant life, decreased risk of postoperative infection, and better implant stability through osteointegration.

The newer non-cemented THR components are covered with a porous titanium surface that has an osteo-inductive ability. Implants are manufactured for

press-fit into well prepared bone beds in the acetabulum and femoral canal. The acetabular cups consist of a high-density polyethylene liner contained inside a metal shell with a porous backing. Short-term stability is achieved via press-fit of the femoral and acetabular components and long-term stability is by the ingrowth of new bone into the porous metal surface of the components.

Materials and Methods:

The BioMedtrixa non-cemented BFX® modular implants were used in 17 client owned dogs, the HELICA® screw-in systemb with threads coated by rough-blasting titanium were used in 14 dogs, and the Zurich THR implants, also known as the KYON® system, were used in 3 dogs. All dogs suffered from hip dysplasia or malunion of a femur neck fracture causing secondary hip osteoarthritis. The KYON® titanium-alloy coated femoral stem contains interlocking, self-tapping screws that can anchor the stem in the proximal femoral canal to prevent subsidence with weight bearing. These implants were used in selected cases with "stovepipe" femoral canal morphology.

Results:

Most dogs were able to bear weight on the operated limb on the second or third day after the operation but had to be confined with restricted exercise for the first 10 to 12 post-operative weeks. Four dogs treated with the BFX® press-fit system developed intra-operative proximal femoral fissure lines that were stabilized with three or four full cerclage wires





before stem impaction. Postoperative subsidence of the femoral stem was encountered with the BFX® press-fit system in two dogs that presented with typical "stove pipe" femoral morphology. Four dogs treated with the HELICA® screwin implants developed radiolucent lines of bone resorption around the acetabular cups after 12 to 16 weeks and two cases needed explantation. In two dogs the HELICA® stem diameter was too big for the femoral neck and an appropriate size BFX® stem had to be used.

Conclusions:

The HELICA® screw-in THR implants were technically easier to apply with shorter surgical times, but more postoperative complications were encountered with this system during the convalescent period of 12 weeks. The KYON® system appeared to be more versatile for use in most cases, but it was technically not easy to insert the self-tapping, interlocking screws through the lateral cortex and stem in some of the femurs. Instrumentation for the BFX® press-fit system was superior and the acetabular reamers could also be utilized for the HELICA® cups.

- a BioMedtrix non-cemented BFX® implants (50 Intervale Road, Boonton, New York, 07005, USA)
- b HELICA® screw-in implants (INNOPLANT veterinary, Höfe strasse 25, Hannover, Germany)
- c Zurich THR implants (KYON®, Technopark strasse 1, CH-8005, Zurich, Switzerland)

As rare as emu 'teeth'

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Extant birds do not possess teeth and true teeth are only known from a few fossil birds such as *lcthyornis*, *Archaeopteryx*, *Parahesperomis* and *Hesperomis*. Psuedoteeth, rhamphothecal-covered bony projections from the jaw, have been identified in *Osteodontornis* and its relatives. It has been proposed that avians maintain the potential for tooth development. Recent observations suggest that serrations in the emu mandibular rostrum may be more likened to 'psuedoteeth' than to lamellae, as previously thought.

The heads of ten 12-14 month old emus were obtained from birds at slaughter. Five heads were immersion-fixed in 10% neutral-buffered formalin and 5 heads were boiled and defatted following removal of the rhamphotheca. The formalin-fixed mandibles were decalcified, relevant regions of the mandibular rostrum cut into appropriate longitudinal and transverse sections, and routinely processed for light microscopy.

Stripping of the rhamphotheca from the rostral mandibular tomium revealed tapered, cylindrical keratinized pegs (Fig. 1) located in a shallow groove in the underlying bone (Figs. 1, 2) between each rhamphothecal serration (Fig. 1). Each rhamphothecal serration corresponded to a small raised region of the lateral edge of the dentary bone (Fig. 2). The keratinized pegs slanted caudally and were positioned on the lateral edge of the dentary bone (Figs. 1, 2). Each bony groove led to a small foramen or pit (Figs. 1, 2), at which point the keratinized peg terminated. Light microscopy revealed that the rhamphothecal serrations were formed by localised thickenings of the *Str. corneum* of the keratinized,

pigmented, stratified squamous epithelium. The underlying Str. germinativum and the supporting dermis formed a small, raised point from which the Str. corneum proliferated, forming the rhamphothecal serrations. The Str. corneum of the keratinized pegs was poorly pigmented and composed of concentrically arranged layers of cells, presenting a lamellated appearance in cross-section (Fig. 3). The underlying Str. germinativum and supporting dermis formed a tapered, cylindrical shell from which the keratinized peg originated.

The rhamphothecal serrations in the emu may represent vestigial 'psuedo-teeth' as the bony projections are much reduced in comparison to those in Osteodontornis species. Whereas the keratinized pegs may serve to anchor the rhamphothecal serrations as well as conduct vibrational stimuli from the rhamphothecal surface to the Herbst corpuscles situated within the dermis and within the pits in the dentary bone, the nature and positioning of the keratinised pegs is also suggestive of arrested tooth development. Thus the 'teeth' of the emu may be modified during early development to form part of an intricate sensory system. In the talpid2 chicken mutant, tooth formation was compared to that in the alligator and it was concluded that the first generation teeth in the avian embryo were markedly archosaurian (crocodilian). The keratinized pegs in the emu are closely associated with a bony groove. Both Ichthyornis and Hesperornis, in the juvenile state, have teeth set in a constricted groove, similar to that reported in young crocodilians. Studies on emu embryos will determine whether the cylindrical keratinized pegs share a similar origin to that of first generation crocodilian teeth.

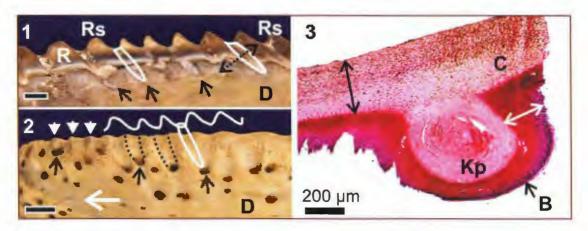


Figure 1. Lateral dentary bone (D) forming the mandibular rostrum with the rhamphotheca (R) partially stripped. Keratinised pegs (two outlined in white) lle in bony grooves which end in pits (arrows). Note the rhamphothecal serrations (Rs) flanking each keratinised peg. The double-headed dotted arrow indicates the plane of sectioning illustrated in Figure 3. Rostral is indicated in Figures 1 and 2 (white arrow). Bars = 1 mm

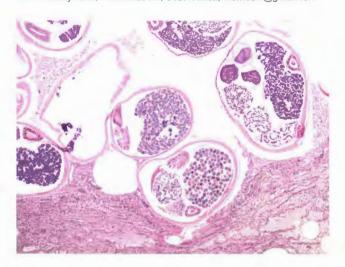
Figure 2. Dentary bone (D) illustrating the bony serrations (arrow heads), bony grooves (black dotted lines) and pits (arrows). The relationship of the thamphothecal serrations is schematically indicated (white wavy line) as well as a keratinised peg (white cylinder).

Figure 3. Transverse section of the epidermis and a keratinised peg (K). The Str. corneum (C) of the epidermis is pigmented and forms the rhamphotheca (↔). Note the concentric, lamellated nature of the keratinised peg which is non-pigmented. Str. germinativum (white ↔) and Str. basale (B).

