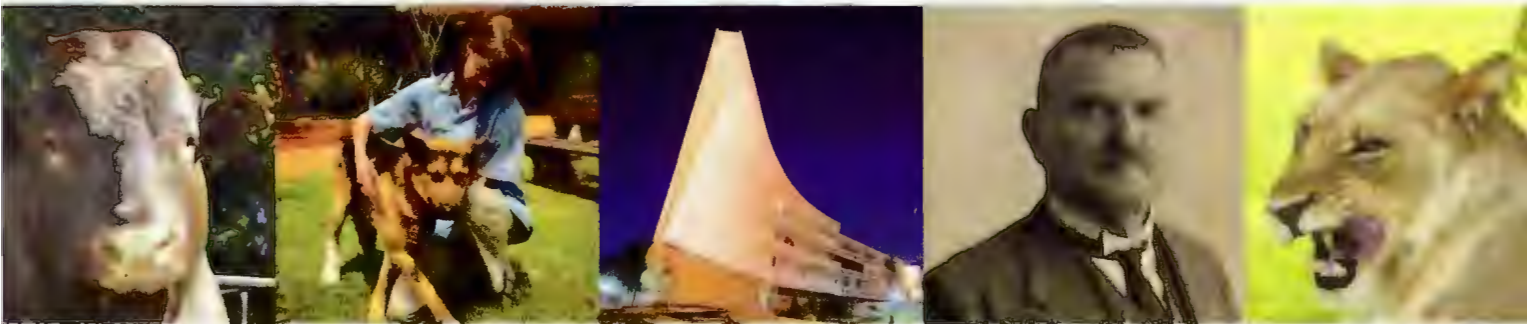




Dr Rebone Moerane
President of the South African Veterinary Council



UNIVERSITEIT VAN PRETORIA
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Faculty of Veterinary Science

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 2010 Faculty Day.



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Faculty Day
Faculty of Veterinary Science
University of Pretoria
2 September 2010





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Prof Gerry Swan

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Sir Arnold Theiler Memorial Lecture

Dr Rebone Moerane:

“The Role of the Veterinary Profession in the Current Developmental Agenda in South Africa”

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– Dr Rebone Moerane, President of the South African Veterinary Council

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Message from the Dean

Innovative and cutting-edge research remains an integral part of the faculty's strategic plan. Part of our mission states the importance of increasing research outputs through effective postgraduate programmes and making research a primary thrust in order to stimulate and focus our research programmes on unique South African animal disease problems. We had an increase in the number of publications in 2009 resulting in 83,88 subsidy units, while all the publications were in ISI-accredited journals, emphasising the high quality of the research.



Prof Gerry Swan, Dean

For the past 26 years, Faculty Day has represented the focal point of our academic year, serving as an event for showcasing the research activities within the faculty to colleagues and peers. However, one needs to ask the question – and this is also applicable to research: Are we able to live up to the expectations of our stakeholders and are our activities and that of the veterinary profession in general aligned with what our country really needs?

We need to continuously revisit our strategic goals and objectives, and determine if these are still applicable and viable. A leading faculty, and any organisation for that matter, is one that recognises and acknowledges change, adapts to it and plans its strategic direction and path accordingly. However, change is not only about certain insufficiencies or a lack of something. It is about the basic realisation and the acknowledgement that change is indeed needed, followed by a noteworthy response. We have to acknowledge the fact that certain important challenges remain and should be part of our focus beyond 2010.

In future planning, the faculty has to take into account the possible influences on the future provision of veterinary services, including changes in the environment of veterinary science brought about by changes in society through major demographic, political and environmental, disease, technological and economic influences, and becoming socially engaged.

Veterinary education of the highest quality provides training that meets the needs of a particular society and remains relevant to changing national, regional and international expectations. The eradication of extreme poverty and hunger is, among others, a principal millennium goal applicable to veterinary education for the immediate future and beyond. The phasing in of a new curriculum with a more comprehensive core-elective approach is one of the faculty's responses to

adapt to new challenges facing the profession and to more effectively incorporate global animal and public health issues into veterinary education.

Another crucial aspect deserving the faculty's continued attention is the increase in the number of African veterinary and veterinary nursing students. Existing awareness and recruitment initiatives have to be strengthened and revisited, and additional measures should be implemented where necessary.

An increase in quality research outputs and the encouragement of active researchers must be undertaken, while steps must be implemented to increase the number of NRF-rated staff members and to improve staff ratings in the faculty. The faculty has to attract, develop and retain excellent academic staff, researchers and support staff. A collaborative effort is also needed to improve the cooperation between the faculty, the Agricultural Research Council (ARC) Onderstepoort Veterinary Institute and Onderstepoort Biological Products (OBP), which is, at the moment, not optimal.

Change is also about social debate and our involvement in those debates. We will have to be involved in social debate and not let debates take place on our behalf. Out of critique(al) dialogue informed by more than one analytical framework can come dialogic imagining about future action. Basic values and basic principles must, however, still be the ship's rudder.

We welcome you to the 2010 Faculty Day. We are looking forward to the Arnold Theiler Memorial Lecture that is this year presented by Dr Rebone Moerane, President of the South African Veterinary Council (SAVC), covering some of the issues that are also contained in this message. This year the format of the Faculty Day programme was adapted slightly and includes, among others, a discussion of the research focus areas of the faculty and a research summary for 2009. We thank the Faculty Day committee for their hard work and dedication in organising this event. May Faculty Day 2010 serve to further stimulate and advocate cutting-edge research and innovation.



Curriculum Vitae: Dr Rebhone Moerane

Dr Rebhone Moerane, currently the President of the South African Veterinary Council (SAVC) and Chief Director: Agricultural Technical Services in the Northern Cape Province, was born in 1965 and matriculated at the Dr WF Nkomo High School in Pretoria in 1982.



Dr Rebhone Moerane

He studied at Medunsa and obtained the Bachelor of Veterinary Medicine and Surgery (BVMCh) in 1990. In 1991, he registered with the SAVC as a veterinarian. Due to his interest in rural development and, in particular, the improvement of the economy within the rural poor communities, and livestock production, he started his career at Agricor (Agricultural Co-operation) as District Veterinary Officer stationed in Mafikeng, where he was involved with animal disease control and surveillance and the supervision of animal health technicians and veterinary public health officers, among others.

From 1994 until 1996 he was acting Deputy Director of Veterinary Services in the North West Province. In 1996 he was appointed as Provincial Deputy Director of Animal Health for the same province, a position that he held until he was appointed Director: Specialist Services in 2002 and subsequently as Chief Director in the Northern Cape. He is responsible for Veterinary Services, agricultural research and sustainable resource management divisions in the province. He is also the Chairperson of the Agricultural Project Committee for the province and assists with agricultural policy analysis and development.

Rebhone Moerane's passion and interest in the animal health and wellbeing, food security and general public health led to him being nominated to participate in various policy developments nationally that included the Livestock Commodity Sector strategy and ultimately being selected to the SAVC in 2003.

Dr Moerane has served on the SAVC for two terms to date, the last one as President. He has also been re-elected to serve as President until July 2013.

Sir Arnold Theiler Memorial Lecture

The Role of the Veterinary Profession in the Current Developmental Agenda in South Africa

Dr Rebhone Moerane

P.O. Box 3455, Diamond 8305

South Africa has, since the 1994 democratic elections, experienced changes in the interest of building a united country with democratic values, social justice and fundamental human rights. In an attempt to develop the country, and promote economic growth and equal participation, a number of policy narratives and plans were deliberated upon and implemented.

However, prior to the 2009 general elections, it became apparent that the majority of South Africans were not satisfied with the impact of the approved/implemented development initiatives and thus, the identification of the five priorities in the manifesto of the ruling party that focuses on decent work, education, health, safety and rural development. The ruling party is therefore very clear on its agenda for development with particular emphasis towards the rural poor.

Pioneers of the veterinary profession, such as Sir Arnold Theiler, had the skill and courage to garner the full support of the then government and to date we are still reaping the benefits thereof. However, during the past two decades, the veterinary profession and other stakeholders have on several occasions and at various levels tried to highlight a plethora of challenges experienced by the profession and we all agree that there is, to date, no tangible improvement or results. Therefore, it is upon the veterinary profession to align its services with the current mandate in order to be relevant to the development agenda of the government. Failure to do so will continuously relegate the profession to the bottom with no consideration or recognition of its impact in rural development and poverty alleviation. A number of proposals on the role of the profession linked to the identified government priorities or outcomes are highlighted to stimulate further debate and consolidation of a common approach on a few key strategies.

Sir Arnold Theiler Memorial Lecture

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	*
1989:	Dr R Swanepoel	*
1990:	Dr A Schutte	"The Impact of Controlled Breeding on the Cattle Industry in Southern Africa"
1991:	Prof DM Joubert	*
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:	*	*
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	"Fumonisin: Historical Perspective and Future Objectives"
2004:	Dr RA Kock	"Wildlife Domestic Animal Disease Interface – Hard or Soft Edge?"
2005:	Prof SS van den Berg:	"The Past, Present and Future of the Clinical Departments in the Faculty of Veterinary Science"
2006:	Dr BD Perry	"The Global Poverty Reduction Agenda: What are the Implications for Animal Health Research and Development?"
2007:	Prof dr AWCA Cornelissen	"What Makes an Excellent Faculty of Veterinary Medicine?"
2008:	Dr G Brückner	"New Challenges for the Veterinary Profession in Global Animal Disease Control and the Trade in Animals and Animal Products"
2009:	Prof P Doherty	"Adventures in Infection and Immunity"

*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)

Research Summary: 2009

The year 2009 was the fourth year of the faculty's four-year strategic plan aimed at increasing its number of postgraduate students and making research a primary thrust in order to stimulate and focus our research programme on unique South African animal disease problems.

A satisfactory growth in research output, mainly measured in terms of scientific publications and the NRF ratings obtained by the faculty's researchers, was achieved during the current year. All four submissions for rating were approved, but unfortunately the faculty also lost two of its previously rated staff members due to retirement. This meant that we had a total of 17 rated researchers in 2009 compared to 15 in 2008. Subsidy units earned for 2007 publications were 62,73, which is lower than the 65,3 attained for 2006 publications but still resulted in a slightly higher research budget of R1,1 million against R1,004 million the previous year. This first decrease in the number of publications in more than a decade was mainly due to the loss of some of our most productive senior staff members. Fortunately it was more than compensated for by an increase in the number of publications in 2008, resulting in 81,58 subsidy units. In 2009, it resulted in 83,88 subsidy units. Again, as in previous years, all the publications were in ISI-accredited journals, reflecting the consistently high quality of our research. According to the faculty's policy, 20% of the research allocation is allocated to the Faculty Research Fund, which is used by our Research Committee to support young or new researchers who have not yet established themselves for external funding purposes. Finally, the amount allocated

to the faculty for postgraduate scholarships was increased to R440 000, which translated into 11 doctoral and 13 master's scholarships, auguring well for the future.

Excellence in research performance was again recognised by the identification of the faculty's Top 10 researchers and of the winner of the 'Researcher of the Year' award (Prof V Naidoo), as measured in terms of productivity reflected by subsidy units earned, and the announcement of the result at the Faculty Day. Similarly two 'Young Researchers of the Year' were selected using slightly different criteria. The two individuals honoured in this way in 2009 were Drs M Saulez (academic staff) and L McGaw (non-academic staff).

For the past 25 years, Faculty Day has represented the focal point of our academic year serving as a platform for showcasing the research activities within the faculty to colleagues and peers. Faculty Day 2009 took place on 27 August. It was a special occasion and a huge success with 25 oral and 15 poster presentations. To make it an even more special event, the Sir Arnold Theiler Memorial Lecture was presented by Prof Peter Doherty of Australia, the first and only veterinarian to have been awarded the Nobel Prize. He shared the prize in physiology or medicine with Dr Rolf Zinkernagel for their discovery of how the immune system recognises virus-infected cells. His lecture with the title "Adventures in Infection and Immunity" served as an inspiration to those present and emphasised that his training as veterinarian provided him with a sound scientific foundation from where he was able to launch his remarkable scientific career.





The output of cutting-edge research by the faculty has been below international standards and has been pointed out in the Soulsby report, during a previous Royal College of Veterinary Surgeons of the UK visitation and during the latest accreditation visitation by the South African Veterinary Council. This could be traced back since the separation of the faculty from the Onderstepoort Research Institute in 1973.

The Faculty of Veterinary Science recently adopted a new vision statement relevant to its research programme. This vision statement declares that the faculty will be involved in the provision of effective veterinary and para-veterinary manpower and skills to promote the welfare of the total socio-economic spectrum of communities in South Africa and other sub-Saharan countries through the improvement and maintenance of animal health, animal welfare, animal production, animal work performance, veterinary public health and the conservation of natural resources. This is to be realised through conducting internationally recognised, ethical research and the development of centres of excellence.

The faculty has selected six research themes to meet these needs and representing the specific strengths within the different departments, including:

- (i) *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
- (ii) *Wildlife and environmental health.* This is an inclusive research focus with contributions from all five departments of the faculty, including, *inter alia*, studies on tuberculosis in buffalo, immune-contraception in elephants, theileriosis in

- roan and sable, toxicity of non-steroidal anti-inflammatories in vultures and endocrine disruptors in the environment
- (iii) *Molecular studies on infectious and parasitic diseases of animals.* A research focus utilising biotechnology for the development of improved diagnostic techniques and vaccines for animal diseases and for the study of their pathogenesis
- (iv) *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
- (v) *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
- (vi) *Anatomical and physiological studies on animals.* Studies on the macro- and micro-anatomy and physiology of a wide variety of animals as required for the teaching of veterinary students

All research within the faculty will be directed within these themes and will draw together related research programmes in the different departments in an attempt to stimulate collaboration and to align limited resources. The faculty's research will

further be focused through the development of specific niche programmes, which are intended to integrate research requirements across themes. Interfaculty, institutional, regional and international collaboration will be promoted through the development of strong, well resourced programmes. Therefore, it is intended to direct limited resources into carefully selected programmes. Application for and securing one or more SARChI chairs is essential for developing very strong veterinary research programmes and will provide a great impetus in strengthening much needed high profile veterinary research in the faculty.

The specific research programmes that will receive focused attention over the period of this plan include:

- Molecular studies on infectious and parasitological diseases of animals with specific attention to heartwater in ruminants, foot-and-mouth disease, malignant catarrhal fever, bovine tuberculosis, theileriosis and trypanosomiasis in domestic stock and wildlife to:
 - develop and validate sensitive, reliable and cost-effective diagnostic tests for the identification of tick-borne blood parasites; and
 - characterise known and undescribed tick-borne parasites, including those that threaten endangered and rare wildlife species.
- Studies on veterinary emerging and neglected diseases in southern Africa:
 - Two Wellcome Trust Grant proposals have been submitted, including the Southern African Centre for Infectious Disease Surveillance (SACIDS) and Neglected Diseases of Africa (NDoA).

The SACIDS is a proposal submitted by a consortium of southern African academic and research institutions in partnership with the universities of London and Edinburgh in the UK together with the International Livestock Research Institute (ILRI) in Kenya. This consortium is known as the Southern African Centre for Infectious Disease Surveillance (SACIDS). Within each of the four neediest countries (i.e. the Democratic Republic of Congo, Mozambique, Tanzania and Zambia), the faculties of medicine and veterinary medicine and national medical and veterinary research institutes will form a national virtual centre to be known as the National Centre for Infectious Disease Surveillance (NatCIDS). Three institutions in South Africa, namely, the Faculty of Veterinary Science, University of Pretoria, the National Institute for Communicable Diseases (NICD), the Onderstepoort Veterinary Research Institute (OVI), and the Medical School of Stellenbosch University, plus the two UK institutions and ILRI, will act as mentors for the scientists and the institutions in the four neediest countries.

