



Prof N James MacLachlan
Distinguished Professor, School of Veterinary Medicine
University of California



UNIVERSITEIT VAN PRETORIA
UNIVERSITY OF PRETORIA
YUNIBESITHI YA PRETORIA
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 2012 Faculty Day.



inqaba biotec™



Animal Health



Bayer HealthCare
Animal Health



Faculty Day
Faculty of Veterinary Science
University of Pretoria
6 September 2012





07:30 – 07:55 Registration and coffee

MASTER OF CEREMONIES: Dr L Le Roux-Pullen

08:00 – 08:10 Welcoming
Dean: Prof GE Swan

08:10 – 08:25 Opening Address
Vice-Principal: Prof S Burton

08:25 – 09:15 First Session (50 min)

SESSION CHAIRPERSON: Dr Dayo Fasina

- 1 **Detection of lumpy skin disease virus in saliva of potentially infected *Amblyomma hebraeum* and *Rhipicephalus appendiculatus* ticks** 7
JC Lubinga, E Tuppurainen, WH Stoltz, JAW Coetzer, EH Venter
- 2 **Molecular characterisation of the fowl adenovirus isolates involved in an outbreak of inclusion body hepatitis in South Africa** 8
HW Joubert, H Aitchison, EH Venter, LH Maartens
- 3 **An African horse sickness virus serotype 4 recombinant canarypox virus vaccine elicits specific cell-mediated immune responses in horses** 9
H El Garch, JE Crafford, P Amouyal, PY Durand, C Edlund Toulemonde, L Lemaitre, V Cozette, AJ Guthrie, JM Minke
- 4 **Efficacy of alpha-cypermethrin treated mesh against *Culicoides* biting midges** 10
PC Page, K Labuschagne, GJ Venter, JP Schoeman, AJ Guthrie

09:20 – 10:10 **Sir Arnold Theiler Memorial Lecture:** Prof NJ MacLachlan
Emerging viral diseases; the example of bluetongue, from Theiler to climate change

10:15 – 10:35 Theiler Memorial Trust Award

10:35 – 10:55 Rescom and Faculty Awards

11:00 – 11:40 Tea

11:45 – 13:00 Second Session (75 min)

SESSION CHAIRPERSON: Dr Darryn Knobel

- 5 **What prevents ratites from choking?** 11
MR Crole, JT Soley
- 6 **A novel structure with phylogenetic implications found in ratite spermatids** 12
L du Plessis, J Soley, J Woodward, T Sewell
- 7 **A comparison of stress physiology and behaviour in beef feedlot calves hot-iron branded on the cheek and the upper hind leg** 13
MJ Grobler, A Ganswindt, AS Shakespeare, PN Thompson
- 8 **The effect of blood urea nitrogen on the reproductive performance of beef heifers on different levels of nitrogen supplementation** 14
T Tshuma, DE Holm, GT Fosgate, DC Lourens
- 9 **Epidemiological investigation of the first reported outbreak of contagious equine metritis in South Africa** 15
CE May, ML Schulman, AJ Guthrie, D Steckler
- 10 **Effectiveness of two topical antimicrobial agents used in the treatment of contagious equine metritis in stallions** 16
B Keys, ML Schulman, K May, C Joone, M Monyai, J Marais, D Mpofo, AJ Guthrie

13:00 – 13:45 Lunch
 13:50 – 15:05 Third Session (75 min)

SESSION CHAIRPERSON: Dr Sarah Cliff

- | | | |
|-----------|--|-----------|
| 11 | Platelet indices in virulent canine babesiosis and their association with outcome | 17 |
| | <i>A Goddard, AT Kristensen, AL Leisewitz, PN Thompson, JP Schoeman</i> | |
| 12 | Evaluation of haemostatic abnormalities in naturally occurring canine spirocercosis | 18 |
| | <i>P Pazzi, A Goddard, AT Kristensen, E Dvir</i> | |
| 13 | Aflatoxicosis causing a hyperfibrinolytic syndrome in 12 dogs | 19 |
| | <i>B Conner, A Goddard</i> | |
| 14 | Effects on coagulation and colloid osmotic pressure of rapid intravenous fluid administration in healthy horses | 20 |
| | <i>E Rioja, L Rubio-Martinez, M Sanz, GT Fosgate, A Goddard</i> | |
| 15 | Use of near-infrared spectroscopy to identify trends in regional cerebral oxygen saturation in horses | 21 |
| | <i>EJ McConnell, MN Saulez, L Bester, GT Fosgate, MG Sanz, E Rioja, RP Raath</i> | |
| 16 | Effects of regional or general anaesthesia on the clinical and pharmacokinetic parameters of amikacin administered by intravenous regional limb perfusion in horses | 22 |
| | <i>AT Mahne, LM Rubio-Martinez, HJ Marais, N Villarino, E Rioja</i> | |