A systematic health assessment of the Indian Ocean bottlenose dolphin (*Tursiops aduncus*) and the Indo-Pacific humpback dolphin (*Sousa chinensis*) incidentally caught in shark nets off the KwaZulu-Natal coast, South Africa

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Coastal dolphin populations are indicators of coastal marine environmental health and may be sensitive to anthropogenic influences. An increase in serosal nodules during necropsies of dolphins incidentally caught in shark nets off the KwaZulu-Natal coast, South Africa prompted the first systematic health assessment. Thirty five *Tursiops aduncus* (Indian Ocean bottlenose dolphin) and five *Sousa chinensis* (Indo-Pacific humpback dolphin), caught between 2010 and 2012, were evaluated using a detailed protocol for necropsy and sampling of small cetaceans developed for this purpose. Samples for histopathology were prepared using standard laboratory methods and stained with Haematoxylin and Eosin; special stains were performed as necessary.

All animals were considered to be in good nutritional condition based on blubber thickness. All animals had lesions with definite or presumed parasitic aetiology, including pneumonia (34/40), bronchiolar epithelial mineralization (33/40)gastroenteritis (28/40), hepatitis (24/39), endometritis (11/26), serosal inflammation of various abdominal and thoracic organs (30/40) and splenic serosal tags (18/40). Five parasites (*Halocercus* sp., *Crassicauda* sp., *Anisarkis* sp., Brachycladiinae, and *Xenobalanus globicipitis*) were recovered. Non-specific meningoencepahalitis (7/18) and

myocardial fibrosis (10/39) was found. Adrenal cortical hyperplasia (18/37), possibly related to chronic stress, was also found. Pulmonary pneumoconiosis and foreign material accumulation in the marginal lymph node of the lung, possibly indicating exposure to polluted air, was seen in three animals. Lesions suggestive of morbillivirus, *Toxoplasma gondii* or *Brucella* spp. tested negative with special stains and immunohistochemistry. The first confirmed cases of lobomycosis and sarcocystosis in dolphins in South Africa were documented.

Most lesions were mild and apparently did not affect nutritional state, although the high (and apparently increasing) prevalence of parasitic lesions may indicate that the host/parasite interface is changing. This may be attributed to anthropogenic factors, stress or environmental pollution, suggesting degradation of the marine environment. A changing marine ecosystem could also negatively impact on the human populations using the same environment for recreation, food and industry. Therefore continued health monitoring of the cetaceans populations and further research into disease pathophysiology and anthropogenic factors affecting these populations is needed

The use of Somatic Cell Count as a valid indicator of Intra Mammary Infections in South African Dairy Cows

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Abstract

The purpose of this study was to determine if a particular somatic cell count (SCC) range can be used as an indicator of intramammary infection (IMI). Mastitis in dairy cows is mostly caused by microbial infections and changes in pathogenicity may be indicated by increased SCC. Cyto-microbiological examinations were performed on 491 464 quarter and composite cow milk samples obtained from South African dairy herds between 2008 and 2012 in the Milk Laboratory at the Department of Production Animal Studies, Onderstepoort. Data of quarter and composite milk samples with SCC ≤400 000 cells/ml and ≤350 000 cells/ml respectively, were analysed using the Milk Sample Diagnostic program (MSD) and Microsoft Excell.

At the Buiatrics Congress (2012) it was decided by the Mastitis Specialist Committee to adopt a SCC of >200x103 cells/ml as an indicator of intra mammary infection (IMI) in dairy cows. In this study, all mastitogenic bacteria isolated from dairy cows in South Africa were found to be present in milk samples with a SCC of ≤200x10³ cells/ml. In quarter milk samples, (Figure 1), 83.17% with no growth, 69.31% of Staphylococcus aureus (STA), 69.89% coagulase negative staphylococci (CNS), 72.45% Staphylococcus pseudintermedius (STI) and 37.23% Streptococcus dysgalactiae (SDY) had a SCC ≤200x103 cells/ml. Similar findings in composite milk samples (Figure 2) indicated that 88.45% with no growth, 70.17% with STA, 74.80% with CNS, 77.61% with STI and 59.54% with SDY had SCC of ≤200x103 cells/ml. The highest percentage of samples with a SCC of ≤50x103cells/ml had no growth, while SDY was the microorganism isolated the least at the same SCC range. Although CNS is regarded as a minor pathogen, it has been shown to increasingly cause mastitis (Harmon & Langlois 1995). As an emerging pathogen in South Africa, STI showed the highest number of isolates found at SCC of ≤50x10³ cells/ml in both quarter and composite milk samples. Staphylococcus aureus is still isolated in high numbers in various countries as an important cause of udder infections (Barkema, Schukken & Zadocks 2006).

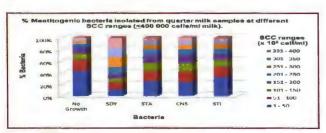


Figure 1 SCC ranges of quarter milk samples



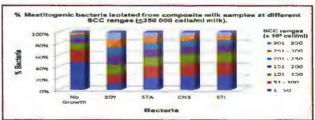


Figure 2 SCC ranges of composite milk samples

This study indicates that the use of a SCC of ≤200x10³cells/ml solely as indicator of IMI is not reliable, as numerous mastitogenic bacteria, including STA were isolated from both quarter and cow milk samples at the indicated level. Future udder health work may therefore be compromised if microbiological examination is not included in the determination of IMI.

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Regulative endocrine mechanisms related to reproductive tactics in free-ranging male giraffes (Giraffa camelopardis)

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Although the social organization of giraffes (Giraffa camelopardalis) imposes a high-cost 'roving male' reproductive strategy, the role of the hypothalamic-pituitarygonadal axis in affecting behavioural tactics used by individual males to deal with the high costs of this strategy has not been examined. In this study, we used faecal steroid analysis for monitoring androgen levels in male giraffes to describe the pattern of sexual activity and its endocrine correlates in giraffe bulls roaming in the Hwange area of Zimbabwe. Giraffes were observed for sexual activity for a total of 188 h using focal animal and ad libitum sampling. Seventy three bulls were individually identified by their unique pelage pattern and categorized into three developmental stages, Class-A, B and -C, based on physical appearance (body size, neck musculature, shape of skull and ossicones). In addition, 66 faecal samples from 39 different bulls were collected. Approximately 0.1g of dried faecal powder was extracted with 3 ml 80% ethanol and analysed using an enzyme immunoassay for epiandrosterone (University of

Veterinary Medicine, Vienna, Austria). The highest frequency of intersexual activity was observed for Class-A bulls, with the presence of other males stimulating this behaviour. Class-A bulls have significantly higher median faecal androgen (fA) levels compared to Class-B and -C bulls, and within classes, sexually active males had on average higher median fA concentrations than inactive individuals. Furthermore, males seem to switch between sexually active/inactive phases at the scale of two weeks, and their fA levels change accordingly (n = 4 cases). These findings suggest that giraffe males have short-term, rut-like periods, not unlike another tropical megaherbivore, the elephant, but at a much shorter time scale. However, further research is needed to assess the frequency of rutting activity, especially in relation to local male dominance and reproductive success.

Distribution of oxytocin receptors in the equine uterus and cervix

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Introduction

Oxytocin plays an essential role in the endocrine regulation of the oestrous cycle as well as in parturition and milk ejection. It is responsible for myometrial contractility which is primarily important for expulsion of the foetus during parturition and for mechanical drainage of uterine content (Nikolakopoulos, Watson 1999). It is reported that oxytocin receptor (OR) concentration in the equine endometrium varies throughout the oestrous cycle with a peak during late dioestrus (Sharp et al. 1997, Stull 1986, Starbuck et al. 1998). There are no reports of OR distribution in the female equine reproductive



tract. This study uniquely provides a detailed description of OR distribution during the oestrous cycle in the equine endometrium, myometrium and the cervix.