The mission of SACIDS is to harness innovation in science and technology in order to improve southern Africa's capacity (including human, financial and physical) to detect, identify and monitor infectious diseases of humans, animals, plants and their interactions in order to better manage the risk posed by them. The overriding concept of the proposal is the One Medicine, intersectoral collaboration for research capacity strengthening for infectious diseases of humans and animals. Some 80% of newly emerging infections of humans over the last 30 years have been shown to originate from animals.

The NDoA proposal brings together individuals and institutions with demonstrable and longstanding commitments to the improvement of education, training and research into human and animal health in sub-Saharan Africa. It also promotes equitable and sustainable south-south and south-north partnerships and promote networking among institutions. Existing partnerships will be consolidated and new long-term partnerships developed between participating universities and research institutes based on a one-health approach. The proposal will contribute significantly to research for the control of neglected infectious diseases in Africa through knowledge transfer, education and training. Target diseases may include human African trypanosomiasis, cysticercosis, rabies, brucellosis, dengue, leprosy, leishmaniasis, trypanosomiasis, lymphatic filariasis, onchocerciasis, schistosomiasis, tuberculosis, Buruli ulcer, West Nile virus and HIV-related infections.

- Epidemiology of animal diseases at the interface between wildlife, domestic stock and humans in the greater Limpopo transfrontier area:
 - Remote sensing tools to study the epidemiology and space/time dynamics of diseases (EPISTIS) to determine the factors contributing to diseases challenge at various types of game/livestock people interfaces in southern Africa (Malawi, Zambia and South Africa). In Africa, there is a general lack of expertise in spatial epidemiology; hence the linkage of the University of Pretoria (UP) with the Institute of Tropical Medicine (ITM), Agriculture and Veterinary Information and Analysis (Avia-GIS) consultancy and other European partners. Through a formal agreement, UP has access to GIS expertise and networks of the Peace Parks Foundation that is a facilitatory NGO in the establishment of transfrontier parks and conservation areas.
- Phytomedicine and ethnoveterinary medicine studies on the development of extracts from plants with antimicrobial and antiparasitic activity. This is a very large and productive programme in the faculty with large numbers of post-graduate students. It is uniquely positioned to be able to participate in the Farmer to Pharma programme within the DST.
- Molecular studies of African horse sickness and development of vaccines
- Development of biomarkers for endocrine disruptors for the measurement of chemical and heavy metal pollution in water sources
- The epidemiology, pathogenesis and control of *Spirocerca lupi* in dogs
- The pathogenesis and molecular studies of *Babesia* spp in domestic and wild felids
- Management and control of resistant nematodes in commercial and rural smallholding small stock. This is a large European-funded project supported by a consortium of European and African institutions (see Department of Veterinary Tropical Diseases)

Research Programme: Oral Presentations

A review of the pathology and pathogenesis of Rift Valley fever in ruminants

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Rift Valley fever (RVF) is a zoonotic viral haemorrhagic fever caused by a mosquito-borne arbovirus belonging to the *Phlebovirus* genus (family *Bunyaviridae*). An extensive outbreak of RVF affecting livestock and humans in South Africa during the summer of 2010 provided the opportunity to re-examine the pathology of RVF. Immunohistochemistry (IHC) and recent significant advances in the understanding of the pathogenesis of viral haemorrhagic fevers provided a framework for the renewed interpretation of the lesions seen in ruminants infected with RVF virus.

The most common gross lesions in ruminants included random multifocal areas of hepatic necrosis disseminated throughout the liver accompanied by widespread haemorrhages; severe lung oedema, mild to moderate hydrothorax, hydropericardium and ascites; and marked enlargement and congestion of particularly the mesenteric and the hepatic lymph nodes. Microscopic lesions in ruminants included varying degrees of random multifocal hepatic necrosis; random foci of necrosis in other organs such as the heart, kidneys, adrenal glands, or the intestines; severe lung oedema and congestion; and depletion of the lymphoid tissues. Viral antigen was demonstrated using IHC in hepatocytes, myocytes, adrenal cells, keratinocytes in the epidermis and the tongue, enterocytes and in trophoblasts in the placenta. RVFV-specific positive labeling was also found in infected mononuclear cells and microvascular endothelial cells in the renal glomeruli, the cortical interstitium, the alveolar septa of the lungs, the thymus, the heart and the lymphoid tissues.

Viral haemorrhagic fevers are caused by RNA viruses from four different families, namely *Arenaviridae*, *Bunyaviridae*, *Filoviridae* and *Flaviviridae*. The genome of RVFV consists

of three RNA genomic segments (L, M, and S). The L RNA segment encodes the RNA polymerase. The M genomic segment encodes the structural glycoproteins, G_N and G_C, as well as NSm. The S segment encodes NSs. The glycoproteins, G_N and G_C, contain the only source of neutralizing epitopes. The NSm protein of RVF virus suppresses apoptosis of target cells, which ensures efficient release of progeny RVF virus within the first 24 hours prior to virus-induced apoptosis. The NSs protein suppresses the transcription of host mRNAs, including interferon- β mRNAs. NSs interacts with specific DNA regions of the host genome. Targeting of these sequences was correlated with the induction of chromosome cohesion and segregation defects. It is postulated that this could account for the high rate of abortions and teratogenic disorders predominantly observed after RVFV infection with neurotrophic RVFV strains in ovines.

Rift Valley fever (RVF) is a zoonotic viral haemorrhagic fever caused by a mosquito-borne arbovirus.

Models representing our current understanding of viral haemorrhagic fevers have been proposed. Studies indicate that macrophages and dendritic cells (DCs) residing in epithelia are the early targets of these viruses. Presumably, endocytosed virus replicates in their cytoplasm and is conveyed by these cells to the regional lymph nodes. From there the virus disseminates to the liver and other organs where tissue macrophages and/or DCs and parenchymal cells become secondarily infected. Widespread necrosis follows. Cytokines and chemokines released from virus-infected macrophages and hepatocytes dysregulate the host immune response causing lymphocytoclasia, increased vascular permeability and coagulopathy which ultimately results in co-infections, disseminated intravascular coagulation, hepatorenal failure and multiple organ dysfunction.



Zoonotic *Brucella* prevalence in livestock in Eastern Zambia and Kenya

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Brucellosis is a neglected zoonotic disease caused by *Brucella abortus*, *Brucella melitensis* or *Brucella suis* and is mainly transmitted to people by cattle, small ruminants and pigs, respectively. In humans, the clinical signs are not specific and the incidence of brucellosis might be seriously underestimated, especially in rural Africa. Brucellosis serology detects principally antibodies directed against epitopes associated to the smooth lipopolysaccharide (s-LPS) which is shared to a great extent by the different smooth *Brucella* species. Spillover of *Brucella* spp. in non-preferential hosts has been documented. It is impossible, therefore, to ascribe which *Brucella* species (wildtype and/or vaccine strains like S19 and Rev1) induced antibodies in a given animal species or in humans. In endemic situations, animals are infected early in life, and females are likely to abort at their first pregnancy. Subsequently, they will develop immunity with apparently normal pregnancies, although the newborn is likely to be latently infected. In the absence of control measures, a state of endemicity is reached at the herd/stock level, which is characterised by a high seroprevalence rate and often by the absence of detectable clinical signs.

The objective of this study was to quantify the prevalence of livestock brucellosis in different types of farming systems in eastern Zambia and Kenya and to identify the livestock groups presenting the highest threat to humans. To this effect, brucellosis serology was applied on sera from 446 cattle, 257 goats and 242 pigs from the Eastern Province of Zambia and 500 cattle, 100 goats and 100 sheep from various districts of Kenya. Our results indicate that in the absence of vaccination, a state of endemicity prevails in cattle (most likely *B. abortus* infection) in most of the study areas, except in arid districts

of north-eastern Kenya. The seroprevalence was particularly high (20–30%) in Turkana and Maasai cattle in Kenya. *Brucella* seroprevalence in Zambian and Kenyan goats and sheep was very low. In the absence of any organised control program, this suggests that *B. melitensis* is probably absent from the area and thus that either a spillover from *B. abortus*-infected cattle or false positive serological reactions have occurred. Finally, the seroprevalence in Zambian pigs was high (16%). Cross-reactions with *Yersinia enterocolitica* O:9 cannot alone explain this observed high seroprevalence. Roaming pigs could therefore play an important role as *Brucella* spp. reservoir in the field. It is impossible, however, to determine whether seropositivity in pigs was induced by *B. abortus* or *B. suis*. Isolation of *Brucella* spp. is therefore recommended, to trace the source of infection.



Anaemia in East African short-horn Zebu cattle

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This project forms part of the IDEAL (Infectious Diseases of East African Livestock) project. The most economically important diseases of livestock in East Africa are tick-borne diseases, in particular East Coast fever (ECF), heartwater, anaplasmosis and babesiosis, also trypanosomosis and helminthosis. A common clinical symptom of many of these diseases is anaemia, yet these infections can also bring about changes in the white blood cells and thrombocytes. Interactions between co-infecting pathogens can influence the course of the resultant infection in the host. These interactions can potentially be synergistic, neutral, as well as antagonistic.

The IDEAL project is based in Busia, western Kenya, and focuses on the sedentary mixed crop-livestock smallholding system. Five hundred calves from 20 sublocations in the district were recruited at birth and routinely monitored at 5-weekly intervals up to 51 weeks. During visits a clinical examination is done, including a FAMACHA® score for anaemia, peripheral blood smears are taken, as well as blood in serum, heparin and ethylenediamine-tetraacetate (EDTA) tubes. Faecal samples are also collected and tick species present are recorded. Further testing include haematology, faecal egg counts and oocyst counts, faecal larval cultures, enzyme-linked immunosorbent assays (ELISA) for *Theileria parva*, *Theileria mutans*, *Anaplasma marginale*, *Babesia bovis* and *Babesia bigemina*, polymerase chain reaction (PCR) for *Trypanosoma brucei*, *Trypanosoma theileri*, *Trypanosoma congolense savannah*, *Trypanosoma congolense forest*, *Trypanosoma congolense killifi*, *Trypanosoma congolense tsavo* and *Trypanosoma vivax*, and reverse line blot technique

(RLBT) for *Theileria parva*, *Theileria mutans*, *Theileria taurotragi*, *Theileria velifera*, *Theileria buffeli*, *Theileria sp.*, *Anaplasma centrale*, *Anaplasma marginale*, *Babesia bovis*, *Babesia bigemina*, *Ehrlichia bovis* and *Ehrlichia ruminantium*.

This PhD study focuses on infectious causes of anaemia of cattle, which include haemoparasites as well as certain gastrointestinal helminths. The study is divided into three parts. Firstly, field evaluation of the FAMACHA® eye colour chart for detecting anaemia, and the laboratory performance of the Sysmex® automated cell counter. The second part focuses on age-related trends in the haematological parameters of the short-horn Zebu calves, as well as distribution of anaemia and its associated risk factors in the population. The third part of the study undertakes to describe the effect of the synergistic and antagonistic interactions between pathogens on the haematological profile of these cattle, in particular the effect on packed cell volume, erythrocyte counts, total white blood cells, lymphocyte and eosinophil counts, and thrombocyte counts.

In the absence of published reference ranges for the East African short-horn Zebu calves, a subset of relatively healthy calves was used as a reference sample to describe the trends in the haematological parameters of the population. A comparison to published reference ranges for European cattle breeds indicated differences in both levels and age-related trends of these parameters. Frequency distribution charts of PCV illustrate how resilient these calves are in terms of controlling clinical anaemia under heavy infectious disease loads.

Mortality in canine babesiosis is associated with a consumptive coagulopathy

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The inflammatory response to infection can activate the coagulation system via complex interactions. If uncontrolled, this may lead to disseminated intravascular coagulation (DIC), which has been identified as a major risk factor for poor outcome in both human and canine medicine. Studies have indicated that patients naturally infected with *Babesia rossi* suffer from Systemic Inflammatory Response Syndrome (SIRS) with possible DIC, without clinical signs of bleeding. We hypothesised that a consumptive coagulopathy is present in dogs with *B. rossi* infection and that the extent of the coagulopathy is related to outcome.

This prospective, cross-sectional, observational study included 72 client-owned dogs diagnosed with canine babesiosis and admitted to the Intensive Care Unit (ICU) of the Faculty of Veterinary Science, University of Pretoria. Infection with *B. rossi* was confirmed by blood smear and PCR. Dogs infected with *B. vogeli*, or *Ehrlichia canis*, and dogs euthanised for reasons other than poor prognosis were excluded. Blood samples were collected at admission to hospital. Coagulation factor-, antithrombin- (AT), protein C activity and fibrinogen were assessed using an automated coagulometric analyzer (ACL top 500, Instrumentation Laboratory). D-dimer was measured using an immunometric flow-through principle (D-Dimer Single test, Nycocard Reader II, Medinor A/S). Mortality during hospitalisation was recorded. Levels between the non-survivors and survivors were compared using the Mann-Whitney U test. $P < 0.05$ was considered significant.



Mortality fraction was 19% (14/72). The individual median coagulation factor activity percentage was significantly lower in the non-survivors than the survivors: FII was 40.5% vs. 76.7% ($p < 0.01$); FV was 56.8% vs. 106.4% ($p < 0.001$); FVII was 46.9% vs. 73.4% ($p < 0.05$); FVIII was 60.3% vs. 109.7% ($p < 0.001$); FIX was 56.5% vs. 101.7% ($p < 0.001$); FX was 35.4% vs. 73.9% ($p < 0.001$); FXI was 43.4% vs. 74.9% ($p < 0.01$); FXII was 48.9% vs. 77.4% ($p < 0.01$). Median AT activity was not significantly different between the two groups. Median protein C activity was significantly lower in the non-survivors than the survivors; 44.0% vs. 74.0% ($p < 0.05$). Median fibrinogen level was not significantly different between the two groups; 3.7 g/L vs. 4.8 g/L ($p = 0.25$). The median D-Dimer value was significantly higher in the dogs that died than in the dogs that survived; 1.6 mg/L vs. 0.4 mg/L ($p < 0.01$).

This study demonstrated that mortality was associated with a consumptive coagulopathy in dogs infected with *B. rossi*. The presence of activated coagulation (decreased coagulation factor activity), inhibitor consumption (low protein C) and increased fibrinolytic activity (high D-dimers) in non-survivors of virulent canine babesiosis are indicative of DIC, possibly secondary to a hypercoagulable state caused by the inflammatory response.

Hypercoagulability in uncomplicated canine babesiosis caused by *Babesia rossi*

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Babesiosis, caused by *Babesia rossi*, is a common cause of morbidity and mortality in dogs. Studies have indicated that patients naturally infected with *B. rossi* suffer from Systemic Inflammatory Response Syndrome (SIRS) with the potential for various coagulopathies. The evaluation of haemostatic abnormalities has never before been conducted on uncomplicated babesiosis cases. We hypothesised that hypercoagulability is present in dogs with uncomplicated *B. rossi* infection.