15:05 – 15:15 Break
 15:20 – 16:20 Fourth Session (60 min)

SESSION CHAIRPERSON: Dr Martina Crole

- | | | |
|-----------|---|-----------|
| 17 | The prevalence of severe combined immunodeficiency, lavender foal syndrome and cerebellar abiotrophy in Arabian horses in South Africa | 23 |
| | <i>CJ Tarr, CK Harper, AJ Guthrie, PN Thompson</i> | |
| 18 | A study on bovine tuberculosis and associated risk factors in humans in Swaziland | 24 |
| | <i>MEM Dlamini, AL Michel</i> | |
| 19 | The brucellin skin test: is it of any value in investigating bovine brucellosis in South Africa? | 25 |
| | <i>N Nyanhongo, M Hansen, A Storm, AL Michel</i> | |
| 20 | Comparative studies on immunogenicity and protective efficacy of a live spore <i>Bacillus anthracis</i> vaccine versus recombinant peptide vaccine candidates in goats | 26 |
| | <i>OC Ndumnego, S Koehler, J Crafford, W Beyer, H van Heerden</i> | |
| 21 | An investigation of the cause of enteritis in ostrich (<i>Struthio camelus</i>) chicks in the Western Cape Province, South Africa | 27 |
| | <i>L Keokilwe, A Olivier, WP Burger, H Venter, D Morar</i> | |

16:20 – 16:30 Break
 16:30 – 17:30 Fifth Session (60 min)

SESSION CHAIRPERSON: Dr Brighton Dzikiti

- | | | |
|-----------|--|-----------|
| 22 | Characterisation of the metabolic acid-base disturbances in canine parvoviral enteritis using the strong ion approach | 28 |
| | <i>RK Burchell, JP Schoeman, HA Demorais, AL Leisewitz</i> | |
| 23 | A comparison of transthoracic echocardiographic measurements to allometric scaling derived values in clinically normal adult dachshunds | 29 |
| | <i>CK Lim, RM Kirberger, GT Fosgate</i> | |
| 24 | An <i>in vitro</i> biomechanical comparison between intramedullary pinning and the use of plates in the dachshund tibia | 30 |
| | <i>F Malan, GL Coetzee, NDL Burger, J Grimbeek, PN Thompson, A Carstens</i> | |
| 25 | Analgesic effects of epidural magnesium sulphate alone and in combination with morphine in dogs | 31 |
| | <i>A Bahrenberg, E Rioja, TB Dzikiti, GF Stegmann, GT Fosgate</i> | |
| 26 | Anaesthetic, analgesic and cardiorespiratory effects of intramuscular medetomidine and ketamine alone or in combination with morphine or tramadol for orchiectomy in cats | 32 |
| | <i>GE Zeiler, E Rioja, GF Stegmann, GT Fosgate, FJ Venter, TB Dzikiti</i> | |