Materials and Methods

Fifteen routinely slaughtered cyclic mares of various breeds and sizes were assigned to two different groups depending on their stage of oestrus, either non-luteal (n = 9) and luteal (n = 6), phases. The assignment of mares to either group was based on findings on inspection of the ovaries and plasmaprogesterone concentration (PPC).

Full thickness uterine samples were obtained from all mares at 3 different sites (uterine body, right and left uterine horn) and one sample from the cervix. Each full thickness sample was fixed in 10 % neutral buffered formalin overnight, embedded in paraffin and sectioned at a thickness of 4-6 µm. Sections were de-waxed and immunohistochemically labelled using a polyclonal antibody against human OR (Sigma O 4389, Sigma-Aldrich, Germany).

Evaluation of the slides was done using an Olympus BX43 microscope and the Olympus cell sens dimension software (Wirsam Scientific & Precision Equipment PTY LD, Johannesburg, South Africa).

Results and Discussion

In the uterus, ORs were found in both luminal and glandular epithelium, in the endothelium of blood vessels and in

the sub-epithelial and periglandular stroma and smooth muscle. The greatest intensity of staining was consistently in the endothelium of blood vessels. The cervix, the luminal epithelium, the endothelium of blood vessels and the muscle layers also showed the presence of ORs. A positive correlation between the appearance of ORs in uterus and cervix was observed.

No difference in distribution could be found between luteal and non-luteal phase.

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The effects of midazolam and butorphanol, administered alone or combined, on the dose of alfaxalone required for induction of anaesthesia and quality of anaesthesia arising therefrom in goats.

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Goats are not among the most commonly anaesthetised animals and consequently, scant information is available on the efficacy of anaesthetic drugs in goats. Administration of sedatives before an injectable anaesthetic agent to calm down a patient is now a widely accepted concept in veterinary practice (Bednarski et al. 2011; Dzikiti et al. 2009). Alfaxalone is a relatively new anaesthetic agent whose efficacy in goats has not yet been studied.

The sedative and alfaxalone-sparing effects of midazolam and butorphanol, administered alone or concomitantly, in goats were assessed. Basic cardiorespiratory effects arising from administration of these drugs were assessed. Eight clinically healthy goats (four does and four wethers) were enlisted in a randomised crossover manner to receive, by the intramuscularly route, sedative treatments consisting of saline 0.05 ml/kg (CONTROL) or midazolam 0.3 mg/kg (MID) or butorphanol 0.1 mg/kg (BUT) or a combination of midazolam 0.3 mg/kg and butorphanol 0.1 mg/kg (MIDBUT) before induction of general anaesthesia with alfaxalone. After induction of general anaesthesia, the goats were intubated and immediately allowed to recover while quality of anaesthesia and basic physiological cardiorespiratory and blood-gas parameters were measured until the goats had recovered from anaesthesia. The degree of sedation obtained, quality of general anaesthesia induction and quality of recovery from general anaesthesia were scored.

Midazolam, administered alone or with butorphanol, caused statistically significant sedation and reductions in alfaxalone required for induction when compared to saline, while butorphanol alone did not show any statistically significant differences. The induction dose of alfaxalone in un-sedated goats was 3 mg/kg. The percentage reductions in the dose of alfaxalone required for induction of general anaesthesia following MID and MIDBUT treatments were 33 % and 42 %, respectively, and were statistically significant while BUT treatment caused a statistically-insignificant reduction of about 24 %. The quality of induction and recovery was good for all treatments including; the control group. Cardiorespiratory and blood-gas parameters were maintained within clinically acceptable limits.



The present study demonstrates that midazolam, administered alone or combined with butorphanol, produces a degree of sedation that significantly reduces the dose of alfaxalone required for induction of general anaesthesia in goats without causing any major adverse cardiorespiratory effects.

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Platelet activation and platelet-leukocyte interaction in dogs naturally infected with Babesia rossi and its association with outcome

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Their unique origin, structure and ability to interact with various cells of the immune system, have positioned platelets in a central role in host immunity. Following activation, platelets express activation markers on their membranes, such as P-selectin (PS). The expression of PS promotes adhesion of platelets to leukocytes, which mediates the adhesion of neutrophils and monocytes to endothelium, as well as upregulate the pro-inflammatory function of neutrophils. Increased platelet-leukocyte heteroaggregates have been reported in dogs and humans with thrombotic diseases, such as myocardial infarction and sepsis. The purpose of this study was to investigate the occurrence of platelet activation and platelet-leukocyte interaction in canine babesiosis and evaluate its association with outcome.

At presentation, prior to any treatment, EDTA blood was collected and processed within one hour. A platelet count was performed on the ADVIA 2120 (Siemens). Leukocytes and platelets were separately harvested according to specifications and washed with phosphate-buffered saline. Platelets were incubated with anti-CD61 PE and anti-CD62P FITC for determination of activated platelets, and leukocytes with anti-CD14 APC and anti-CD62P FITC for determination

of platelet-leukocyte heteroaggregates. Flow cytometric analysis was performed on the FACSCalibur (Becton Dickinson). Platelets were identified using forward/sideward scatter and CD61 positivity, and further analysed for CD62P, indicating the extent of platelet activation. Platelet-leukocyte heteroaggregates were determined using CD14 to identify monocytes and sideward scatter to identify neutrophils, and further analysed for CD62P, indicating the extent of platelet-monocyte and platelet-neutrophil interaction.

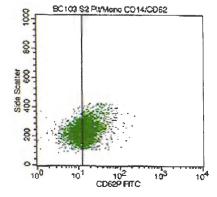
Thirty-four Babesia-infected and 14 control dogs were included. Of the Babesia-infected dogs, 27 survived and 7 died (mortality rate 26%). Compared to controls, the Babesia-infected dogs had significantly lower platelet count (P<0.001), and significantly higher circulating platelet activity (P=0.026) and platelet-monocyte heteroaggregation (P=0.027). Dogs that survived had significantly higher circulating platelet activity (P=0.02) and platelet-monocyte heteroaggregation (P=0.013), but no significant difference in platelet-neutrophil heteroaggregation (P=0.074), compared to controls. Dogs that died had values that were not significantly different from either controls or survivors.

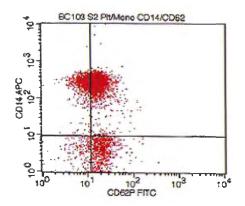
Variable	Controls ¹	Babesia ¹	Survived ¹	Died¹
Platelet count (×10°/L)	295 (254-383)	42 (20-71)	43 (20-77)	38 (20-48)
Circulating platelet activity (%)	0.4 (0.2-1.4)	2.5 (0.4-7.3)	3.2 (0.6-8.1)	1.5 (0.2-4.6)
Platelet-monocyte aggregates (%)	34.5 (22.9-47.2)	54.0 (31.3-69.9)	58.9 (32.7-71.0)	39.2 (20.3-55.1)
Platelet-neutrophil aggregates (%)	16.1 (11.2-41.6)	28.3 (14.9-76.4)	31.6 (16.2-76.7)	16.8 (2.0-63.6)

¹Median (Inter quartile range)

In conclusion, *Babesia*-infected dogs, specifically dogs that survived, had a significantly up-regulated response with regards to platelet activation and platelet-monocyte heteroaggregation. Moreover, the dogs which died showed no difference compared to the controls, except for platelet count. These findings may indicate altered a-granule content,

as has been suggested in humans with sepsis, or platelet "exhaustion" secondary to the severe systemic inflammation present, resulting in immune "paralysis" and possible death. Further studies and correlations with other markers of inflammation such as IL-6 and cortisol are needed to investigate this interesting phenomenon.