Preliminary results of this prospective study included 14 client-owned dogs diagnosed with uncomplicated canine babesiosis at the Onderstepoort Veterinary Academic Hospital. Infection with *B. rossi* was confirmed by PCR. Blood samples were collected at the time of diagnosis. A group of 10 healthy control dogs were included for comparison. Antithrombin (AT) was measured using an automated spectrophotometric analyzer (Cobas Integra 400, Roche, South Africa). D-dimer was measured using an immunometric flow-through principle (D-dimer Single test, Nycocard Reader II, Medinor A/S). PT, aPTT and fibrinogen assays were performed on the ST art® 4 analyser (Diagnostica Stago, Roche, South Africa). Thromboelastography analysis was performed using the TEG® 5000 Thrombelastograph®

The evaluation of haemostatic abnormalities has never before been conducted on uncomplicated babesiosis cases.

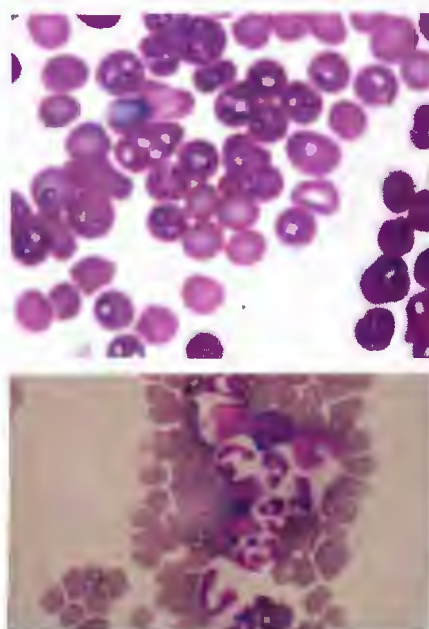
Haemostasis System (Haemoscope, Pro-Gen Diagnostics (Pty) Ltd, South Africa). A complete blood count was performed on the ADVIA 2120 (Siemens, South Africa).

The results between the control and infected dogs were compared using the Mann-Whitney U test. $P \leq 0.01$ was considered significant. The median platelet count was significantly lower in the infected group; $26 \times 10^9/l$ vs. $284 \times 10^9/l$ ($p < 0.01$). Median aPTT was significantly prolonged in the infected group; 13.65 sec vs. 11.5 sec ($p < 0.01$). Median AT activity was significantly lower in the infected group; 108.85 mg/ml vs. 128.8 mg/ml ($p < 0.01$). The median fibrinogen level was significantly higher in the infected group; 5.82 g/l vs. 2.6 g/l ($p < 0.01$), and median d-dimer value was higher in the infected group; 0.3 mg/l vs. 0.1 mg/l ($p < 0.01$). The 30 and 60 minute clot lysis values of the TEG were significantly different with the median LY30 AND LY60 values of the infected dogs being 0.0 (for both) vs. 1.5 and 5.8 respectively ($p < 0.01$).

This study demonstrated that dogs with uncomplicated babesiosis, caused by *B. rossi*, are hypercoagulable compared to healthy controls. The lysis values further demonstrate that the clots formed are more resilient to normal lysis.



A dog receiving a blood transfusion



Babesia blood smears

Ranging behaviour of threatened Gyps vultures in southern Africa determined by GPS tracking technology

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Vultures in the genus *Gyps* are declining rapidly both globally and regionally in southern Africa. Multiple threats have caused the two species of *Gyps* vultures found in South Africa to be listed as 'Vulnerable' in regional assessments. The Cape vulture (*G. coprotheres*) is listed as globally threatened on the IUCN red list, owing to its limited breeding range and decline in numbers during the 20th century. A modern threat to *Gyps* vultures has been identified following the collapse of three Asian vulture species after they consumed tissue from livestock that had been treated with the veterinary non-steroidal anti-inflammatory drug (NSAID), diclofenac. This highlighted the importance of veterinary drug contamination of the food source with regards to species survival. In South Africa, we were lucky in that diclofenac was not available. It has also been argued that the large number of wildlife preserves in South Africa provides a more natural source of food, thereby preventing the entry of a veterinary drug into the vulture food-chain. We were of the opinion, however, that the local vulture population may not necessarily be exclusively feeding within these wildlife preserves. In order to answer this question, GPS tracking technology was used to investigate ranging and foraging behaviour of *Gyps* vultures caught in the Pilaanesberg region of South Africa.

Six immature African white-backed vultures (AWbVs, *G. africanus*) and eight Cape vultures (*G. coprotheres*) (four adults and four immatures) were caught at a vulture feeding site using a walk-in cage trap. The GPS-GSM devices

recorded three GPS location readings at specified times each day. The vultures' altitude and speed and direction of travel were also recorded. By the end of June 2010 the devices had been transmitting data for between 4 and 8 months.

Home range analysis has been possible for five African white-backed vultures and seven Cape vultures. Home ranges calculated using the 95% Minimum Convex Polygon (MCP) method are between 25,661 km² and 517,579 km² for the AWbVs, and between 49,017 km² and 209,304 km² for the Cape vultures. The vultures ranged across a wide area, with the immature individuals of both species regularly travelling long distances and crossing several international borders. Two of the AWbVs entered at least four southern African countries. Three of the adult Cape vultures remained relatively close to their presumed breeding site on the Kransberg cliffs in Limpopo Province, whereas the immature Cape vultures travelled up to 750 km from the capture site.

Key vulture foraging areas have been identified, and the tracking data suggest that *Gyps* vultures do not forage preferentially in officially protected wildlife conservation areas in southern Africa, a finding that contrasts with previous studies. The presence of power-lines appears to strongly influence the location of Cape vulture foraging areas as they regularly utilise the structures for roosting.

Immunisation of roan antelope (*Hippotragus equinus*) using *in vitro* cultured *Theileria* species (sable) schizonts

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Theileria species (sable) causes significant mortalities in roan (*Hippotragus equinus*), and to a lesser extent, sable antelope (*Hippotragus niger*) yearly. Treatment of the condition and an 'infect and treat' vaccination method using a tick-derived stabilate both rely on the availability of buparvaquone, a naphthoquinone with anti-theilerial activity. As buparvaquone is a controlled drug which is not commercially available in South Africa, a viable commercial alternative prevention or treatment method is necessary to control this disease.

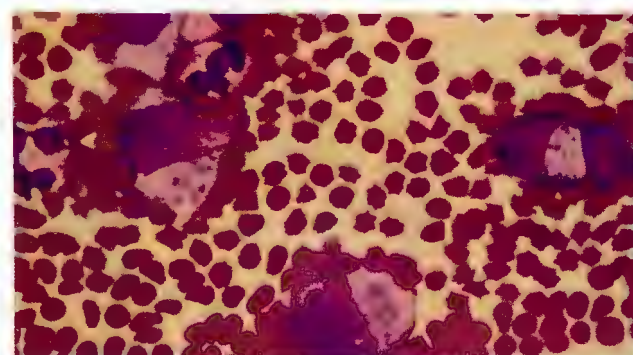
This study explores the effectiveness of an alternative vaccination method using *Theileria* sp. (sable) infected *in vitro* cultured leukoblasts.

A *Theileria* sp. (sable) containing cell line was initiated from lymphnode biopsy material of an infected roan antelope

and the parasite was successfully propagated *in vitro*. Attenuation is believed to have been achieved by 16 cycles of passage.

Real time PCR suggests that the parasite was successfully transmitted via subcutaneous inoculation with this cell line to two naïve roan antelopes. These two inoculated animals remained clinically unaffected by challenge with a tick stabilate used in the 'infect and treat' vaccination method. In contrast, the two unvaccinated control animals became clinically ill and required buparvaquone treatment after challenge.

This pilot study provides enough evidence to encourage further investigation in the use of *Theileria* sp. (sable) infected cells as a potential vaccine. A field study involving more animals which are challenged by natural infection after inoculation is the proposed next step.



Roan theileriosis - schizonts and piroplasmids.



Lymph node biopsy from Roan to harvest theileria infected lymphoblasts.

Pansteatitis associated mortalities in Nile crocodiles from the Olifants River – a retrospective overview

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Nile crocodile (*Crocodylus niloticus*) mortality due to pansteatitis was confirmed in Loskop Dam and the Olifants Gorge, South Africa. Both localities are in the Olifants River: Loskop Dam (upper Olifants River) in the Loskop Dam Nature Reserve and the Olifants Gorge (lower Olifants River) in the Kruger National Park (KNP). These are the first confirmed cases of pansteatitis in wild Nile crocodiles in South Africa.

A multi-species fish die-off in Loskop Dam during August 2007 was caused by acid mine drainage released in the upper catchment of the Olifants River. Dead crocodiles were found 2–3 weeks after the start of the fish die-off. Gross and histopathological lesions in the crocodile carcasses were typical of pansteatitis. The trigger for the pathological changes in the fat depots of the crocodiles was speculated to be the preceding fish die-off, seeing that confirmed cases of pansteatitis in southern Africa have been linked to the feeding of rancid fish material to farmed crocodiles.

Lower down in the Olifants River (Olifants Gorge), large numbers of crocodiles died during the winter months of 2008 (high mortality), 2009 and 2010 (low mortality). A

Concerns have been raised that wild Nile crocodiles could disappear completely from South African aquatic ecosystems in the near future.

diagnosis of pansteatitis was made based on the typical pathological changes observed in all the fat depots of numerous crocodiles that were necropsied. In the pansteatitis outbreaks (2008, 2009 and 2010) in the Olifants Gorge, no fish die-offs were reported before the start of the crocodile deaths. It was confirmed that most of the sharptooth catfish (*Clarias gariepinus*) in the Gorge were obese, however, with pathological lesions consistent with steatitis. Catfish collected from the Gorge were caught alive and, except for obesity and steatitis, no obvious clinical signs were observed. Catfish collected from other rivers in the KNP during the same periods, did not show any pathological changes as observed in the catfish from the Gorge.

The large number of crocodiles that died during the various outbreaks, in the upper (2007) and lower parts (2008, 2009 and 2010) of the Olifants River, raised concerns that wild Nile crocodiles could disappear completely from South African aquatic ecosystems in the near future. The crocodile pansteatitis outbreaks in Loskop Dam and the Olifants Gorge are reviewed, with special reference to the history of the outbreaks, observed pathology, the suspected pathogenesis and future research.

Causes of hatch failure in artificially incubated emu (*Dromaius novaehollandiae*) eggs in South Africa

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The emu (*Dromaius novaehollandiae*) industry in South Africa is about 10 years old, and as such it is a relatively new industry when compared to the established ostrich (*Struthio camelus*) industry. It is still fraught with uncertainties as far as feeding, breeding and artificial incubation are concerned. This study set out to investigate problems emu farmers are facing with artificial incubation as indicated by hatchability; especially from an infection point of view.



Eggs that failed to hatch were collected from African Emu Ranch in Muldersdrift, Gauteng, South Africa, as and when the incubation period had expired. The eggs were visually inspected for obvious malformations or other abnormalities before they were opened. Swabs were taken for bacteriology and mycology at the bacteriology laboratory of the Department of Veterinary Tropical Diseases, Faculty of Veterinary Science, University of Pretoria. The samples were cultured on MacConkey agar, *Salmonella*-XLD agar, and horse blood agar and incubated aerobically at 37 °C for 72 hours. For *Salmonella*, swabs were pooled (about five swabs in a pool) and the positive pool was investigated further. Isolates were identified using routine staining and biochemical tests.

According to the farmer's records the hatchability between the years 2004 and 2006 had dropped as follows, 82 %, 72 %, and 64 % respectively. In 2007, hatchability was 54 % and

it fell precipitously in 2008 to 27 %. This disastrous outcome was allegedly traceable to change of feed and feed supplier in 2008. The other challenge that emerged during this investigation was the high infertility rate, at 60 % and 58 % for 2007 and 2008 seasons respectively.

On standard bacterial cultures from egg contents' swabs, the following isolates were found: *Staphylococcus* species (4/187); *Escherichia coli* (2/187); *Enterococcus* spp (8/187); *Bacillus* spp (2/187); *Corynebacterium* spp (1/187); *Acinetobacter calco* variant *anitratu*s (1/187); *Pseudomonas fluorescens* (1/187); mixed culture of *Escherichia coli* and *Pseudomonas aeruginosa* (1/187); *Pseudomonas chlororaphis* (1/187); *Pseudomonas alcaligenes* (1/187), and *Flavobacterium* spp. (5/187). Only one egg (out of 120) tested positive for fungi and only one pool tested positive for *Salmonella*, which on typing was found to be *Salmonella typhimurium*.

The large number of eggs with dead-in-shell chicks that tested negative for infection suggested that physical factors such as humidity fluctuations affecting moisture loss and egg weight loss, turning of the eggs and possibly nutrition, may be more important factors than infection. These bacteriology test results show that the farm routine at African Emu Ranch is effective at preventing infection in artificially incubated emu eggs.

Surface structure of the emu (*Dromaius novaehollandiae*) tongue

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There are few reports on the surface features of the avian tongue using scanning electron microscopy (SEM). The few existing SEM studies focus mainly on the underlying connective tissue papillae and the macroscopic papillae emanating from the tongue surface. The only ratite tongue previously examined by SEM is that of the ostrich although little detailed information is provided. This study examined the surface structure of the tongue of the emu, a commercially important ratite.

Five emu heads obtained from birds at slaughter were rinsed with phosphate buffer and immersion-fixed in 10% neutral-buffered formalin for at least 48

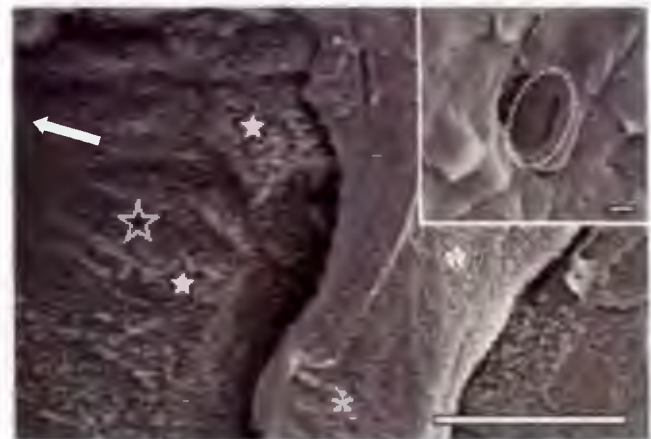
hours. The tongues were removed, halved and routinely processed for SEM. Specimens were examined and the surface features digitally recorded.

The emu tongue consisted of two basic components, the body and root. The surface cells of the tongue body dorsum were flattened and polygonal-shaped and demonstrated a high degree of desquamation, giving the surface a "flaky" appearance. The surface of the cells revealed a complex pattern of microplicae and the cell boundaries were clearly demarcated. The only other notable feature of this region was the presence of large, similarly sized circular openings. The openings were shown histologically to emanate from large, mucus-secreting glands in the underlying connective tissue. The rostral and caudo-lateral aspects of the ventral tongue body displayed similar features to that of the dorsum, although the caudo-lateral part also revealed small gland openings, mostly occurring in rows, randomly distributed amongst the larger openings. The cells immediately surrounding the small gland openings characteristically displayed densely packed microvilli. The ring of microvilli-adorned cells made an abrupt transition to the surrounding surface cells demonstrating microplicae. In contrast, the mid caudo-ventral tongue body had an uneven appearance and was composed of cells which were not clearly demarcated from each other due to a dense covering of microvilli. Interspersed between these cells were randomly distributed ciliated cells and globular protrusions. The mid region of the tongue root appeared similar to that of the dorsal tongue body. Small areas displaying a pattern similar to the mid caudo-ventral area of the tongue body were also present in this region.