17:45

COCKTAIL



POSTER PRESENTATIONS

P1	<p>The value of the Onderstepoort Feedlot and AI challenges as educational tools</p> <p><i>DE Holm, L Strydom, M van Aarde, CA Poggenpoel, SG Beukes, D Bester</i></p>	33
P2	<p>The role of the centriolar complex in the formation of sperm defects in the emu (<i>Dromaius novaehollandiae</i>)</p> <p><i>L du Plessis, JT Soley</i></p>	34
P3	<p>Steroid profiling in crocodilian urine by gas-chromatography/mass spectrometry</p> <p><i>LC Bekker, JG Myburgh, LG Guilliette, CJ Botha</i></p>	35
P4	<p>Non-invasive monitoring of stress in Nile crocodiles: validation of a faecal glucocorticoid assay</p> <p><i>SB Ganswindt, A Ganswindt, EZ Cameron, JG Myburgh</i></p>	36
P5	<p>Urban habitat moderates seasonality in the stress physiology, movement ecology and foraging behaviour of free-ranging banded mongooses</p> <p><i>P Laver, A Ganswindt, SB Ganswindt, M Williams, K Alexander</i></p>	37
P6	<p>Facing the challenge: rewilding South China tigers (<i>Panthera tigris amoyensis</i>) in South Africa</p> <p><i>MC Fàbregas, HJ Bertschinger, G Koehler</i></p>	38
P7	<p>The postpartum endometrial inflammatory response: a normal physiological event with potential implications for bovine fertility</p> <p><i>A Chapwanya, KG Meade, C Foley, F Narciandi, ACO Evans, ML Doherty, JJ Callanan, C O'Farrelly</i></p>	39
P8	<p>Endometrial epithelial cells: sentinels of innate immunity and potent producers of tumour necrosis factor, tracheal antimicrobial peptide and serum amyloid A3 gene expression in response to <i>E. coli</i> stimulation</p> <p><i>A Chapwanya, KG Meade, ML Doherty, JJ Callanan, C O'Farrelly</i></p>	40
P9	<p>Global endometrial transcriptomic profiling during involution: transient immune activation precedes tissue proliferation and repair in postpartum healthy beef cows</p> <p><i>C Foley, A Chapwanya, C Creevey, F Narciandi, D Morris, E Kenny, P Cormican, JJ Callanan, C O'Farrelly, KG Meade</i></p>	41
P10	<p>The survival of eight different mastitis pathogens in milk after being frozen at -196°C and -20°C for 18 and 30 weeks respectively</p> <p><i>JC Watermeyer, IM Petzer, J Karzis, TJ van der Schans</i></p>	42
P11	<p>Antibacterial sensitivities of the primary respiratory bacterial pathogens in the bovine respiratory disease complex in feedlot cattle in South Africa</p> <p><i>CAP Carrington, GH Rautenbach, JA Picard, PN Thompson</i></p>	43
P12	<p>Bacterial isolations in bovine respiratory disease complex in feedlot cattle in South Africa</p> <p><i>CAP Carrington, GH Rautenbach, JA Picard, PN Thompson</i></p>	44
P13	<p>The role of <i>Mycoplasma spp.</i> in bovine respiratory disease complex in feedlot cattle in South Africa</p> <p><i>CAP Carrington, GH Rautenbach, JA Picard, PN Thompson</i></p>	45