Metabolic derangements in dogs naturally envenomed by African puffadder (Bitis arietans) and snouted cobra (Naja annulifera)

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Snake venoms contain a complex mixture of proteins that may result in several metabolic derangements. Envenomation Viperinae by snakes vipers, (e.g. adders) are known to induce myocardial injury; either a direct cardiotoxic effect, or secondary to systemic inflammation. Renal injury inflicted by snake venom has been reported to involve the glomeruli, tubules, interstitium and vasculature of the





kidney. Traditional laboratory assays are known for their insensitivity to determine renal damage and their inability to determine the location of the damage. The purpose of this study was to investigate the occurrence of myocardial and renal damage in dogs envenomed by African puffadder (*Bitis arietans*) and snouted cobra (*Naja annulifera*).

Serum samples were collected at presentation, 12-, 24- and 36 hours post-envenomation. Cardiac Troponin I (cTnl) was measured using a high-sensitivity immunoassay, and C-Reactive protein (CRP), a marker for inflammation, using a turbidimetric immunoassay. Urine samples were collected at presentation and 24 hours post-envenomation. For glomerular damage urine albumin (uAlb), CRP (uCRP) and IgG (uIgG) were determined using canine-specific sandwich enzyme-linked immunosorbent assay (ELISA) kits and, for proximal tubular damage, urine retinol binding protein (uRBP) using a human sandwich ELISA kit. All were reported as a ratio against urine creatinine (Cr). Traditional assays for renal damage [i.e. urea, creatinine, urine specific gravity (SG), urine protein:creatine (UPC)] were also measured. Ten healthy control dogs were included for all analysis.

Of the 5 dogs envenomed by *B. arietans*, [cTnI] was significantly higher than the controls at 24 hours postenvenomation (*P*=0.039) with no correlation between [CRP] and [cTnI]. One dog died after 24 hours and had the highest [cTnI] in the group. Necropsy revealed several small paintbrush haemorrhages in the endocardium of the left

ventricle. Of the 9 dogs envenomed by *N. annulifera*, [cTnI] was significantly higher than the controls at presentation (*P*=0.04), 12 (*P*=0.005), 24 (*P*<0.001), and 36 hours (*P*=0.0014) postenvenomation. Increased [CRP] was significantly correlated with [cTnI] only at presentation (r=0.77, *P*=0.025). One dog was euthanised due to a poor prognosis. Although it had no signs of systemic inflammation, it had the highest [cTnI] of any dog. Necropsy revealed no macroscopic cardiac lesions.

At presentation all snake-envenomed dogs demonstrated a significant increase in uALB/Cr, ulgG/Cr and uRBP/Cr (P<0.05 for all) compared to the controls. At 24 hours post-envenomation a significant increase, compared to the controls, was only noted in ulgG/Cr (P<0.01) and uCRP/Cr (P<0.001). Serum urea, creatinine and urine SG remained within reference limits at presentation and 24 hours post-envenomation, with no significant difference among groups. A significant difference was noted for UPC at presentation and 24 hours post-envenomation.

In conclusion, myocardial injury was shown to be a frequent finding in dogs envenomed by *B. arietans* and *N. annulifera*, but systemic inflammation did not appear to play a role in the pathogenesis. Transient glomerular and tubular renal dysfunction was also shown using novel urinary biomarkers such as uAlb, ulgG, uCRP and uRBP, which appeared to be more sensitive than traditional serum and urinary markers.

The use of partial-opioid antagonism combined with oxygen insufflation to support the physiology of chemically immobilized white rhinoceros (Ceratotherium simum)

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White rhinoceros immobilized with a combination of etorphine, azaperone and hyaluronidase experience adverse physiological changes including severe hypoxia, hypercapnia, respiratory and metabolic acidosis and tachycardia. With escalating levels of rhino poaching, conservation activities have accelerated in an effort to ensure the long-term survival of this species. Immobilization of rhino is a necessary and integral part of these conservation efforts. However, this procedure places an animal at risk and may result in morbidity and mortality. The goal of this study is to identify the best available intervention(s) to improve the cardiorespiratory function of immobilized white rhinoceros. Eight subadult male white rhino were captured in Kruger National Park and taken to bomas

where they were housed for the duration of the experiment. Boma-kept animals were used in order to limit confounding variables. After a period of boma adaptation, all the rhino were immobilized with a combination of etorphine, azaperone and hyaluronidase on seven occasions at two-week intervals so that each rhino received the same seven experimental interventions in a randomized order. The experimental interventions, administered at 6 minutes after lateral recumbency, included various combinations of butorphanol, diprenorphine and oxygen insufflation via nasotracheal intubation. Oxygen was administered at a constant flow rate of 30L/min. The partial opioid-antagonists were administered as butorphanol alone (15 x etorphine dose) or butorphanol combined with diprenorphine (3.3 and 0.4 x etorphine dose respectively). A control experiment was also conducted in



each rhino whereby no supportive treatment was given after immobilization. Physiological measurements including heart rate, respiratory rate, blood pressure, haemoglobin oxygen saturation, and arterial blood gas samples were taken at 5 min intervals throughout a 20 min immobilization period. Thereafter, the rhino were stimulated to stand and walk. The quality of arousal was scored according to predetermined criteria based on ease of arousal and use of stimulation. The findings of this study will be presented to show the better intervention to support an immobilized rhinoceros' cardiorespiratory physiology. This supportive treatment can be applied immediately in the field, thus assisting conservation efforts in which immobilization of this iconic species are a necessity.

The effect of thoracic injury severity on blood gas and acid base balance in dogs sustaining blunt trauma

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The aim of this work was to estimate the association between thoracic injury severity as evaluated by computed tomography (CT) and arterial blood gas and acid base status in dogs sustaining natural, blunt trauma in motor vehicle accidents. Twenty four dogs were assessed by thoracic CT scan and arterial blood gas analysis between 4 and 24 hours after the traumatic incident. All dogs had a TRIAGE score performed at admission to the Onderstepoort Veterinary Academic Hospital. The score was on a scale of 0 (normal) to 3 (severely abnormal) and a total score out of a potential of 18 was calculated. The mean, SD and median of scores for this group was 4.5, 1.6 and 5 respectively thus indicating that extra-thoracic injury did not play a large part in the status of this group. Fifteen clinically normal dogs were sampled as a comparison group for the trauma cases. The median weight of the traumatised group was 6.1kg (range 2-36kg) and median age was 13 months (range 3-120 months). Thorax injuries were classified as pleural space, rib cage or pulmonary and each of these three components of the thorax were scored according to severity. Dogs blood gas and acid base status

was evaluated for statistical difference from normal. There was a significant difference between normal controls and the trauma group with respect to arterial PO2 (P<0.001) and the calculated variables of arterial blood oxygenation: PO/ FIO, (P=0.033) and PAO,-PaO,(P=0.015) There was also a significant correlation (Spearman rank correlation, P<0.05) between the composite lung score and pleural score and the variables of arterial oxygen status. PCO2 was not significantly different to any of the thoracic injury variables indicating normal alveolar ventilation. Acid base imbalances were variable and considered clinically insignificant. Pulmonary and pleural injury significantly affected blood oxygen status and this was strongly correlated with what was observed on CT imaging. Trauma thus did not affect alveolar ventilation (PCO, was unaffected) but did cause a clinically significant ventilation perfusion mismatch (oxygen status was abnormal). The pulmonary contusion caused by blunt trauma is a serious injury especially as there is little that can be done to treat it and the pathology it causes is progressive over the first 48 hours after which changes generally start resolving.