The tongue body dorsum displayed similar features to those described for the ostrich tongue. The presence of microplicae observed on the surface cells of the emu tongue has also been noted in the ostrich where microridges are reportedly present on all surfaces of the tongue. Microridges function to trap, retain and spread mucus on the cell surface. These structures are therefore found on surfaces where it is advantageous to maintain a mucus layer. The epithelium of the emu tongue is non-keratinised and would thus be protected by the copious amounts of mucus secreted by the ubiquitous underlying glands, a similar finding to that in the ostrich. The desquamating

surface cells fulfil a mechanical protective function, whereas the microplicae, microvilli and cilia appear to be adaptations for the trapping and spreading of mucus which also fulfils a protective function. The importance of the restricted areas on the tongue body and root containing cells rich in microvilli with interspersed ciliated cells and globular elements, remains unknown. A region with comparable features has been described in the crocodile pharynx where it was associated with underlying lymphoid tissue. There are some indications that a similar situation may exist in the emu.



Rostro-ventral tongue body. Enlargement of encircled part in the inset demonstrates the vertical lining cells of a gland opening (arrow). The lining cells have a mixture of microplicae (black star) and microvilli (white star). The desquamating cells adjacent to the opening display microplicae (*). Adult. Bars = 10 mm.

Bacteria profile and antibiogram of the bacteria isolated from exposed pulp of the canine teeth of cheetahs (*Acinonyx jubatus*)

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Root canal treatment is a procedure commonly performed in veterinary dentistry in an attempt to retain periodontally sound strategic teeth that are affected by pulpal injury. Many studies have been done in human dentistry to isolate the bacteria from injured pulp, revealing some interesting results, but in veterinary dentistry no such studies have yet been conducted. This study was attempted to address some of these issues and, in future, may help specialists in veterinary dentistry as the findings will serve as predictors for the treatment outcome.

Pulp-exposed samples from the canine teeth of cheetahs of two different population were taken using the following protocol. The root canal was first opened using a sterile Haedstrom-file or K-file ranging from 15 to 40. Once opened, a sterile H-file or K-file, ranging from 45 to 70, was used to instrument the canal and a few drops of sterile lactated Ringer's solution was introduced into the canal while filing in order to suspend the bacteria present. A sterile paper tip was introduced into the canal with dressing forceps of which the working area had been sterilised in a bead steriliser. The paper tip was left in the canal for 1 minute after which it was removed and was placed in an anaerobic culture medium. In the laboratory the samples were cultured for aerobic and anaerobic bacteria. Once the bacteria grown and were isolated, evaluation of sensitivity/resistance to antibiotics was performed.

The results show a high prevalence of aerobic bacteria (87,5%). Just a few anaerobic bacteria (12,5%) were isolated, in contrast to the human literature which affirms a high prevalence of anaerobic bacteria. According to the author, the reason is that root canal treatment in humans is always performed when the crown is intact, and in our case the samples were always taken when the pulp had been exposed, changing the environmental conditions in which that bacteria are growing. The bacteria isolated with the higher prevalence



are the aerobic bacteria *Bacillus* spp. and *Pseudomonas aeruginos*. Anaerobic bacteria isolated were *Clostridium sordelli* and *Clostridium septicum*. The results from the evaluation of the sensitivity/resistance to antibiotics revealed that the antibiotics which showed the best effectiveness are Amikacin and Gentamycin, while the highest resistance was against Lincomycin. The results confirm the use of Amikacin or Gentamycin as coadjuvant antibiotic treatment to increased the success in the outcome of root canal treatment.

Learn, pass and have practical fun: A case study of assessing veterinary nursing practical procedures (AVP111)

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Rationale: There is a general lack of assessment resources such as certified veterinary nursing assessors, clinical specimens, patients, equipment, facilities and time to accommodate the evaluation of veterinary nursing practices and procedures on an individual basis.

Educational framework: Assessment for Learning (AFL):

In this intervention for learning I use assessment to improve the student's achievement level. AFL is founded on the premise that learning will improve if learners understand what the aim of their learning is, what their progression is in relation to achieving their aims, and how they will be able to achieve their learning goals. (<http://www.qcda.gov.uk>)

Learning Intervention:

Since 2008 a unique way of assessing practical skills obtained during the course of the General Nursing (AVP111) module has been applied with great success by means of group video recordings. For this evaluation students are divided into groups of 3 to 5 members to record a specific practical procedure taught during the course module. This intervention uses a blended approach with live learning sessions supported by online learning activities in ClickUP. The following specific learning activities are included:

1. Face-to-face theoretical and practical lessons of procedures are conducted
 - The Learner is made aware of learning outcomes
 - The specific outcomes of each practical procedure is discussed
 - Theoretical and practical knowledge of each practical procedure is conveyed to the learners
 - A workshop on video recording and video editing is held
2. Group work to capture video clips of practical procedures in an authentic setting is undertaken.
3. Group members reflect on learning in threaded group discussion with the video clips attached as well as and in face-to-face discussions



4. Peer-to-peer evaluation of practical procedure and video clips amongst groups is undertaken
5. The lecturer and more knowledgeable peer/s mediate/s learning in the discussion forum, by means of telephonic communication and face-to-face sessions
6. The groups improve clips according to the feedback they have received from the lecturer and their peers
7. Classroom seminars where groups present their recorded procedures are held, wherein lively discussions ensue amongst learners and the lecturer
8. The lecturer evaluates group videos and provides final feedback

Feedback from learners and lecturer:

A questionnaire was completed by learners. Learners learnt that planning is essential before executing the recording process. They learnt the terminology and rehearsed the procedures numerous times. They learnt how to collaborate to produce a common output by means of active participation by all group members. As a group they learnt how to support each other in the learning process. Specific skills such as crisis management, leadership skills, building of a positive self-image, sensitivity to diverse learning needs

of others, problem-solving, listening skills, planning skills, stress handling, group management and positive attitudes to peers were built in the process.

The lecturer felt that the information contained in the group product was correct and better researched than in the past. The learning intervention served to whet the appetite of the learners to enquire further about veterinary procedures and terminology. Meta-cognitive thinking skills, and examination skills were acquired by learners as they engaged with the rubric criteria, and learning outcomes.

Learners learnt that learning can be challenging and fun!

Constructing real-time experiences in the classroom

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Students about to graduate as veterinarians are not empowered to deal with flock / herd emergencies, even when these have been mentioned or discussed during lectures in the classroom. The challenge is to circumvent short-term memory by creating a "real-time" experience. Assessment techniques are also outdated and need to be amended in some cases and drastically changed in others. Students seem to believe that the more written tests they successfully complete, the better their subject knowledge will become. Unfortunately, this is all short-term memory and will not contribute to practical application of such knowledge during their careers. For these reasons, lectures and assessments should become more interactive and meaningful with regard to case studies and the flock / herd approach.

Academics often lose contact with the practical aspects of veterinary science and convince themselves that the education and assessment formula that has apparently been successful and has been internationally acceptable for the past 40 years should not be tampered with.

Lecturing philosophy and innovation at universities must be compared to those in business and industry. The question of what is needed to survive and function efficiently in a practical career needs to be addressed. Graduate veterinarians

need to be empowered to fit into practice and thrive in an ever-evolving system.

In order to optimally train and develop their students, lecturers need to accept that traditional didactic teaching and assessment practices are outdated. More practical and modern teaching methods have been developed and need to be implemented.

In large classes, an interactive PowerPoint presentation can be used, while in smaller classes or groups, miniature animal models will facilitate discussion and make the scenario more relevant and realistic.

Structured group assessment within large classes can also be very successful. Academics are able to evaluate each group as well as individual students. Students observing have to evaluate each group in a structured way (written evaluation form). Benefits include excellent class attendance (groups are chosen at random during dedicated assessment sessions), students learn from each others' mistakes and effective team work is encouraged. A staff member supplies feedback and leads a discussion immediately after each group has presented and the student evaluation forms have been collected.

Student feedback received after implementation of these methods has been positive.

Lectures and assessments should become more interactive and meaningful with regard to case studies and the flock/herd approach.

Curricular community engagement: An ethology experiment

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Curricular community engagement or academic service-learning refers to community engagement that forms part of an academic module whereby students are assessed on their work done in a community as part of the assessment of the module. This implies that the community engagement satisfies one or more learning outcomes in the module. It must be meaningful to the community i.e. it must meet identified community goals. Experiential learning and structured reflection form part of the theoretical framework of curricular community engagement.

Community engagement in the curriculum is already in existence at the Faculty of Veterinary Science, University of Pretoria, South Africa, in some final year modules. In these examples, students are involved in offering veterinary services to communities in the form of clinical services. In 2010, the CPE 400 module (Companion Animal Ethology, Handling and Welfare for BVSc 1) for the first time incorporated a community engagement project into the module. Ethology is a fundamental module taught before clinical modules. The class consists of 140 students.

First, students have to identify an appropriate community (this can be a non-profit organisation or an indigent community) and in participation with the community, perform a needs analysis. They then choose an appropriate need that can be linked to the learning outcomes of the module and present a proposal that has to be approved by the organisation or community as well as the lecturer. The proposal has to include a needs analysis, a research question, motivation for the chosen project, proposed budget, explanation of how it links to module learning outcomes (including critical outcomes) and a reflection on the process thus far. The proposal is assessed and this formative assessment forms part of the year mark.

Students are involved in offering veterinary services to communities in the form of clinical services.

Next, the student implements the projects and at the end has to produce a final report. The final report contains a full description of the project and the budget, an explanation of how the identified learning outcomes were achieved, challenges encountered and solutions found, evidence of collaboration and a reflection on the whole experience. This is also assessed as part of the year mark for the module. Factors such as level of community participation, impact on the community, sustainability of the project and innovation are considered in assessing the projects. The best projects are selected and exhibited at Faculty Day.

As this was a first time exercise, several areas of improvement have been identified. It is envisaged for the next year, to make this a group project rather than an individual project. The nature of the project lends itself very well to group work and this would promote cooperative learning. It would also reduce the marking workload for the lecturers. In order to avoid the project being mainly a fundraiser for welfare organisations, the focus in future will be on identifying a research question and presenting a participatory research proposal so that the emphasis is more clearly on the learning outcomes of the module. As the students currently fund the projects themselves, innovative ways of making financial and other resources available to students should be sought. Although one lecture period was devoted to the theory of community engagement, students would benefit from more guidance particularly with reference to structured reflection. This exercise has shown that community engagement can be successfully incorporated into a fundamental module in the veterinary science programme.

Rapid spatial and temporal outbreak investigation using cellular phone technology: A pilot study

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The cellular telephone has become an affordable and appropriate means of communication for all levels of society in South Africa, including those with little formal education. Recently, in 2009, constraints in rapid mobilisation during outbreaks of foot and mouth disease were experienced, when state veterinary staff could not reach or communicate with affected livestock farmers because of service delivery protests in the area.

Cellular telephone technology has been used elsewhere for monitoring and surveillance of disease outbreaks, as well as for recording field data and transmitting it to a distant server for analysis. This technology, however, requires a well trained technician and equipment that would be too costly for a small scale farming enterprise. In this suggested model a key person (community animal health worker) at each dip tank or village in a veterinary district will be appointed. This person will be directly linked via cellular phone to an Animal Health Technician (AHT) and serves as a conduit; both for information required by the state to monitor and control diseases and also information to help the farmers in the area cope with an outbreak. It can also be used to arrange appointments or notify communities of information days. Linking this to the geographical positioning system (GPS) will enable the state veterinarian or AHT to go directly to the farm concerned, which is currently difficult because of the lack of road signs and road infrastructure in communal areas.

A central data point was created using cellular phones and a computer. The personal details of the farmers and the GPS coordinates of their farms were recorded in Microsoft Excel 2007 linked to Garmin Mapsource 6.15.4. Data was received from 24 farmers paired with 48 students. In total 121 report messages were received via cellular phones from both students and farmers. The data was grouped into cases, treatment, number of animals and species. Maps were compiled of incidence of cases, vaccination coverage and distribution of species.



The results indicate that there is a difference in the preferred method of reporting by that farmers compared to the students. Farmers preferred calling and the students preferred text messaging (SMS). However, signal coverage in certain areas was a problem and prevented the calling in of the information probably due to the geographical position of these farms. In more than 60% of cases text messaging was more successful than calling. The study maps were compiled in real time as cases from different farms were received over time and data analysis indicated areas of concern that needed to be addressed.

The pilot study shows that cellular phones can be an effective communication tool in disease surveillance and reporting, but the geographic positioning of the farms will determine which method will be effective for reporting the disease.

Canine ecology in rabies endemic to KwaZulu-Natal Province: Community surveys

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Canine rabies has been endemic to KwaZulu-Natal (KZN) Province, South Africa, since 1976 after the disease was reintroduced through migration of peoples from Mozambique. The KZN Department of Agriculture and Environment estimates that less than 40% of the dog population is vaccinated annually, but it should be noted that the domestic canine population of KZN has never been surveyed and documented on a provincial level. Apart from accurate data on population size, factors affecting accessibility and presentation of dogs to animal health technicians during government-sponsored rabies vaccination campaigns must be understood for effective planning and execution rabies control. The main objective of this study is to elucidate the ecology of the dog population of KZN and the characteristics of communities that support them.

Six communities identified as urban, peri-urban or rural and with known figures of canine rabies prevalence were selected for household demographics and canine ecology surveys. This selection was made in collaboration with KZN Department of Veterinary Services, based on past experiences and knowledge related to rabies and rabies control throughout the province. As one of the internal controls, a peri-urban community that has been rabies negative for decades was included. In order to conduct these extensive surveys, animal health technicians, human and environmental health workers and local SPCA employees were trained to assist. Surveys were conducted in

either Zulu or English at all households within the randomly chosen clusters, provided that a respondent over the age of 14 years was present. The surveys posed a set of socioeconomic and opinion questions to each household surveyed; individual dog statistics and related information were additionally collected from those households that owned dogs.

Preliminary results have clearly indicated significantly larger populations of dogs in rural areas, compared to urban and peri-urban communities. The majority of survey respondents revealed some knowledge of rabies as well as an interest in learning about animal husbandry. The inability to handle the owned dog population, rather than owner's lack of desire for rabies vaccination, appears to be one major factor in the failure of vaccination campaigns to achieve the 70% vaccination coverage recommended by the World Health Organization.

While this study is ongoing, with conclusive data and analysis pending, it is expected that these surveys will provide Government Veterinary Services with descriptive statistics of the owned dog population, as well as an understanding of current hindrances to achieving vaccine coverage goals. Along with improved awareness and control of rabies, educators and welfare organisations should benefit from the information gathered towards appropriate and effective targeting of socio-economic needs and program development.

Significance of chemiluminimetric assessment of plasma endotoxin and neutrophil oxidative activity to prognosticate parvoviral enteritis in puppies

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Parvoviral enteritis (PVE) is known to cause viral damage to the intestinal epithelium of infected puppies with increased risk of translocation of bacteria (coliform bacteraemia) and endotoxin to the bloodstream, likely to be part of morbidity and mortality. Decreased leukocyte counts and increased plasma endotoxin activity (EA) has been suggested to correlate with disease severity and outcome in puppies suffering from PVE. Observations of severely affected non-surviving puppies without leukopaenia generated the hypothesis that relative dysfunction of leukocytes may provide prognostic information. The aim of this study was to assess the significance of chemiluminimetric assessment of plasma endotoxin and neutrophil oxidative activity to prognosticate outcome of PVE based on relative leukocyte dysfunction.