P14	Evaluation of medicinal turpentine used for the prevention of bovine babesiosis in southern KwaZulu-Natal and the eastern Free State	46
	<i>LJ Biggs, CAP Carrington, V Naidoo</i>	
P15	Cytotoxicity and mutagenicity investigation of extracts of common South African ethnoveterinary plants	47
	<i>LJ McGaw, EE Elgorashi, JN Eloff</i>	
P16	<i>Mycobacterium tuberculosis</i> complex specific antigens for use in serodiagnosis	48
	<i>BM Modise, J Fehrsen, AL Michel</i>	
P17	The occurrence of non-tuberculous mycobacteria (NTM) in natural habitats of cattle and African buffalo in South Africa and immunologic reactivity caused in these species by natural NTM exposure (preliminary results)	49
	<i>N Gcebe, VPMG Rutten, N Gey van Pittius, AL Michel</i>	
P18	Evidence of TH1/Th17 immune responsiveness and cross reactivity to PPD-F in mice sensitised with atypical mycobacteria	50
	<i>AO Jenkins, AL Michel, VPMG Rutten</i>	
P19	Occurrence of tick-borne haemoparasites in the African buffalo (<i>Syncerus caffer</i>) in northern Botswana	51
	<i>D Eygelaar, F Jori, M Mokopasetso, EM Debeila, NE Collins, I Vorster, M Troskie, MC Oosthuizen</i>	
P20	Molecular phylogeny of novel <i>T. buffeli</i>-like and <i>T. sinensis</i>-like genotypes of the African buffalo (<i>Syncerus caffer</i>) based on their 18S rRNA gene and internal transcribed spacer sequences	52
	<i>ME Chaisi, L He, NE Collins, MC Oosthuizen</i>	
P21	Coagulation abnormalities in dogs undergoing elective and traumatic orthopaedic surgery	53
	<i>E Rioja, A Bahrenberg, B Conner, PN Thompson, A Goddard</i>	
P22	Thromboelastographic evaluation of haemostatic function in dogs with natural envenomation by South African snakes	54
	<i>SS Nagel, A Goddard, B Wiinberg, JP Schoeman</i>	
P23	The effects of repeated intravenous iohexol administration on renal function in healthy beagles	55
	<i>N Cassel, RM Kirberger, A Goddard, A Carstens</i>	
P24	The effects of hydroxyethyl starch 6% 130/0.4 on thromboelastography in healthy horses	56
	<i>A Viljoen, MN Saulez, PC Page, GT Fosgate</i>	
P25	Evaluation of serum amyloid A, haptoglobin, nucleated cell count, total protein and haemolysis in peritoneal fluid for differentiation of medical and surgical colic in horses	57
	<i>E Scheepers, T Holberg Pihl, M Sanz, P Page, A Goddard, S Jacobsen</i>	
P26	Post mortem survey of equine dental disorders	58
	<i>DC Vemming, G Steenkamp, A Carstens, P Page</i>	
P27	Overground endoscopy for diagnosis of upper airway abnormalities in thoroughbred racehorses in South Africa	59
	<i>J Mirazo, P Page, L Rubio-Martinez, J Marais, C Lyle</i>	

Message from the Dean

The Faculty aspires to be widely recognised for the quality of its staff and graduates, unique postgraduate opportunities, and innovative research that makes an impact.

Our new Faculty Plan for 2013–2017 that is fully aligned with the strategic plan of the University of Pretoria is unequivocal in specifying objectives and clear directives for the next five years and beyond, contributing to becoming a research-intensive University.

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.

Our research output per individual academic member remains one of the highest at the University. The number of publications in 2010 resulted in 83,74 subsidy units, with virtually all the publications in the higher category of ISI-accredited journals, emphasising the high quality of the research. Furthermore, the number of NRF-rated staff members increased from 19 in 2010 to 23 in 2011.

For the past 27 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. During this period, Faculty Day has undoubtedly contributed enormously to establishing a culture of research within the Faculty.

Having said that, we have to continuously revisit and evaluate our strategic goals to determine if our activities – and this also pertains to our research programmes – and that of the veterinary profession in general are aligned with what our country needs.

As a leading Faculty and keeping our local and global responsibilities in mind, we will always be faced with new challenges and new mind-sets that will have to be integrated in our thought processes in order to grow as a leading and renowned veterinary institution. It is thus of crucial importance that the Faculty continues to increase its research outputs and ensure that these are locally relevant and keep pace with research worldwide.

It will also require professionals and experts to work together in multidisciplinary teams and institutions within the University, across continents and over boundaries, ultimately emphasising the One Health approach. The Faculty can play a pioneering role in this regard and is already engaged in various initiatives such as the Institutional Research Themes and discussions to



Prof Gerry Swan, Dean

find new collaborators and partners to enhance high-quality research and relevant postgraduate training. This should increasingly be part of our focus beyond 2012.

In future planning, the Faculty also has to take into account the possible influences on the future provision of veterinary services, including changes in the environment of veterinary science. These include changes in society through major demographic, political and environmental developments as well as technological, economic influences and becoming socially engaged.

Global animal and public health issues will increasingly be integrated into veterinary education. Therefore, we must ensure that 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.

It is my pleasure to welcome you to Faculty Day 2012. The Sir Arnold Theiler Memorial Lecture reflects the spirit of Faculty Day and this year we are also pleased to welcome Prof N James MacLachlan, an esteemed veterinary scholar and researcher in our midst who will be delivering the Arnold Theiler Memorial Lecture. Prof MacLachlan is Distinguished Professor in the Department of Pathology, Microbiology and Immunology in the School of Veterinary Medicine of the University of California and Extraordinary Professor in our own Department of Veterinary Tropical Diseases. We are looking forward to being enlightened by Prof MacLachlan on his thought-provoking lecture title *Emerging viral diseases; the example of bluetongue, from Theiler to climate change*, a focus on what we as a global community might expect in the future in terms of the emergence of vector-borne diseases driven by climate change.