A comparison of the in vitro replication kinetics and cytopathogenicity of reverse genetics parental and reassortant strains of bluetongue virus serotypes 1, 6 and 8

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Bluetongue virus (BTV), a segmented dsRNA virus, is the type species of the genus Orbivirus in the family Reoviridae and the aetiological agent of bluetongue, an economically important arthropodtransmitted disease of domestic and wild ruminants(2). Bluetongue virus demonstrates substantial antigenic, genetic and phenotypic diversity and has the capability to reassort its genome segments in host or vector cells that have been co-infected with more than one strain of the virus. Genetic reassortment may potentially lead to the generation of viruses that display unique properties biological enhanced either pathogenicity or an altered capacity to be transmitted by Culicoides vectors in the field). Several reassortant strains

have recently been isolated in Europe, including both wild-type/wild-type and wild-type/modified live-virus vaccine reassortants (1,3).

In order to clarify the effects of reassortment on the phenotype of parental BTV strains, parental as well as reverse genetic reassortant strains of BTV-1, -6 and -8 were assessed in vitro in regards to their replication kinetics and cytopathogenicity in mammalian cell cultures. African green monkey kidney (Vero) cells were infected with 100-1000 tissue culture infectious dose fifty (TCID₅₀) of parental or reassortant strains. Virus yield, virus induced cytopathic effect, apoptosis/ necrosis induction and the effect of viral infection on infected cell viability was subsequently measured at different time intervals post infection utilising either classical virological techniques and/or commercially available assays. The results from the study indicate that genetic reassortment can affect the replication kinetics, the induced level of cytopathic effect and the level of cell death of BTV in infected cell cultures. Importantly, it was demonstrated that reassortment between reverse genetics strains derived from vaccine (BTV-1 and -6) and field origin (BTV-8) can result in the generation of reassortant strains that demonstrate increased rates of cytopathic effect induction relative to their parental strains.



Although increased rates of cytopathic effect induction for certain reassortant strains may indicate increased virulence, further experimental infection studies in mice and/or ruminants should be conducted in order to evaluate whether these results have significant implications *in vivo*.

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Non-invasive assessment of reproductive function in the southern African spiny mouse (Acomys spinosissimus)

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The genus Acomys raises increasing interest in science, as spiny mice show a comparatively long oestrus cycle for a rodent of such small size, raising immediately questions regarding their reproductive strategies. Unfortunately, detailed long-term data on reproductive function and especially endocrine correlates is still lacking, as limited blood volumes make frequent collections of individual plasma samples for hormone monitoring impractical. As an alternative approach, the overall aim of this study was to examine the suitability of enzyme immunoassays for measuring hormones using faeces as matrix. Faecal androgen metabolites (FAM) and faecal progestagen metabolites (FPM) were measured to monitor male and female reproductive function in the southern African spiny mouse (Acomys spinosissimus). Fourteen non-pregnant

and one pregnant female and 24 male spiny mice were wild-caught and subsequently monitored under controlled conditions. Faecal samples for hormone monitoring were collected every second day for up to six weeks. Minimum sample mass for hormone monitoring required as well as the rate of respective hormone metabolism post-defecation was additionally investigated using separately collected faecal material. Thirteen out of the 14 non-pregnant females exhibited elevated FPM concentrations with eight individuals showing indications of a luteal phase. Two females showed two post-ovulatory luteal phases with estimated cycle lengths



of 16 and 18 days, respectively. The pregnant female showed an elevation of 231% in mean FPM levels compared to the overall mean baseline hormone concentration determined for the 14 non-pregnant females. Males exposed to a long photoperiod, simulating summer-related breeding activity, exhibited a 47.8% increase in FAM levels compared to males exposed to a short photoperiod. Collectively, the data clearly demonstrate that reproductive endocrine function can be reliably monitored in male and female spiny mice by measuring respective faecal hormone metabolites.

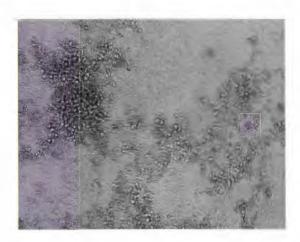
An improved method for determining virucidal efficacy of a chemical disinfectant using an electrical impedance assay

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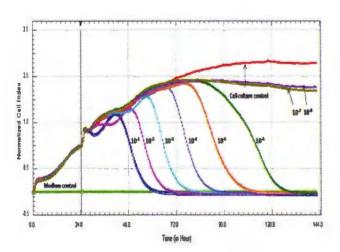






Regular cleaning and disinfection of animal enclosures to reduce the level of bacterial and viral pathogens is essential in the domestic farming environment, veterinary and also medical practises. Using chemical disinfectants that are proven to be effective against particular disease causing micro-organisms is of extreme importance to the farming community, and in particularly the poultry and dairy industries (3). A major problem with the testing of virucidal efficacy using current cell culture based protocols is that scoring of virus-induced cytopathic effect (CPE) is dependent on the subjective visual interpretation of cell death by using light microscopy (2). The following report details the use of an electrical impedance assay (xCELLigence, Roche) for its utility in virucidal

efficacy testing. The system measures the development of viral induced CPE in real-time and provides several data parameters that allow for inter-laboratory comparison and standardization. In this study the xCELLigence system was evaluated in a procedure developed from guidelines given by the Deutsche Vereinigingzur Bekämpfung der Viruskrankheiten (DVV) (German Association for the Control of Virus Diseases) (1) in order to demonstrate the inactivation of infection bursal disease virus (IBDV, genus Avibimavirus, family Birnaviridae) using a commercial virucide. Although the modified DVV assay using the xCELLigence system yielded identical results as the traditional DVV assay, it allows virucidal efficacy and cytotoxicity to be measured in a more precise and reproducible fashion.



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Isolation of Bacillus anthracis from pachyderms in Kruger National Park, South Africa

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Bacillus anthracis is a gram-positive spore forming bacterium and the causative agent of the disease known as anthrax. Bacterial sporulation usually occurs on exposure to oxygen and spores can persist in the soil for decades or longer. It is a zoonotic and epizootic disease, endemic in the Kruger National Park (KNP) in South Africa where it primarily affects wildlife.

It is an OIE reportable disease and the opening of a carcass from a suspected death by anthrax is prohibited in the field. During anthrax outbreaks, swabs and smears of the bloody discharge are collected for diagnostics and when appropriate, exposed tissue or bone samples as well. The most widely practised methods of confirmation are firstly, stained blood smears and thereafter bacteriologic methods using selective media, penicillin and gamma phage.

While the culture of *B. anthracis* on selective media from biological samples of most terrestrial animals has met with a high success rate, samples from pachyderms have proven quite difficult at times. This is due to the thick skins of animals like the elephant (*Loxodonto africana*), rhinoceros

(Ceratotherium simum; Diceros bicornis) and hippopotamus (Hippopotamus amphibius) which are not easily opened by scavengers and hence allows putrefaction to destroy the vegetative B. anthracis cells before they are able to sporulate in significant quantities to be detected. We report on the successful isolation of B. anthracis from the tusks, blood smears and a tissue sample of pachyderm cases in the KNP.

Perception of communal farmers on foot and mouth disease control at the wildlife/livestock interface of the Kruger National park South Africa

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Areas surrounding the Kruger National Park (KNP) are part of the Foot-and-mouth disease (FMD) control zone with KNP being an infected area due to the presence of African buffalo (*Syncerus caffer*), which serve as the major reservoir to the Southern African Territories (SAT) serotype viruses. The aim of this study was to evaluate the perceptions of communal farmers concerning the current FMD control interventions at the wildlife/livestock interface.

A structured questionnaire was used to gauge farmers' opinion as they presented their cattle at dip tanks within the Mnisi Tribal Area. The questionnaire was applied through in-person interviews using the local language (Shangaan). Questions addressed owner demographics, herd management practices, general disease control and knowledge of FMD epidemiology. Cross-sectional cluster sampling of cattle within herds was used to estimate the proportion of cattle with high titres against FMDV-structural proteins. Positive titres were assumed to indicate an immunological response to routinely administered vaccination (every 6 months) in the absence of recently recorded outbreaks.