A rapid assay for the detection of endotoxin (LPS) activity in whole blood based on *in vitro* neutrophil activation (SpectralDx, Toronto, Canada) was applied. This assay uses the priming effects of complement opsonised immune complexes on the respiratory burst activity of neutrophils as an analytical chemiluminiscence (CL) platform. In short, tube 1 (blank) reflects baseline neutrophil activation (BA). Tube 2 (test) contains specific anti-LPS IgM that stimulate neutrophil activity in proportion to the concentration of LPS in the blood. Tube 3 (max) contains specific anti-LPS IgM and an excess of LPS so that the CL reflects the maximum inducible response of the neutrophils. The assay was previously validated for use

in dogs. EA is assessed as a ratio (test-blank)/ (max-blank). A ratio max/blank was calculated to reflect the inducible residual oxidative capacity (RC). It was hypothesised that decreased BA and RC, reflecting relative dysfunctions, and increased EA were correlated to poor outcome, independent of leukocyte count.

Sixty-seven client-owned puppies, diagnosed with PVE were included. Stabilised (EDTA) whole blood was obtained at admission and analysed within 180 minutes, as recommended, and a complete cell count was obtained. Mann-Whitney test was applied to test differences between survivors (S) and non-survivors (NS), and multiple logistic regression analysis (MLG) was used to determine independency of prognostic markers to neutrophil and leukocyte counts.

Twelve dogs (18%) died. EA did not differ between groups ($P=0.19$). Median levels of BA and RC was significantly lower in NS compared to S ($P=0.01$ and $P<0.001$, respectively). MLG revealed BA and RC as prognosticators independent of neutrophil and leukocyte count.

A combination of decreased baseline neutrophil oxidative activity and decreased residual oxidative capacity improved the prognostic sensitivity identifying non-survivors without leuko- and/or neutropenia. Observations support relative dysfunction of neutrophils being a risk factor for poor outcome in PVE.

Serial C-reactive protein concentrations as a predictor of outcome in puppies infected with parvovirus

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Canine parvovirus (CPV) remains a leading cause of enteritis in young dogs. To date no agent-specific treatment exists, so treatment remains symptomatic and supportive. Without treatment CPV infection is often fatal. Because of the high cost associated with treatment, early and more effective prediction of outcome will have both an economic and clinical impact. Objective and easily accessible parameters for outcome is preferred. C-reactive protein (CRP) is a major positive acute phase protein in dogs. It has been used extensively in human and animal medicine as a quantitative marker for inflammatory activity in disease processes and is often useful as a prognostic indicator, especially when serial measurements are used.

The aim of this study was to evaluate serial CRP values as a predictor of outcome in puppies suffering from CPV enteritis. Seventy-nine client-owned puppies, diagnosed with CPV and admitted to the isolation ward of the Onderstepoort Veterinary Academic Hospital, were included in the study. Serum for CRP measurements was collected at admission and after 12- and 24 hours. CRP concentrations were determined using an automated human CRP turbidometric immunoassay, previously validated for use in dogs.

Association of CRP concentrations and changes in CRP concentrations with survival, were estimated using logistic regression, adjusting for age, weight and sex. Clinical performance was evaluated by means of receiver-operating characteristic (ROC) curves. Mortality fraction was 20% (16/79). Median CRP concentrations on admission, 12 h and 24 h after admission for survivors were 104.8 mg/l, 89.2 mg/l and 68 mg/l, and for non-survivors 155 mg/l, 151.3 mg/l and 128.5 mg/l respectively. There was a significant negative association between survival and CRP concentration on admission ($p=0.04$), 12 h after admission ($p=0.005$) and 24 h after admission ($p=0.003$). Survival was not significantly associated with change in CRP between admission and 12 h ($p=0.33$), admission and 24 h ($p=0.62$) and 12 and 24 h ($p=0.99$).



Despite the significant association between CRP and survival, ROC analysis demonstrated that discriminative ability of CRP alone predicting survival was not acceptable (area under the ROC curve for CRP on admission, 12 h and 24 h was 0.69, 0.78 and 0.79 respectively). However, together with other known prognosticators, like blood leukocyte changes, CRP may prove to be a useful early predictor.

Sensitivity of a modified faecal flotation method to differentiate between non-neoplastic and neoplastic *Spirocerca lupi* oesophageal nodules in dogs

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Spirocerca lupi, a nematode with an indirect life cycle, has the dog as predominant definitive host. Canine spirocercosis typically results in nodule formation in the caudal oesophagus. It has been found that these nematodes shed eggs intermittently. Dogs may become re-infected, presenting with multiple nodules in various stages. Nodules can undergo neoplastic transformation into fibrosarcomas, osteosarcomas and sarcomas. It is reported that worms within these neoplastic nodules do not exist, are not viable or do not shed eggs. The goal of this study was to determine if faecal examination using a novel modified centrifugal flotation method was sensitive in differentiating neoplastic from non-neoplastic patients.

Faeces were collected from 42 dogs which were diagnosed with spirocercosis using oesophageal endoscopy at the Onderstepoort Veterinary Academic Hospital. A modified centrifugal flotation method was used as it has been shown to have the highest sensitivity and egg count. This method required centrifugation (1400 G) of the faecal solution (1 g faeces mixed with 5 ml of sodium nitrate flotation solution, SG 1.22) for 10 minutes after which 0.1 ml of the supernatant was aspirated from the surface for microscopic examination. Nodules were classified as neoplastic based on histopathology (n=6) and non-neoplastic based on regression in response to therapy determined by endoscopy or histopathology of the entire nodule (n=30). Dogs with multiple non-neoplastic and neoplastic nodules were excluded (n=6). A total egg count

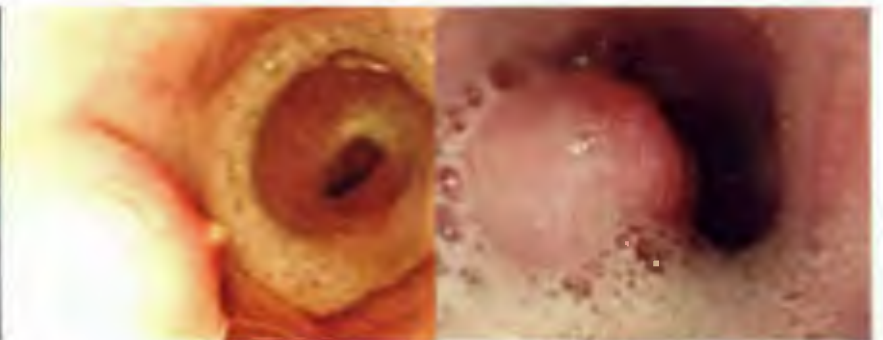
on the 0.1 ml supernatant was also performed on the first 27 cases (5 neoplastic and 22 non-neoplastic).

Faecal examination was positive in 67% of the non-neoplastic group which was not significantly different from the neoplastic group, where 33% were positive (P=0.13, chi-square test). The overall sensitivity of detecting *S. lupi* eggs was 64%. The mean egg count was 1 ± 1.73 in the neoplastic group and 61 ± 95 in the nonneoplastic group (p=0.049, Mann-Whitney U Test).

This may indicate that neoplastic nodules may contain fewer worms or that these worms shed fewer eggs.

The results show that dogs with neoplastically transformed *S. lupi* nodules do shed eggs but egg shedding was higher in dogs with non-neoplastic nodules. This difference was not however significant. The number of eggs per case was significantly lower in the neoplastic group. This may indicate

that neoplastic nodules may contain fewer worms or that these worms shed fewer eggs. The negative results in some of the non-neoplastic cases might be due to intermittent shedding and immature worms, factors which complicate such an analysis. The use of faecal examination as a tool to differentiate between dogs with neoplastic and non-neoplastic spirocercosis does not appear to be useful, although non-neoplastic cases tend to shed more eggs. The presence of multiple benign nodules concurrently with neoplastically transformed nodules in the 6 dogs (14%) which were excluded makes faecal examination unsuitable as a diagnostic tool in this group.



Cementless total hip replacement in the dog: A South African perspective

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Cemented total hip replacement (THR) in dogs with coxofemoral degenerative joint disease has been performed since the early 1970s. Indications for canine THR include painful hip dysplasia in mature dogs, osteoarthritis secondary to hip dysplasia, traumatic luxation of a dysplastic hip joint, and failed femoral head and neck excision arthroplasty. Cemented THR is widely used in dogs with good success, but the reported complication rates varied between 12.1% and 22%. Complications that need revision are aseptic implant loosening at the cement-implant interface, latent bacterial infection at the cement-bone interface with endosteal bone resorption, luxation, malpositioning of the femoral stem, poor cement fill, cracks in the cement and femoral fracture.

New-generation, osteoinductive titanium alloy-coated, non-cemented THR prostheses were developed as modular systems by BioMedtrix® in the USA in 2004; the Helica® Screw Prosthesis in Germany has been in clinical use since 2006. The objective of this clinical study was to locate the technical differences during placement of these two non-cemented THR systems, as well as the therapeutic efficacy and potential complications that could develop with each of these systems over the short-term period (ten weeks) postoperatively. Long-term follow-up radiographic and clinical evaluations (ranging between 4 and 18 months) were also performed for each case. Eight dogs were operated with the BioMedtrix® implants and six dogs received the Helica® screw THR prosthesis. One dog had the BioMedtrix® prosthesis in the right hip joint and the Helica® screw prosthesis in the left hip.

A steep initial learning curve was experienced with the application of both non-cemented THR systems, as it was technically demanding for correct positioning of each implant. The acetabular component should be in 15° to 18° retroversion and between 40° and 50° ventroversion in the sagittal pelvic plane. Reaming and broaching of the femoral canal had to be done with extreme caution and precision when the BioMedtrix®

femoral stem had to be compacted into position (with a 15° angle of anteversion) to prevent fissure fractures to occur in the proximal femur. Cerclage wires had to be applied in two cases where fissure lines became evident.

The Helica® femoral stem was easier to insert when compared to the BioMedtrix® stem. It should fill most of the inner femoral neck diameter and turned tight to seat into position with a 145° angle of inclination in relation with the femoral weight-bearing axis. There should be sufficient bone stock on the ventro-medial aspect of the femoral stem to prevent neck-bone fracture during normal weight bearing.

Full functional limb use was detected in 10 of the 12 medium and large breed dogs following THR within the first 3 postoperative weeks. The mean age at the time of THR surgery was 4.1 years (range: 1.2–8.0 years) and the mean body weight was 38.3 kg (range: 23–62 kg). An overall short-term success rate was estimated at 83% over the period of 18 months with no detectable cup loosening on follow-up radiographic examinations. The position of one Helica® femoral stem had changed by 25° and penetrated the lateral cortex following radiographic evaluation in one case 3 weeks after THR due to extreme weight being placed too soon onto the operated pelvic limb. All dogs should be kept confined in a blocked-off area for 12 weeks after the operation to prevent potential complications of implant loosening. One case developed a femoral diaphyseal fracture distal to the BioMedtrix® femoral stem 2 days after THR. The femur was stabilised with a plate and screws and this dog made a complete, uneventful recovery with full limb use 10 weeks after rigid internal stabilisation.

Both the BioMedtrix® and Helica® THR systems, although technically challenging procedures, were successful for the alleviation of chronic pain in the affected hip joints and should be considered a promising surgical alternative to femur head and neck excision arthroplasty, especially in larger dog breeds.



Parentage verification of the domestic dog (*Canis familiaris*) in a multisire insemination trial using an international microsatellite test panel

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Parentage verification in the domestic dog is finding increasing application in dog breeding societies as well as in research. The aim of parentage verification is to correctly assign both parents to the offspring by excluding non-parents. For accurate parentage verification informative microsatellite markers have to be identified.

The aim of the current study was to establish an accurate method for parentage determination from domestic dog conceptuses during early pregnancy in a multi-sire insemination trial. Semen from 10 male dogs was used in each of 12 females for artificial insemination. Additional males, some being siblings to the selected sires, were used to test the sensitivity of the microsatellite panel. Blood and uterine tissue for DNA extraction was collected from males and females, and embryonic material was collected after ovariohysterectomy between D16 and D30 after the onset of cytological dioestrus.

Twenty-four microsatellite markers were used for parentage verification, including AHT121, INRA21, AHTh171, AHTk252, CXX279, FH2001, FH2054, AHTk211, FH2328, REN105L03,

INU030, Amelogenin, LEI004, REN169D01, AHTh260, REN247M23, REN162C04, INU005, AHTh130, REN64E19, FH2328, REN54P11, FH2848, and AHT137. Mean observed heterozygosity (HObs), mean expected heterozygosity (HExp), and mean PIC were high (0.6753, 0.6785, and 0.628, respectively). Locus AHTk252 was the least informative.

The aim of the current study was to establish an accurate method for parentage determination from domestic dog conceptuses.

Parentage could be assigned in 64 out of the 66 conceptuses. In two conceptuses parentage could not be assigned due to contamination with maternal material during sample collection. CERVUS 3.0.3 assigned parentage in 66% (42 out of 64) of the cases without difficulty. Another 33% of the cases (21 out of 64) could be resolved using the number of exclusions, LOD scores or manual verification of genotyping errors. In one conceptus, paternity could not be established because its sire may have been either of two siblings.

As shown in the current study, early embryonic material can be used for DNA extraction. The panel of 24 microsatellite markers used in the current study provides a high information content suitable for parentage verification in multi-sire litters.

Pathology of recently diagnosed fatal neurotropic arboviral infections in horses in South Africa

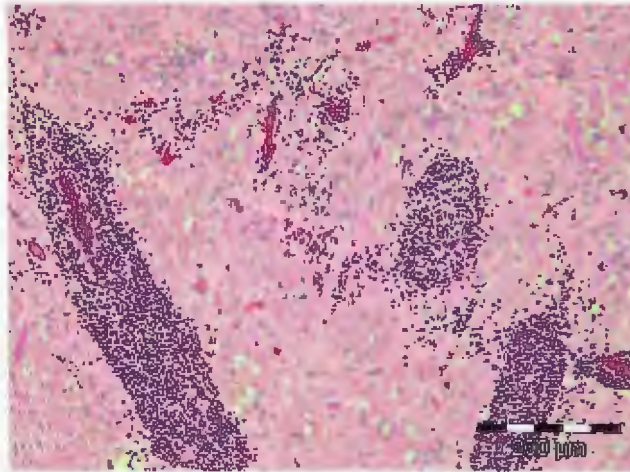
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WNV, a flavivirus, and Sindbis, an alpha virus, are mosquito-borne neurotropic arboviruses, that may cause potentially fatal infections in accidental mammalian hosts, especially horses and humans. Recently, endemic lineage 2 strains from Southern Africa have been reported in neurological horse cases of which 5/7 died or had to be euthanased. Shunivirus, a Bunyavirus of the Simbu group, is transmitted by mosquitoes or midges, and was previously isolated from a horse brain in Zimbabwe

and from a South African mare which died with neurological: both had severe meningoencephalitis and were negative for rabies. For the past three years neurological cases in horses were screened for zoonotic alpha, flavi and bunyaviruses and several positive cases identified. The gross pathology and histopathology of four fatal single lineage 2 West Nile virus (WNV) infections, two horses with WNV-Sindbis virus co-infections, and two horses with Shuni virus infection, from the Gauteng region of South Africa, during the summers of 2007–2010, are described and illustrated.

All diagnoses of the current cases were confirmed by RT-PCR on various CNS tissues and some also by virus isolation and electron microscopy. Gross necropsy lesions were non-specific but most commonly included pulmonary oedema and congestion, visceral congestion, variable subcutaneous oedema, serosanguinous hydropericardium, and occasional especially cardiac haemorrhages. Supraorbital fossae were in some cases oedematous, mimicking some cases of African Horsesickness. The WNV and WNV-Sindbis co-infected horses from the 2007–2009 late summer seasons had relatively mild central nervous system (CNS) lesions which were not grossly visible, were generally more severe in the caudal than cranial spinal cord, and multifocal and mild in the brain, especially in brain stem and midbrain. The ventral horn grey matter of the spinal cord was most commonly affected, with predominate lesions including asymmetrical mild perivascu-



lar mononuclear cuffing with occasional presence of neutrophils, vascular congestion, some perivascular petechiae, mild pericentral canal to diffuse gliosis, occasional glial nodules, some neuronal degeneration or necrosis, and mild intermittent meningitis.

Three horses from the current 2010 late summer have shown macroscopically visible, variably distributed, moderate to severe lesions, including extensive spinal haemorrhage and distended blood vessels; microscopic lesions

were generally similar to but much more severe than the previous cases, with focal unilateral poliomalacia observed in the spinal cord of one. This array of lesions is similar to those listed in northern hemisphere lineage 1 WNV equine non-suppurative polioencephalomyelitis. Shuni viral lesions, similar in several aspects and indistinguishable from those of WNV, are described and illustrated for the first time in two euthanised horses, one severe and one mild; they presented with neurological signs during January and June of 2009, respectively.



Changes in serum cardiac troponin I concentration after semen collection in stallions

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Increased concentration of cardiac troponin I (cTnI) in horses is associated with piroplasmosis, ventricular tachycardia and short-term high intensity exercise. Anecdotal reports exist of acute death in stallions during and immediately after breeding and this could be attributed to myocardial injury. We hypothesised that cTnI and fractional shortening (FS) would increase following semen collection.

Fourteen clinically healthy, phantom-trained stallions were studied. Echocardiography was performed prior to semen collection and heart rate (HR) recorded prior to, during and after semen collection using cardiac telemetry. Fractional shortening was determined within five minutes following semen collection. All ejaculates were collected using a Missouri-model artificial vagina following successful mounting of the phantom. cTnI concentrations were measured using a chemiluminescent immunoassay pre-semen collection and at 4, 6, 12 and 24 hours post-semen collection. Linear regression analysis was used to determine the associations

between the predictors (HR, age, weight and FS) and change in cTnI concentration.

cTnI concentrations were available for eleven stallions. Compared with pre-semen collection cTnI concentrations (0.001 [interquartile range 0.001-0.03] ng/ml), cTnI concentration was significantly increased at 4 (0.01 [0.001–0.09] ng/ml, $P = 0.016$) and 6 hours (0.01 [0.001–0.11] ng/ml, $P = 0.010$), but not at 12 (0.01 [0.001–0.06] ng/ml, $P = 0.396$) and 24 hours (0.001 [0.001–0.06] ng/ml, $P = 0.623$) post-semen collection. No significant association was found between the measured predictors and change in cTnI concentration.

Data suggests that cTnI concentration may increase after semen collection. This may not be of clinical significance, but should be considered in stallions with suspected cardiac disease.



Catastrophic musculoskeletal injuries associated with four racetracks in Gauteng, South Africa during 1998–2004

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A retrospective investigation was performed of Thoroughbred racehorses euthanased due to catastrophic musculoskeletal injury (CMI) at four racetracks in Gauteng, South Africa during the period 1998–2004. The incidence of CMI per 1000 starts was 0.53, similar to the incidence reported worldwide. Fifty-five cases of CMI from 103 603 starts were evaluated. The affected limb of 32/55 horses with a CMI was evaluated by radiography, ultrasonography and macroscopic dissection.

CMI occurred unilaterally and predominantly in the forelimbs, the left forelimb (LF) was affected most frequently (LF 77.3%, vs right fore (RF) 22.7%). Damage to the suspensory apparatus, particularly the proximal sesamoid bones was the most common injury (56.4% cases), with increased incidence in the left forelimb. Sixty-nine percent of the proximal sesamoid bone fractures occurred biaxially, the medial proximal sesamoid bone being most commonly affected. Proximal sesamoid bone fractures were often associated with extensive damage to the flexor tendons and ligaments of the metacarpophalangeal joint.

Five condylar fractures occurred, with the lateral condyle affected in four forelimbs, and the medial condyle in one

forelimb; three right forelimbs and two left forelimbs were affected.

A retrospective investigation was performed of thoroughbred racehorses euthanased due to catastrophic musculoskeletal injury (CMI) at four racetracks in Gauteng, South Africa during the period 1998–2004.

Risk factors for CMI were evaluated by univariable screening followed by a mixed-effects logistic regression model, with race as the random effect. Significant risk factors were gender, racing interval, and weight carried. Intact males were more at risk of CMI than females (Odds ratio = 14.8 [95% CI: 6.2, 35.4]; $P < 0.001$) and than geldings (OR = 5.3 [2.8, 9.9]; $P < 0.001$), and geldings were more at risk than females (OR = 2.8 [1.3, 6.2]; $P = 0.01$). Horses carrying >59 kg were more at risk than horses carrying 54–59 kg (OR = 3.4 [1.4, 8.2]; $P = 0.007$) and than those carrying <54 kg (OR = 5.9 [1.7, 19.7]; $P = 0.004$). Horses racing less than 7 days since their last race were more at risk than those with a longer interval (OR = 2.8 [1.2, 6.6]; $P = 0.02$). Factors found not to be significant were racing year, going, distance, racetrack, age, size of field and draw.

Risk factors identified in this study may be useful in the development of intervention strategies to lower the incidence rate of CMI in Gauteng. This would have value in both improving equine welfare and in minimising the economic and social impact of catastrophic injury.

Radiographic changes in thoroughbred yearlings in South Africa

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A radiographic examination forms part of the pre-purchase examination for Thoroughbred yearlings in many countries. This has provided data of prevalence of radiographic changes in Thoroughbred populations around the world. These data assist veterinarians in advising their clients on the risk of their potential purchase, which has financial implications for the client as well as the racing industry.

Radiographs of 269 Thoroughbred yearlings were lodged at the 2008 National Yearling Sales in Germiston, and evaluated. The prevalence of radiographic changes was recorded and compared to other yearling populations in the world where similar studies had been done.

Differences relative to similar studies were: a lower prevalence of pedal osteitis (1.26%), metatarsophalangeal joint dorsal osteochondral fragmentation (1.60%), sagittal ridge changes (15.7%), ulnar carpal bone lucencies (8.33%), carpal osteophytes (1.19%), distal intertarsal and tarsometatarsal joint radiographic changes (9.92%), tarsal osteochondrosis lesions (4.40%) and stifle joint osteochondrosis lesions (0.4%). The prevalence of dorsal osteochondral fragments in the metacarpophalangeal joint was similar to other studies (1.60%). A higher prevalence of vascular channels was visible in the proximal sesamoid bones as well as irregular borders and lucencies. There was generally a higher prevalence of palmar metacarpophalangeal and plantar metatarsophalangeal osteochondral fragments (2% and 7.10%, respectively). There was an absence of palmar metacarpal disease, supracondylar lysis, proximal sesamoid bone fractures and carpal osteochondral fragmentation in the current study. Additional findings recorded were: proximal interphalangeal joint hyperextension (left front 15.13%, right front 18.91%), the solar angle (right front 2.38°, left front 2.79°), the prevalence of carpal bone one (30.95%) and carpal bone five (1.59%).

The study population was limited to the radiographs lodged at the sale repository. As radiographic changes influence sale price of the yearling, yearlings with severe radiographic changes may not have been lodged at the repository; therefore the prevalence of radiographic changes recorded may not be a true reflection of the 2006 Thoroughbred foal crop. The incidence of many radiographic changes recorded was found to be lower when compared to similar studies done elsewhere. This may be a result of differences in management, pre-sale exercise programmes or genetics.

The radiographic changes recorded will serve as a reference for future research to evaluate their effect on the racing career of the yearling. Management, nutrition and genetics in the various groups of Thoroughbred yearlings should be further investigated in order to explain the reasons behind the differences recorded in the current study.



The comparison of bolus tracking versus time delay studies (utilising a test bolus) for computed tomography thoracic angiography in healthy beagles

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Thoracic computed tomography (CT) is superior to conventional radiography by eliminating superimposition and enhancing resolution, thus improving detection of pathology. Acquisition timing during computed tomography angiography (CTA) is critical to optimise contrast medium enhancement. Due to the large differences in veterinary patients optimal timing of contrast enhancement cannot be standardised. This study serves to determine if there is any statistically significant difference in the two CTA techniques, namely bolus tracking and time delay utilizing a test bolus in terms of enhancement, scan time and CT dose index (CTDI).

Six healthy beagles (36–48 months) underwent dual slice thoracic CTA using the bolus tracking and time delay techniques. The time interval between studies was 6 weeks and the order was randomised. Images were interpreted at the level of the cranial aspect of the 8th thoracic vertebra (T8), mid and caudal T8, where the aorta, pulmonary artery and caudal vena cava were visualised simultaneously. Images were viewed in a mediastinal window. In this within-subject study design the bolus tracking and time delay techniques were compared with respect to the attenuation (Hounsfield units) in these vessels during the arterial and venous phases, respectively. Findings were compared using the students paired t-test and in each case the result was confirmed by the non-parametric Wilcoxon's method pairs signal-ranks test. The Shapiro-Wilkes test for normality was done. Additionally, the 95% confidence interval for the difference of the means between the bolus tracking and time delay techniques was rewritten to a 95% confidence interval for the bolus tracking as a ratio of the time delay studies, where the latter was considered the gold standard for an individual.

During the arterial phase bolus tracking values were between 0.97 and 1.77 of time delay values for the aorta, 0.58 and 2.25 for the pulmonary artery and 0.15 and 1.14 for the caudal vena cava. During the venous phase bolus tracking values were between 0.79 and 1.17 of time delay values for the aorta, between 0.49 and 1.29 for the pulmonary

artery and between 0.78 and 1.05 for the caudal vena cava. Mean CTA scan time for the bolus tracking (8.1minutes \pm 1.8) was significantly different to time delay scan time (16.2minutes \pm 7.5). Mean bolus tracking CTDI (17.68 \pm 7.4) was significantly different to time delay CTDI (44.84 \pm 0.35). Data distribution was normal.

Due to the large differences in veterinary patients optimal timing of contrast enhancement cannot be standardised.

During both the arterial and venous phases, bolus tracking compares favourably to time delay techniques when interrogating the aorta, however, results were variably when looking at the pulmonary artery and caudal vena cava. It can be speculated that results for the pulmonary artery and caudal vena cava would compare more favourably if the

trigger for the bolus tracking technique be placed in the vessel of interest. Bolus tracking however results in less exposure and scan time. It is concluded that thoracic CTA using bolus tracking gives reliable results.



Research Programme: Poster Presentations

The characterisation of *Babesia* spp. in felids in southern Africa

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Babesia is an intracellular erythrocytic haemoparasite of mammals but has also been reported in reptiles and birds. The two most frequently reported *Babesia* species in felids are *B. felis*, which causes clinical babesiosis in domestic cats, and *B. leo*, primarily reported from asymptomatic lions. In this study, DNA was extracted from blood collected from various domestic, captive and free-ranging felids from various countries in southern Africa. The hyper variable region of the 18S rRNA gene was amplified. The PCR products were analysed using the Reverse Line Blot (RLB) hybridisation assay, sequencing and phylogenetic assays. RLB probes to detect *B. felis*, *B. leo* and *B. lengau* were designed,



and used to screen samples collected from various felid species. Results showed that *B. felis* and *B. leo* occurred more frequently in the host from which they had initially been described, namely domestic cats and lions respectively, but were also detected in other felid species. A large number of samples reacted only with the *Babesia* / *Theileria* genus-specific probe. These samples were further analyzed using sequencing and phylogenetic analysis. Phylogenetic studies showed a new *Babesia* species in cheetahs (*B. lengau*), confirmed *Hepatozoon* in lions in the Kruger National Park and revealed the possibility of a novel *Babesia* species in domestic cats.

Evaluation of a "pan" FRET real-time PCR test for discrimination of *Theileria* species in cattle and African buffalo (*Syncerus caffer*)

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Theileria parva is the causative agent of cattle theileriosis in East and Southern Africa. It is transmitted by ticks of the genus *Rhipicephalus* and buffalo are healthy carriers. It usually co-occurs with non-pathogenic *Theileria* species in infected buffalo, and although these parasites do not have any economic significance, they can interfere with the diagnosis of *T. parva*. Diagnostic methods used for the detection of *T. parva* should therefore be sensitive and specific to ensure accurate diagnosis. A real-time PCR assay based on the 18S ribosomal RNA (rRNA) gene was developed and is currently used for the diagnosis of *T. parva* in cattle and buffalo in South Africa. More recently, a nested fluorescence resonance energy transfer (FRET) real-time PCR based on the cytochrome oxidase (Cox) III gene has been developed and used for simultaneous detection and differentiation, by melting curve analysis, of seven *Theileria* spp. from cattle samples using the Rotor Gene 3000 (Corbett Research). The aim of the study was to evaluate this assay for use in the identification and differentiation of *Theileria* spp. in mixed infections in buffalo. Results obtained were compared to those obtained by the reverse line blot (RLB) hybridisation assay. Both assays can simultaneously detect and differentiate different *Theileria* spp. in mixed infections.

Cattle and buffalo samples originated from South Africa and Mozambique. A nested PCR protocol was used for the

amplification of the coxIII gene of the parasite. Melting curve analysis was used for species identification. Selected samples which had unpredicted melting points were cloned and sequenced. The sequences were assembled and edited using GAP4 of the Staden software package (v1.6.0 for windows), aligned using Mafft (v5), and manually edited using BioEdit (v7). Phylogenetic trees were constructed using MEGA 4.0, PAUP* (v4b10) and MrBayes v.3.1.2.

Of the 224 samples, 83% were positive for of *T. parva*, 56% for *Theileria* sp. (buffalo), 18% for *T. taurotragi*, 6% for *T. buffeli* and 2% for *Theileria mutans*. The *T. parva* and *Theileria* sp. (buffalo) were also identified as the most commonly occurring species by the RLB assay. The RLB detected more infections of *T. mutans* and *T. buffeli* than the real-time PCR assay. Unlike results obtained using the real-time PCR assay, 23.7% of samples were positive for *T. velifera* and no *T. taurotragi* infections were detected by the RLB. Of the 14 samples that only had a *Theileria/Babesia* genus-specific signal on the RLB assay, 11 showed mixed *Theileria* spp. infections and 3 were negative on real-time PCR. A total of 27 new sequences were obtained, which can be used for the development of new primers and probes in order to increase the specificity of the coxIII real-time PCR in the diagnosis of mixed *Theileria* infections in the South African buffalo population.