One hundred and four farmers responded to the questionnaire with 73%, (76/104) being cattle owners. The majority of the respondents, (79%; 95% confidence interval, 70% - 80%) indicated a high level of satisfaction with the current animal

health programmes at the dip tank. The education level of farmers varied over levels of satisfaction with the median education level being standard 9 (interquartile range, 2 – 12) for non-satisfied respondents, standard 3 (0 – 6) for little satisfied and standard 7 (2 – 11) for very satisfied respondents (P = 0.036). Non-satisfied respondents were more likely to treat sick animals themselves rather than seek veterinary assistance (P = 0.002). The majority of respondents identified the African buffalo as a risk for FMD outbreaks (92%, 95% confidence interval: 85% - 96%).

Two hundred and eighty-six cattle were sampled within six months post vaccination and relative to an antibody titre of ≥1.6Log₁₀ (1:40 dilution), 20% (95% confidence interval: 15% - 27%) of sampled cattle had serologically converted to SAT 1, 38% (95% confidence interval: 32% - 45%) to SAT 2 and 24% (confidence interval: 18% - 13%) to SAT 3. This indicates an unsatisfactory immune response to the current vaccination schedule (six-monthly vaccination) and therefore that the level of satisfaction expressed by cattle owners in the animal health programme may be misplaced, at least as related to FMD control. The higher education level in the nonsatisfied respondents further suggests that more educated farmers were able to correctly recognize inadequacies in the animal health programmes.

Differentiation of Brucella species by multiplex PCR assays, Bruce-ladder and AMOS

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Brucella species are zoonotic pathogens and causative agents of the widespread disease brucellosis. The disease has a negative impact on the global economy and public health as it causes substantial losses of livestock and affects the livelihood of communities relying on their livestock for survival. Brucella species are genetically homogenous and mainly classified on the basis of pathogenicity, host preference and phenotypic/ genotypic characteristics. Due to this reason various assays were developed in an attempt to differentiate them.

The main objective of the study was to use multiplex PCR assays, Bruce-ladder and AMOS, to characterize and distinguish between *Brucella* spp. strains collected from

different farms and hosts in Zimbabwe, to provide the insight into Brucellosis, a zoonosis affecting livestock- farming communities and wildlife. Sixteen *Brucella* strains isolates, 3 vaccine strains and 3 reference strains were characterized and out of the sixteen, 8, 5 and 2 were found to be *Brucella suis*, *B. abortus* and *B. canis* respectively. Of the *Brucella* species identified, *B. canis* and *B. suis* have not yet been formally reported in Zimbabwe. The use of these multiplex PCR assays together, enable identification of *Brucella* species which is of great importance since some of the laboratories in Africa lack adequate resources, expertise and infrastructure to do biotyping of *Brucella* species and biovars under proper biosafety and biosecurity conditions.

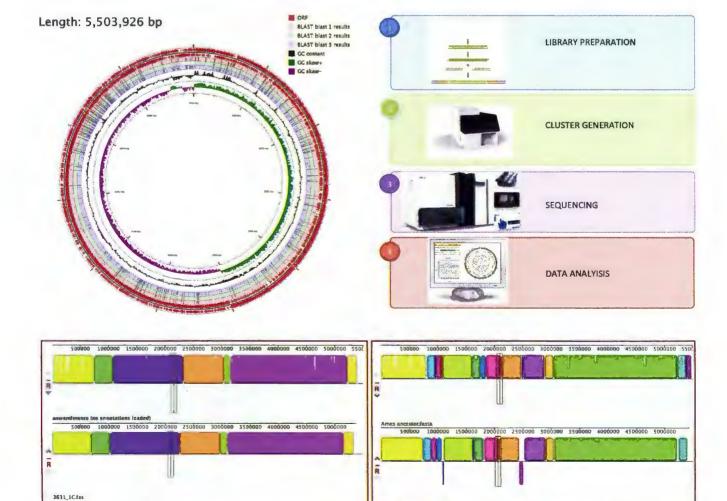
Whole genome sequencing and genetic variant analysis of two South African Bacillus anthracis strains

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Bacillus anthracis is the causative agent of anthrax, which is zoonotic, although it primarily affects animals both wild and domesticated. Genome-wide characterization through whole genome sequencing is becoming more relevant in diagnostics monitoring and control of anthrax outbreaks since it facilitates the identification of the infecting strains. In this study, complete genomes of two South African B. anthracis strains were sequenced and further characterized relative to B. anthracis Ames ancestor genome using Single Nucleotide Polymorphism (SNPs), Insertion and Deletions (INDELs), and functional comparison of metabolites. The genome sequencing was performed using single and paired end massive parallel sequencing of the Illumina HIScan SQ platform. Over 2.0 and 3.0 million reads were generated

for *B. anthracis* 20SD and 3631 1C strains respectively. Approximately 99% of the reads generated for the 20SD strain mapped to the Ames ancestor reference chromosome and virulence plasmids pXO1 and pXO2. Over 98% of the reads from 3631 1C strain mapped to the chromosome and the plasmid pXO1. Using a stringent filtering assay, 458 SNPs and 149 INDELs were identified in strain 3631 1C, while 403 SNPS and 126 INDELS in strain 20SD. A comparison of the annotated strains indicated that 3631 1C lacked poly-gammaglutamate biosynthetic genes. Both strains were considered virulent but showed different features in comparison with *B. anthracis* Ames Ancestor. The impact of the observed genetic variants on virulence and diagnosis of *Bacillus anthracis* will be discussed



The prevalence of zoonotic tuberculosis in cattle at the livestock/human interface in the Mnisi community, South Africa

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In African countries, including Sub-Saharan Africa, numbers of informal animal slaughters are observed. In addition, many rural households consume animal by-products, such as milk, untreated. This is of great public health concern as often there is no adequate inspection of the meat and the animal by-products consumed. and few of these countries have policies implemented to control zoonotic diseases. The aim of this investigation is to determine the presence and, if applicable. the prevalence of bovine TB (BTB) in cattle within the Mnisi community in Mpumalanga. It is also to investigate possible risk factors for TB transmission of bovine and human TB between livestock and humans.



livestock using a cross-sectional as well as a targeted survey at the 15 dip-tanks. The cross-sectional survey entailed random sampling at the 15 dip-tanks, whereas the targeted survey entailed selecting cattle from the HDSS-live database that were recorded with respiratory syndrome reported by the farmer (e.g. chronic cough). The target number of cattle to be tested using the cross-sectional survey is 1 200, whereas approximately 300 cattle are to be tested using the targeted approach. To date, 1 066 cattle were tested as part of the cross sectional survey, while 37 cattle were identified and tested in the targeted approach. Thus far, a total of 4/1 066 cattle (0.37%) tested positive for BTB, three of which also tested positive on the interferon gamma assay (Bovigam). None of the 37 cattle tested using the targeted approach were positive.

The four BTB positive cattle were slaughtered and inspected for TB lesions. Two of the four slaughtered cattle had lesions



suspect of BTB in some lymph nodes and lung lobes. These lesions and lymph nodes from the head, thorax and carcass were collected and cultured for mycobacteria in the BSL 2+ laboratory. Mycobacterium cultures obtained will then be genetically characterized.

A human study is also to be performed in order to screen for BTB in humans and possibly isolate and characterize the mycobacteria. The mycobacterial isolates obtained will be genetically characterized using spacer oligonucleotide types (spoligotyping). The genetic profile obtained will be compared to genetic profiles of the livestock study group, the human study group, the milk study and reported cases in Kruger National Park to determine genetic relatedness.