Comparative distribution of Herbst corpuscles within the oropharynx of the ostrich (*Struthio camelus*) and emu (*Dromaius novaehollandiae*)

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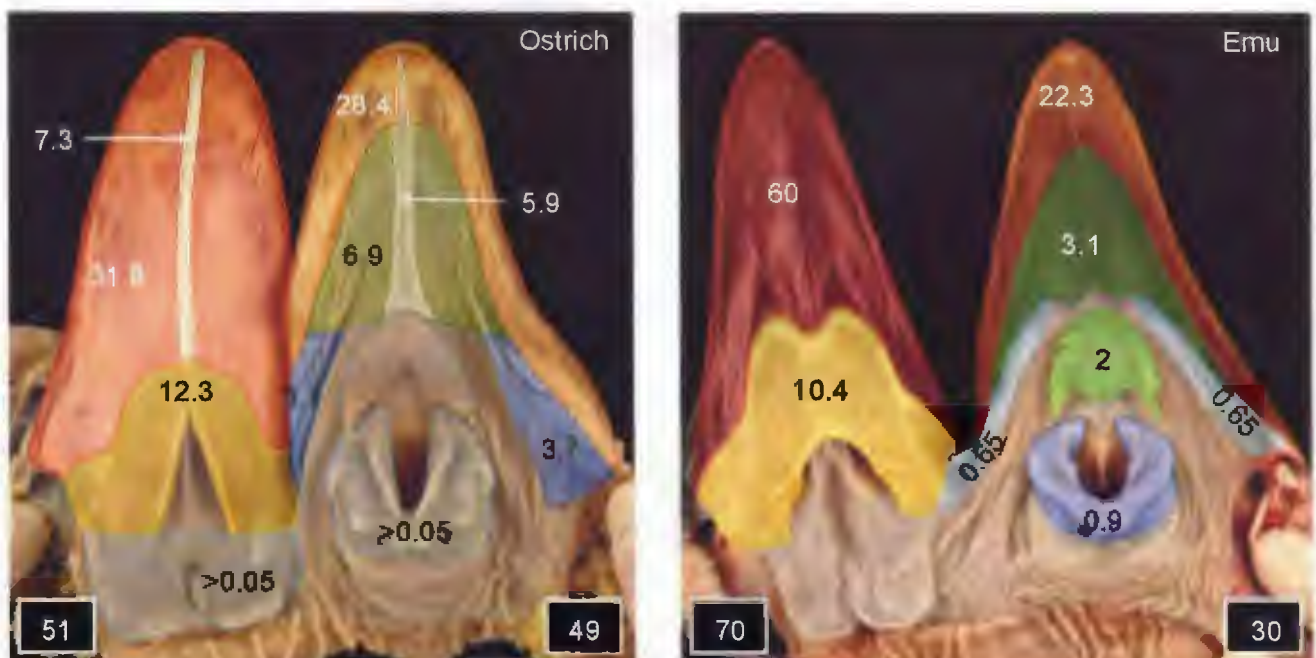
Herbst or Pacinian corpuscles are lamellated sensory structures sensitive to vibratory stimuli and their presence in the oropharynx has been reported in a number of avian species including ratites. Although identified in the oropharynx of the ostrich, emu and greater rhea, the distribution and relative density of Herbst corpuscles in this region remains unknown. As this information may provide important data regarding food selection and/or manipulation, and assist in relating structures involved in feeding to function in paleognathous birds, this study compares the distribution of Herbst corpuscles in two commercially important ratites, the ostrich and emu.

Three ostrich and three emu heads were obtained from birds following slaughter at a commercial abattoir. The heads were rinsed in water to remove mucous and blood and fixed in 10% neutral-buffered formalin. Samples representing all anatomical regions of the oropharynx were removed and routinely prepared for light microscopy. Sections containing bone were decalcified in 8% formic acid for at least 1 month prior to processing. Corpuscles were identified, counted and averaged, their distribution determined and the relative percentage obtained for each region.

The ostrich revealed a similar density of corpuscles in the oropharyngeal roof and floor, although they were concentrated in the median longitudinal mucosal folds typically present in the rostral part of both regions. In the emu the roof displayed a higher density of corpuscles than the floor and there was no preferential concentration of corpuscles in the median longitudinal folds. Herbst corpuscles were absent from the ostrich tongue but present in the emu, whereas they were absent from the pharyngeal folds in the emu but present in the ostrich.

Herbst or Pacinian corpuscles are lamellated sensory structures sensitive to vibratory stimuli and their presence in the oropharynx has been reported in a number of avian species including ratites.

The differences in distribution and relative density of Herbst corpuscles between structurally comparable regions of the ostrich and emu oropharynx may indicate differing functions for related structures. For example, the emu tongue (presence of corpuscles) appears to function as an organ of touch while that of the ostrich lacks this capability (absence of corpuscles). To what extent these findings indicate species subtleties in food selection and subsequent manipulation remains to be determined.



Ostrich and emu oropharynx openly displayed. Numbers are expressed as a percentage and reflect the relative density of Herbst corpuscles in the outlined regions relative to its area. Numbers in the bottom corners reflect the percentage of Herbst corpuscles in the roof (left) and floor (right) of the oropharynx of each species. Non-shaded areas were not sampled and the grey shading represents sampled regions where corpuscles were not observed.

Detection of *Ehrlichia* species of the African buffalo (*Syncerus caffer*) by means of the Reverse Line Blot (RLB) hybridisation assay

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The African buffalo is known to be the reservoir host of numerous important tick-borne pathogens, most of which are carried asymptotically, but which can cause disease if transmitted to susceptible animals. Of these, *Theileria parva*, the causative agent of East Coast fever, Corridor disease (a controlled disease in South Africa) and January disease; and *Ehrlichia ruminantium*, the causative agent of heartwater, are considered to be the most important tick-borne disease agents of livestock in sub-Saharan Africa. No mortalities due to heartwater have yet been documented in the African buffalo, but it is known that a subclinical carrier state can occur in buffalo. This suggests that buffalo could play an important role in the epidemiology and spread of heartwater and therefore could serve as reservoirs of infection which may represent a threat to the livestock industry. As little is known about the *Ehrlichia* spp. infection status of African buffalo, the primary objective of this study was to determine the occurrence of *Ehrlichia* species in buffalo samples collected from two game parks in South Africa. These samples were simultaneously screened for the presence of *Anaplasma*, *Theileria* and *Babesia* spp.

DNA was extracted from 200 buffalo blood samples originating from the Kruger National Park and the Hluhluwe-iMfolozi Park (KwaZulu-Natal province), South Africa and subjected to the Reverse Line Blot (RLB) hybridisation assay. *Ehrlichia* sp. Omatjenne, *Anaplasma marginale* and *Anaplasma centrale* were detected in 70% of the samples. The presence of these parasites has not been previously reported in South African buffalo populations. None of the samples tested positive for *E. ruminantium*. Of the piroplasm parasites included in the assay, *Theileria parva*, *Theileria* sp. (buffalo), *Theileria mutans*, *Theileria buffeli* and *Babesia occultans* were identified. The detection of *B. occultans* is of interest since this is mostly a cattle parasite that has not been reported before in buffalo. The PCR products of five samples only hybridised with the genus-specific probe, suggesting the presence of novel species and/or variants of species in the buffalo population and will be further investigated by cloning and sequencing of the PCR products.

Molecular detection of cheetah-associated *Babesia* species in field-collected ticks (Acari: Ixodidae) in South Africa

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Ticks are of vast importance due to their ability to transmit an impressive variety of infectious agents. Emerging arthropod-transmitted parasites of wildlife are potential disease threats and the worldwide picture of ixodid tick-transmitted parasitic diseases is an example of this dynamic situation. The present work describes the molecular detection of cheetah-associated *Babesia* species in field-collected ixodid ticks which could serve as potential vectors for feline babesiosis in cheetahs at various breeding centres in South Africa.

The vegetation at three cheetah breeding centres (The Ann van Dyk Cheetah breeding Centre-De Wildt/Brits, Ann van Dyk Cheetah breeding Centre-De Wildt/Shingwedzi, The Hoedspruit Endangered Species) was dragged for ticks over a year period. A total number of 4214 ixodid ticks of five species, namely *Amblyomma hebraeum*, *Amblyomma marmoreum*, *Haemaphysalis (Rhipistoma) elliptica*, *Rhipicephalus simus* and *Rhipicephalus zambeziensis* were collected and identified taxonomically. Subsequently, DNA was extracted from homogenised immature and adult ticks. The V4 variable region of the parasite 18S rRNA gene was amplified and subjected to the Reverse Line Blot (RLB) hybridisation assay for the detection and differentiation of *Babesia* and *Theileria* species.

Results showed that *Babesia* species could only be detected from immature and adult developmental stages of *Haemaphysalis (R.) elliptica* ticks. The RLB primers successfully amplified a fragment of ~500 bp of DNA of the 18S rRNA gene spanning the V4 variable region. The 18S rRNA complete gene sequences (1700 bp) of cheetah-associated *Babesia* in ticks, *Babesia canis rossi*, *Babesia felis*, *Babesia lengau* and *Babesia leo*, were then compared. The alignments resulted in 100% identity with *Babesia lengau* in cheetahs.

The key elements involved in vector-borne infectious diseases are the infectious micro-organisms, the vector and the reservoir from which the vector obtained the infection. Cheetahs with subclinical form of babesiosis can be sources of infection for ticks since they carry *Babesia* species. Circumstantial evidence of existence of *Babesia* parasites in the immature stages of the life cycle as well as the adults is indicative of a possible transstadial transmission of the parasite. Since *Babesia* species are transmitted by ixodid ticks, the results suggested that *Haemaphysalis (R.) elliptica* might play an important role as a natural vector in the field-transmission of cheetah-associated *Babesia* species.

Influence of seminal plasma on fresh and post-thaw parameters of stallion epididymal spermatozoa

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Cryopreservation of epididymal spermatozoa may be the only opportunity to preserve valuable genetics of males in cases of unforeseen injury or death. Stallion epididymal spermatozoa have been cryopreserved successfully. It has been demonstrated that stallion epididymal spermatozoa are fertile, and pregnancies as well as live foals have been produced. As spermatozoal quality parameters like motility, morphology and viability have a major influence on fertility and pregnancy rates, it is of great interest to describe these and investigate the influence of seminal plasma on these parameters.

Fresh and post-thaw parameters (motility, morphology and viability) of stallion epididymal spermatozoa that have been and that have not been exposed to seminal plasma were evaluated, and directly compared to fresh and post-thaw parameters of ejaculated spermatozoa from the same stallions.

Six sperm categories of each stallion (n= 4) were evaluated for motility, morphology and viability. These categories were fresh ejaculated spermatozoa (Fr-E), fresh epididymal spermatozoa that had been exposed to seminal plasma (Fr-SP+), fresh epididymal spermatozoa that had never been exposed to seminal plasma (Fr-SP-), frozen-thawed ejaculated spermatozoa (Cr-E), frozen-thawed epididymal spermatozoa

that had been exposed to seminal plasma prior to freezing (Cr-SP+) and frozen-thawed epididymal spermatozoa that had never been exposed to seminal plasma (Cr-SP-).

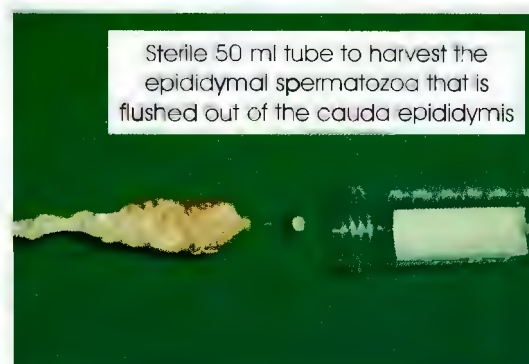
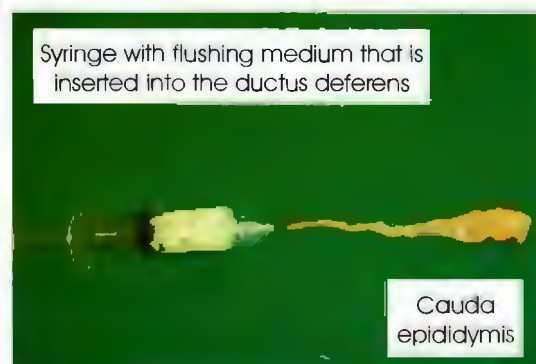
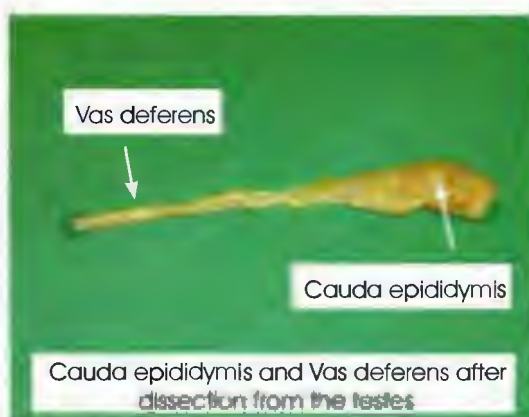
Results show that seminal plasma stimulates initial motility of fresh epididymal stallion spermatozoa while this difference in progressive motility is no longer present post-thaw. Progressive motility of fresh or frozen-thawed ejaculated stallion spermatozoa is not always a good indicator for post-thaw progressive motility of epididymal spermatozoa.

Results show that seminal plasma stimulates initial motility of fresh epididymal stallion spermatozoa

This study shows that seminal plasma has a positive influence on the incidence of overall sperm defects, midpiece reflexes and distal cytoplasmic droplets

in frozen-thawed stallion epididymal spermatozoa while the occurrence of midpiece reflexes is likely to be linked to distal cytoplasmic droplets. Furthermore, we could show that seminal plasma does not have an influence on viability of fresh and frozen-thawed morphologically normal epididymal spermatozoa.

In conclusion, we recommend seminal plasma as flushing medium to harvest and freeze stallion epididymal spermatozoa.



Evaluation of the discriminatory power of variable number of tandem repeat (VNTR) typing of *Mycobacterium bovis* isolates from South Africa

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Mycobacterium bovis is the causative agent of bovine tuberculosis (BTB) and belongs to the *Mycobacterium tuberculosis* Complex (MTC), a group of closely related bacteria causing tuberculosis in various mammalian hosts. The disease has a major economic impact on livestock productivity, can persist in wildlife and thus affect entire ecosystems and it is of public health concern due to its zoonotic potential. For better understanding the transmission of the disease, reliable, reproducible, molecular methods which allow standardisation between laboratories are required to determine the spread of individual *M. bovis* strains. Molecular epidemiological studies on bovine tuberculosis are changing towards Variable Number of Tandem Repeat (VNTR) typing, which is reported to meet the above criteria. VNTR analysis is not yet standardised in *M. bovis*, however, and allelic diversity can vary among countries.

In this study, we have assessed the discriminatory power of 29 published VNTR markers on a panel of 23 *M. bovis* isolates from previously characterised cases with varying degrees of relatedness and one *M. bovis* BCG ATCC 1173P2 strain. The main aim was to identify a set of markers suitable for typing South African *M. bovis* isolates for epidemiological studies.