A short questionnaire will also be administered to identify possible risk factors of bovine and human TB at the livestock/human interface.

Development of a real time polymerase chain reaction assay for equine encephalosis virus

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Introduction

Equine encephalosis virus (EEV) is the cause of equine encephalosis. The disease is usually mild or subclinical. Clinical signs include fever for one to five days, listlessness, inappetence, congestion of the mucous membrane of the conjunctiva and swelling of the eyelids, heads and supraorbital fossae. Equine encephalosis is an important disease, as it is similar to mild forms of African horse sickness (AHS) (Erasmus et al., 1970; Howell et al., 2004), a listed disease of the World Organisation of Animal Health (OIE) and for this reason it is necessary to differentiate the two diseases. Traditional methods of viral identification, such as viral isolation on tissue culture are time-consuming and a rapid diagnostic assay for EEV is needed to identify EEV and distinguish it from African horse sickness virus (AHSV).

Methods

The S7 (VP7) gene from 38 EEV isolates, representing all seven serotypes, was amplified and sequenced. A conserved region at the 5' end of the gene was identified and used to design a group specific, one-step, real-time, reverse transcription polymerase chain reaction (RT-PCR) assay using a TaqMan®MGB™ hydrolysis probe.

Results

The efficiency of the EEV real-time RT-PCR assay was 81%. The assay was specific, as it did not detect any of the nine serotypes for AHSV, nor 24 serotypes of bluetongue virus (BTV) and sensitive, with a 95% limit of detection of 10^{2.9} TCID₅₀ (95% confidence interval: 10^{2.7} – 10^{3.3}).

Conclusion

The EEV real-time RT-PCR assay is efficient, specific and sensitive. The real-time format is convenient, sensitive and rapid.

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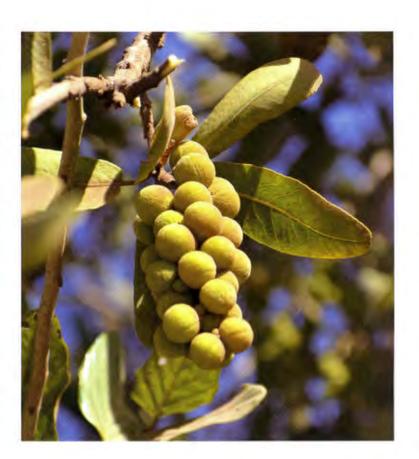
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Opportunistic feeding behaviour in free-ranging Galago moholi

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The African lesser bush baby, Galago moholi, is described as a food specialist, feeding exclusively on small arthropods and gums of primarily Acacia karroo trees. We studied a population of G. moholi in a highly fragmented habitat in the most southern part of its natural distribution range in South Africa. In this habitat we opportunistically observed bushbabies (N=4) feeding on fruits of the winter fruiting tree Pappea capensis. Plot counts of tree composition revealed that although the dominant tree species in the area belonged to the genus Acacia, accounting for 73% of the 285 trees counted, A. karroo trees, preferred by G. moholi for gum feeding, were widely absent, representing only 1.5% of all Acacia trees. Only about 8% of Acacia trees and about 22% of Ziziphus mucronata trees showed small quantities of gum exudates. The analysis of P. capensis fruits showed high levels of proteins and fats. The fruits also showed a high energy content (26.26 MJ/kg compared to ~16MJ/kg found in gum) making the fruits an important food source during winter when insect availability is low. Our observation is the first documented case of fruit feeding in G. moholi, suggesting that the species is not a food specialist as so far reported, but rather a food opportunist, presumably exploiting any available food source in its habitat.

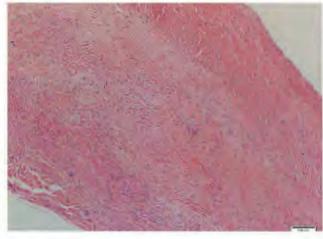


The comparative pathology of spirocercosis in the black-backed jackal (Canis mesomelas) versus the dog (Canis lupus familiaris) in South Africa

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Introduction:

Spirocerca lupi (S.lupi) is a spiurid nematode of Canidae, of worldwide distribution but most prevalent in the tropics and subtropics. Larvae penetrate the gastric mucosa and migrate in the wall of the gastric and celiac arteries to the caudal thoracic aorta where they moult to L4 and finally to adults. Young adult worms then migrate from the aorta to the oesophagus.

Lesions considered pathognomonic for spirocercosis in dogs include: Scarring and mineralization of the caudal thoracic aorta with aneurysm formation, spondylitis of the caudal thoracic vertebrae and the formation of caudal oesophageal nodules (1).

Microscopic aortic lesions include smooth muscle and elastic fiber degeneration with replacement by collagen and occasional foci of mineralization and heterotopic bone formation (2). Histologically, early oesophageal nodules are inflammatory and collagenous, older nodules are predominantly fibroblastic with foci of inflammation, and in 25% of cases, proceed to sarcomatous neoplasia (3).

The present study targeted black-backed jackal since they are widely distributed throughout Southern Africa and are closely associated with domestic dogs, particularly in rural and semi-rural areas.

Materials and Methods:

Necropsies were conducted on fresh black-backed jackal carcasses that were routinely culled. Worms and larvae were preserved in 95% ethanol for genotyping and molecular characterization by DNA extraction, PCR amplification and sequencing. All lesions were collected in 10% buffered formalin for histopathology.

Results:

<u>Pathology:</u> Of the 93 jackals that were necropsied, 17% had aortic aneurysms, of which 25% had larvae present. Thirteen

larvae were collected. Only one jackal with aortic aneurysms (no larvae present) had an oesophageal nodule which contained no worms and no communication with the oesophageal lumen. The histological appearance of the nodule was similar to what has been described for the early inflammatory nodule in dogs. Aortic pathology was also similar to what has been described in the dog except that eosinophils were far more abundant in the jackal aortas. One of the jackals with aortic lesions and larvae had a single nodule within the wall of the greater curvature of the stomach. Histologically the nodule was centrally calcified with a peripheral rim of mature collagen and foci of mononuclear inflammatory cells. Another jackal had multifocal larvacontaining nodules throughout the lungs (aberrant migration). None of the jackals had spondylitis.

Larval genotyping: The worms were identified as S.lupi.

Conclusion:

It has now been established that *S.lupi* is present within the jackal population in South Africa. In our study the parasite did not complete its life cycle in the jackal and the most parsimonious explanation is that host-parasite co-adaptation exists. Another possible explanation is that the jackal is a novel host.

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Distribution of oxytocin receptors in the equine uterus and cervix

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Introduction

Oxytocin plays an essential role in the endocrine regulation of the oestrous cycle as well as in parturition and milk ejection. It is responsible for myometrial contractility which is primarily important for expulsion of the foetus during parturition and for mechanical drainage of uterine content (Nikolakopoulos, Watson 1999). It is reported that the oxytocin receptor (OR) concentration in the equine endometrium varies throughout the oestrous cycle with a peak during late dioestrus (Sharp et al. 1997, Stull 1986, Starbuck et al. 1998). There are no reports of OR distribution in the female equine reproductive tract. This study uniquely provides a detailed description of OR distribution during the oestrous cycle in the equine endometrium, myometrium and the cervix.

Materials and Methods

Fifteen routinely slaughtered cyclic mares of various breeds and sizes were assigned to two different groups depending on their stage of oestrus, either non-luteal (n=9) and luteal (n=6), phases.The assignment of mares to either group was based on findings on inspection of the ovaries and plasmaprogesterone concentration (PPC).

Full thickness uterine samples were obtained from all mares at 3 different sites (uterine body, right and left uterine horn) and one sample from the cervix. Each full thickness sample was fixed in 10 % neutral buffered formalin overnight, embedded in paraffin and sectioned at a thickness of 4-6 μ m. Sections were de-waxed and immunohistochemically labelled using a polyclonal antibody against human OR (Sigma O 4389, Sigma-Aldrich, Germany).