Of the 29 markers tested, 13 showed genetic diversity whereas 14 markers did not give any variation among the *M. bovis* isolates. Two markers could not be amplified under our set conditions. The most discriminatory marker was Qub 11a, which identified 5 VNTR profiles. ETR A and MIRU 26 also showed high discriminative power (4 profiles each). We therefore described these 3 markers as highly discriminative, 3 markers (Qub 18, Qub 11b and ETR E) as moderately discriminative, 7 markers (Qub 26, MIRU 23, ETR B, ETR C, Mtub 21, MIRU 16 and Mtub 12) as slightly discriminative, and those that did not differentiate the isolates as poorly discriminative.

Our results showed that the combination of the 13 VNTR markers was able to distinguish the *M. bovis* strain types from epidemiologically related and unrelated cases from both wildlife species and cattle, deeming VNTR typing a very useful tool for studying molecular epidemiology of bovine tuberculosis in South Africa. It was found to be less discriminative amongst IS6110 C8 variant strains, however, and therefore needs further improvements in this regard.

Development of a real-time PCR assay for detecting *Babesia rossi* genotype

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Babesia rossi induced canine babesiosis is associated with severe clinical manifestations and mortalities. Further information is required regarding the relationship between parasite genotype and disease phenotype. The current techniques of genotyping include: PCR, RLB (RLB primers) second PCR with different primers, sequencing, sequence analysis and phylogenetic analysis. These methods are time consuming. In this study, a highly sensitive real-time polymerase chain reaction (PCR) assay will be developed to detect *Babesia rossi* genotype in dogs.

A total of 106 blood samples were obtained from *B. rossi* infected dogs at Onderstepoort Veterinary Academic Hospital (OVAH). DNA was extracted from 200µl of whole blood. Samples were screened for the presence of parasites in DNA using the reverse line blot (RLB) hybridisation assay. Fifty

two positive *B. rossi* samples were selected and the *BrEMA1* gene was amplified and sequenced using FrepBrEMA1 (5'-CCA ACA TTG ATG ATG ACA A-3') and RrepBrEMA1 (5'-CTG CAT GTC AGC TTA ATC A-3') primers. These primers were also used for Real-Time PCR to specifically amplify the *BrEMA1* gene using SYBR Green.

Based on sequence analysis, we have identified 9 genotypes in agreement with previously published results and the existence of an additional 2 new genotypes.

Although we were successful in detecting *BrEMA1* genotype using SYBR Green, the assay failed to reliably differentiate amongst the various genotypes. A new approach using hybridisation probes is currently being explored.

Optimising and testing of plant-made RANTES analogues as topical microbicides

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Heterosexual contact accounts for the majority of all human immunodeficiency virus (HIV) infections worldwide. Women increasingly bear a disproportionate burden of the pandemic, an urgent need to develop new strategies to reduce HIV-1 transmission that could be controlled by women themselves. Microbicides which are chemicals that act to impede transmission of HIV have become a compelling target for HIV prevention. They have the potential to benefit HIV positive women by enhancing their sexual lives and helping reduce risk of infection with new or resistant strains of HIV and other sexually transmitted infections (STI). RANTES analogues, which are natural ligands for the CC chemokine receptors such as CCR5, block HIV infection and have been found to be effective ingredients for microbicides. The current study investigated the expression of RANTES analogues in plants as an alternative production system for the microbicides. Two RANTES analogues with histidine residues were transiently expressed in *Nicotiana benthamiana* leaves via agrobacterium-mediated transfection.

The expression was assayed by ELISA, dot and western blotting techniques. Expression levels ranged between 5.2–63.4 and 1.4–54.4 mg/kg fresh weight tissue as measured by ELISA for 5P12-RANTES and 6P4-RANTES proteins, respectively. The protein size was confirmed by western blotting. The sizes of the expected 8.8 kDa RANTES proteins were found to have migrated at 10 kDa which corresponded with the RANTES positive control (expected 7.8 kDa). Our results highlight the potential usefulness of plants for the production of RANTES in tobacco, a non-food and non-feed crop, for preventive intervention of HIV in humans. Ongoing work involves stable transformation of *Nicotiana tabacum* and characterisation of the expressed RANTES proteins for efficacy testing.

Blood parasites of African rhinoceroses and their impact on conservation

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Circumstantial evidence suggests that endemic stability to vector-borne protozoal diseases occurs in wildlife populations, similar to the well-studied endemic stability to babesiosis in cattle. Trypanosomosis and babesiosis have been implicated in mortalities in both black (*Diceros bicornis*) and white (*Ceratotherium simum*) rhinoceroses in East and southern Africa. Subclinical carrier states have recently been demonstrated by molecular techniques. There is mounting evidence that endemic stability, which may exist to both diseases, can be disrupted by stress, e.g. capture and translocation. Furthermore, rhinoceroses

Trypanosomosis and babesiosis have been implicated in mortalities in both black (*Diceros bicornis*) and white (*Ceratotherium simum*) rhinoceroses.

growing up in areas where specific vectors are rare or absent (e.g. ticks in arid areas) may be fully susceptible if infected later in life. (Black rhinoceroses from Etosha National Park and Damaraland, Namibia, were shown to be free of haemoprotozoa.) Rhinoceroses occur in geographically dispersed populations. Metapopulation management, which implies translocation of individuals between populations to maintain genetic diversity, may result in individuals succumbing to either of the two diseases. Controlled exposure and chemoprophylaxis are possible preventative measures.

Preliminary results from a prospective multicentre study of the association of lactate concentration with survival in sick neonatal foals

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The concentration of lactate [LAC] in the blood of sick equine neonates at admission ($[LAC]_{ADMIT}$) and other time points is associated with survival in several retrospective studies, with larger [LAC] associated with non-survival. We hypothesised that this association would remain when examined prospectively.

Thirteen university and private equine referral hospitals on 3 continents enrolled 643 foals over the 2008 foaling season of both hemispheres. Signalment, historical, clinical and clinicopathologic data were entered into a standardised spreadsheet by a single veterinarian at each institution. Spreadsheets were unified into a single data set for analysis. Not all data were available for all foals. Data were analyzed using Kruskal-Wallis and regression analysis, $P \leq 0.05$.

More colts ($N=354$) than fillies ($N=277$) were enrolled; there was no difference in $[LAC]_{ADMIT}$ by sex. Neither gestational age nor age at admission was significantly different between survivors and non-survivors. Median $[LAC]_{ADMIT}$ was significantly larger in non-survivors ($N=120$, 5.79 mmol/L, $P < 0.001$) compared to survivors ($N=466$, 3.55 mmol/L). The difference in [LAC] between groups remained statistically significant through 120 hrs post-admission. $[LAC]_{ADMIT}$ was significantly greater in foals born following premature placental separation ($P < 0.001$) and those with a history of dystocia or caesarian section delivery ($P < 0.001$). Mean arterial pressure had a small ($R^2 = 19.1\%$) but significant ($P < 0.001$) association with $[LAC]_{ADMIT}$. $[LAC]_{ADMIT}$ was not significantly different in blood culture positive vs. negative foals.

This large prospective study supports the findings of several smaller retrospective studies regarding the utility of initial and repeated [LAC] measurement in the management of sick foals.

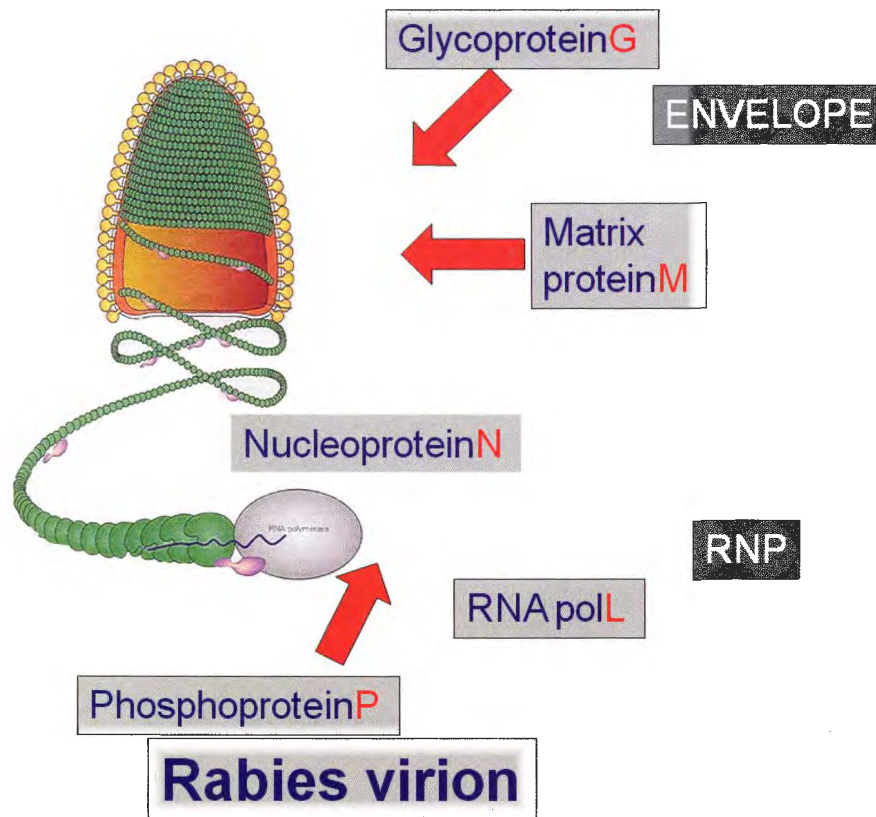


The pathogenesis of rabies virus (RABV) canid strain and mongoose strain in South Africa

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Rabies is an important public health and veterinary threat in South Africa and is constantly diagnosed in domestic and wild carnivore species in this country. Presently, there are two biotypes within genotype 1 in South Africa – the canid strain and mongoose rabies strains. These two RABV biotypes are independently maintained and transmitted by species of Canidae and Herpestidae, and are capable of jumping species boundaries which are referred to as infectious “spillover”. This study was undertaken to evaluate the pathogenicity of the three RABV strains (canid, mongoose and spillover).

Three rabies positive brain tissues of the selected strains were retrieved from the rabies archive of the Onderstepoort Veterinary Institute (OVI). Ten percent brain suspension of each brain tissue was prepared and then passaged in suckling mice twice and the Fluorescent Antibody Test (FAT) undertaken to confirm presence of rabies virus antigen in mouse-infected brain tissues. Total viral RNA extractions were performed using Trizol and reverse transcription – PCR

reactions performed. The three amplicons of the complete glycoprotein genes of the three virus isolates were purified and sequenced.

Preliminary nucleotide analyses of partial regions of the two sequences (22/7 and 198/8) compared to three other previously characterised viruses isolates (381/06 mongoose strain, 385/06 canid strain and 221/07 mongoose strain) demonstrate some differences (100 out of 633; 15.79%) which could influence the pathogenicity of Southern African canid and mongoose rabies strains.

These two RABV biotypes are independently maintained and transmitted by species of Canidae and Herpestidae.

However, in order to obtain a complete picture, pathogenesis assays of these rabies genotype 1 variants will be undertaken in mice to understand disease progression. In addition, reverse genetics for apoptosis induction and survival pathway (phAKT/AKT) will also be performed. This study will constitute the first description of virulence using reverse genetics of the different rabies strains in South Africa.

Faculty of Veterinary Science: Photo Competition 2009

Novices



Above: People - Melinda Devenish

Below: Birds - Andre Hanekom





Above: Animal Human interaction -
Katelyn Conlong

Below: Best Novice & First Scenic -
Freude Bertram



Novices (continued)



Above: Flowers - Kylene Kelbe

Faculty of Veterinary Science: Photo Competition 2009

Experienced



Above: People's Favourite & First Scenic - Johan Marais

Below: Birds - Jaco Goosen



Faculty of Veterinary Science: Photo Competition 2009

Experienced (continued)



Above: *Best Experienced & First Wildlife - Jaco Goosen*

Below: *Domestic Animals - Leon Venter*



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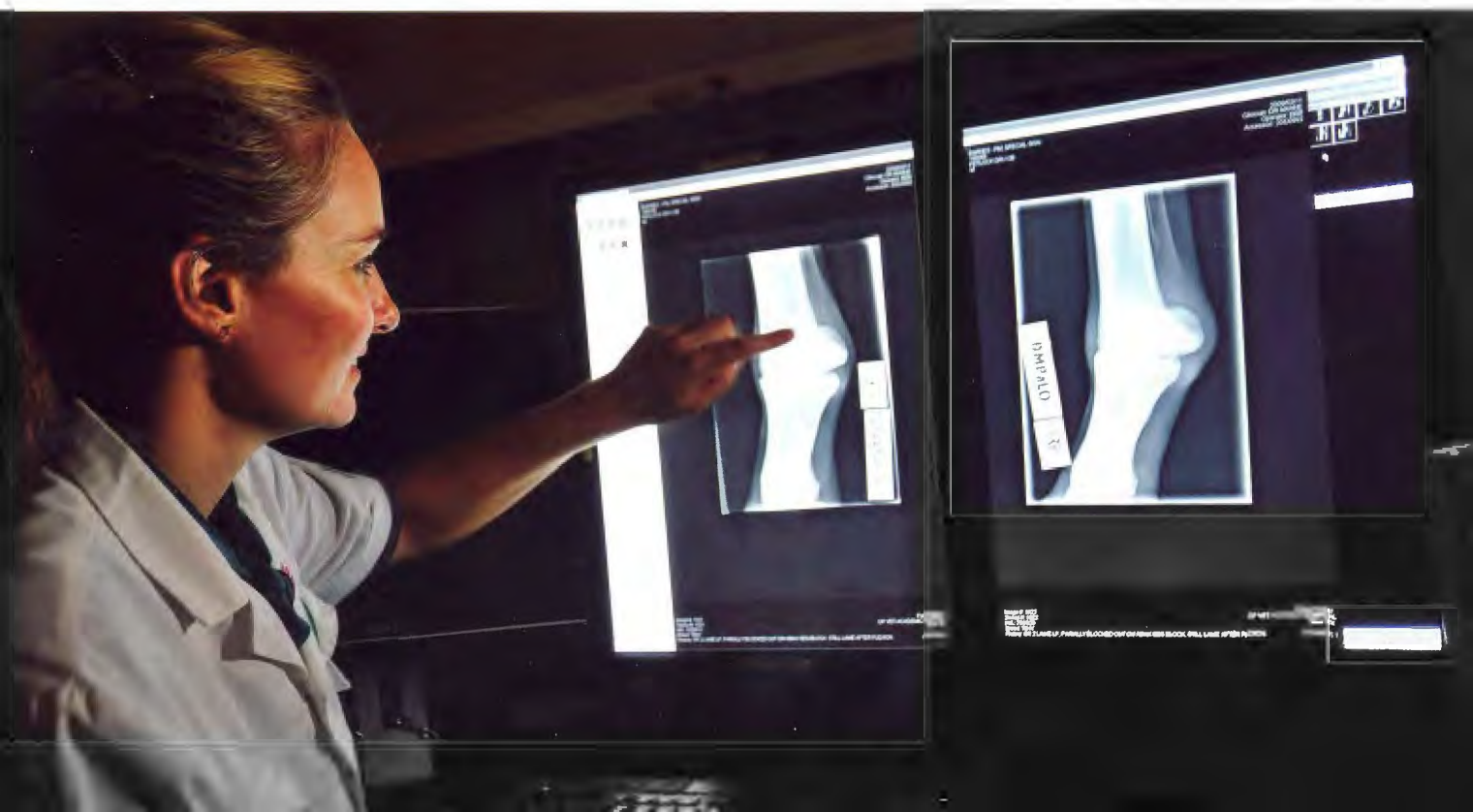
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