Evaluation of the slides was done using an Olympus BX43 microscope and the Olympus cell sens dimension

software (Wirsam Scientific & Precision Equipment PTY LD, Johannesburg, South Africa).

Results and Discussion

In the uterus, ORs were found in both luminal and glandular epithelium, in the endothelium of blood vessels and in the sub-epithelial and periglandular stroma and smooth muscle. The greatest intensity of staining was consistently in the endothelium of blood vessels. The cervix, the luminal epithelium, the endothelium of blood vessels and the muscle layers also showed the presence of ORs. A positive correlation between the appearance of ORs in uterus and cervix was observed.

No difference in distribution could be found between luteal and non-luteal phase.

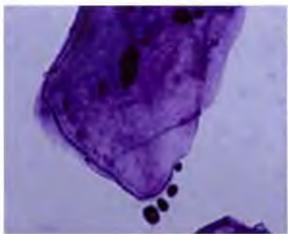
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Anti-adhesion effect of Ptaeroxylon obliquum acetone leaf extract on Candida albicans

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Background:

Candidiasis is the most common oral manifestation associated with immuno-compromised patients such as patients suffering from Acquired Immuno Deficiency Syndrome (AIDS), which is of high burden globally, and in South Africa in particular. Cost of current therapies limits access to care. Therefore, there is a need for investigations on cheaper alternative drug therapies. Despite the critical importance of candidal adhesion to human buccal epithelial cells (HBEC) in the pathogenesis of oral candidiasis, only limited amount of information is available on alternative drug therapies to limit adhesion.

Objective:

This study investigated the ability of *C. albicans* standard strain (ATCC 10231) and two clinical isolates to adhere to human buccal epithelial cells (HBEC) in the presence of *P. obliquum* acetone leaf extract.

Methods:

250 mg/ml *P. obliquum* extract was used as the starting material. HBEC were collected from 20 consenting healthy volunteers aged between 20 and 40-years-old. Amphotericin B was used as a positive control and acetone as negative control. The subcidal concentration values for amphotericin B

were the concentration slightly below the minimal fungicidal concentration (MFC) of the drug. Adhesion abilities were compared using adaptation to a previously published method. Further-

more, the cytotoxicity of P. obliquum extract was determined.

Results

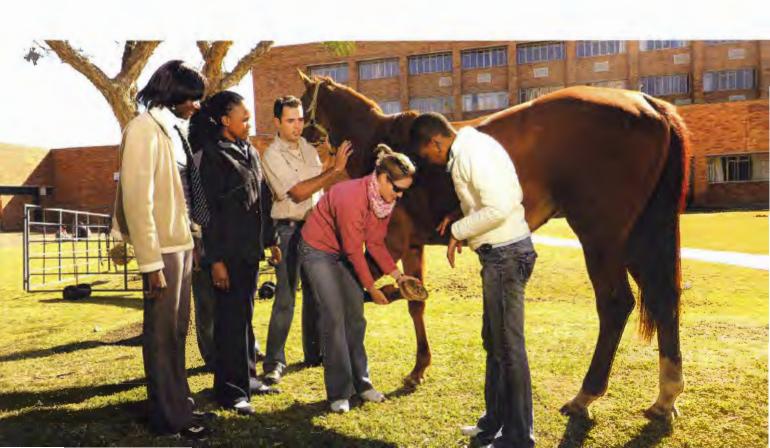
P. obliquum extract at a concentration ≤250 mg/ml suppressed candidal adhesion to HBEC as effectively as subcidal concentrations of amphotericin B. Graphical illustration of LC $_{50}$ values demonstrated that 90% candidal adhesion was reached with 20 mg/ml and 50% of candidal adhesion was inhibited by concentrations between 5 and 20 mg/ml depending on the clinical isolates used. Based on the cytotoxicity of *P. obliquum* extract (LC $_{50}$ =35.58 µg/ml), <20 mg/ml may be of use for clinical application.

Conclusions:

This study demonstrated a non-cytotoxic dose-dependent inhibitory effect of *P. obliquum* extract on the ability of *C. albicans* strains to adhere to HBEC.

Scientific peer-reviewed articles published in 2012 where a member or student of the Faculty of Veterinary Science was an author (alphabetical according to the name of the first author)

Authors	Title	Journal
Abdullah J, Fasina FO, Jibril A, Mohammed	Sub-clinical mastitis and associated risk factors on lactating cows in	BMC Veterinary Research
A, Shittu A	the Savannah Region of Nigeria	
Abolnik C, Bisschop SPR, Bosman A-M,	Molecular characterisation of Newcastle disease virus isolates from	Onderstepoort Journal of Veterinary
Ebersohn K, Fringe R, Venter EH	different geographical regions in Mozambique in 2005	Research
Abolnik C, Burger CE, Fosgate GT	Antibody Response and Viral Shedding Profile of Egyptian Geese	Avian Diseases
	(Alopochen aegyptiacus) Infected with Low Pathogenicity H7N1 and	
	H6N8 Avian Influenza Viruses	
Abolnik C, Cappelle J, Caron A, Cattoli	Investigating Avian Influenza Infection Hotspots in Old-World Shore-	PLoS One
G, Cumming GS, Fereidouni S, Fofana B,	birds	
Gaidet N, Gil P, Grosbois V, Hammoumi S,		
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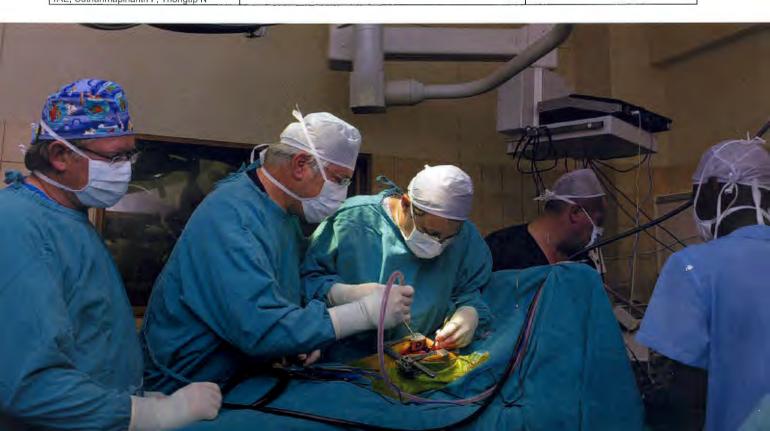
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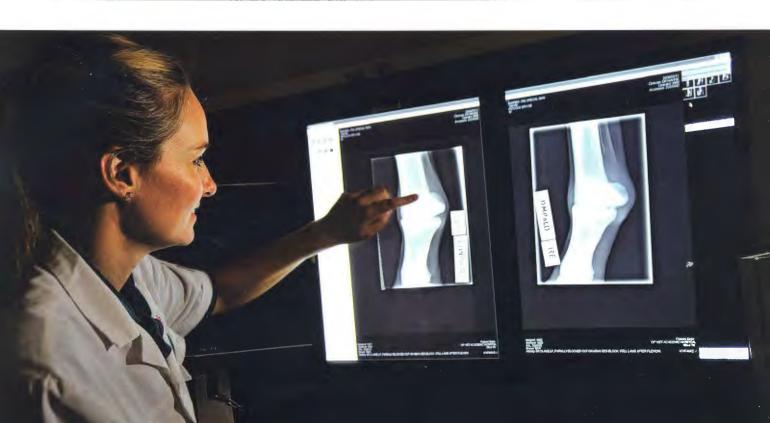
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