We thank the Faculty Day Organising Committee for its hard work and dedication in organising this event. Congratulations also to the 2012 teaching and research award winners. May Faculty Day 2012 once again serve to further stimulate the Faculty's pursuit for excellence, distinction, innovation and creativity in giving effect to the vital role this Faculty has to play locally and internationally.



Curriculum Vitae: Prof N James MacLachlan

N James MacLachlan is Distinguished Professor in the Department of Pathology, Microbiology and Immunology, School of Veterinary Medicine, University of California and Extraordinary Professor, Department of Veterinary Tropical Diseases, Faculty of Veterinary Science, University of Pretoria.



Prof N James MacLachlan

He received his veterinary degree (BVSc) from Massey University, New Zealand in 1976, an MS in Microbiology (Veterinary Virology) from the University of Missouri in 1979, and a PhD in Comparative Pathology from the University of California in 1983. Prof MacLachlan is a Diplomate and past-President of the American College of Veterinary Pathologists (ACVP), and he served for 10 years as inaugural Chair of his home department at UC Davis.

Prof MacLachlan studies viral diseases of livestock that impact international commerce, including bluetongue, African horse sickness and other emerging diseases, and he is author or co-author of some 250 peer-reviewed publications, reviews, chapters, and books. Prof MacLachlan has served as an expert advisor to numerous organizations including the World Organization for Animal Health (OIE), the United States Departments of Agriculture and Homeland Security, and the European Union (EU).

Some of his awards include the Norden Distinguished Teaching Award (1993), the Pfizer Award for Research Excellence (2003), the Sophomore Class Teaching Award (1993, 2003, 2006 and 2008) and the Alumni Achievement Award (2007), all from the School of Veterinary Medicine, University of California. Among other responsibilities, he currently chairs the United States Animal Health Association Committee on Bluetongue and Related Orbiviruses and serves as co-editor-in-chief of *Comparative Immunology Microbiology of Infectious Diseases*.

Sir Arnold Theiler Memorial Lecture

Emerging viral diseases; the example of bluetongue, from Theiler to climate change

Prof N James MacLachlan

History is replete with examples of new diseases that have appeared, or known ones that have reappeared, often with devastating consequences to animal and/or human populations. The challenge has been to identify the anthropogenic and environmental drivers responsible for these disease events, a challenge that has become increasingly complicated in an era of rapid increases in human populations, blurring of the urban/rural interface, international travel, and alterations in the global climate. It has been widely proposed that recent changes in the earth's climate will especially favour the emergence and spread of insect-transmitted (vector-borne) diseases, but definitive evidence of such events is surprisingly lacking. Dramatic recent changes in the global distribution of bluetongue, an insect-transmitted disease of livestock first described in South Africa over a century ago, offer perhaps the best current example of what we as a global community might expect in the future in terms of the emergence of vector-borne diseases driven by climate change.

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	"Important Research Requirements for Future Animal Production-Orientated Research with Particular Reference to Veterinary Science"
1989:	Dr R Swanepoel	*
1990:	Dr A Schutte	"The Impact of Controlled Breeding on the Cattle Industry in Southern Africa"
1991:	Prof DM Joubert	*
1992:	Dr CM Cameron	"The Environment – Whose Responsibility?"
1993:		Opening of the Onderstepoort Veterinary Academic Hospital – No lecture
1994:	Dr W Plowright	"Rinderpest and Cell-Culture Revolution"
1995:	Prof WL Jenkins	*
1996:	Prof PV Tobias	"Premature Discoveries in Science"
1997:	Prof DL Block	"Our Universe: Accident or Design?"
1998:	*	*
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"
2010:	Dr R Moerane	"The Role of the Veterinary Profession in the Current Developmental Agenda in South Africa"
2011:		World Veterinary Congress in SA – no Faculty Day

** We do apologise that the above list is not complete. It would 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: 2011

The research programme of the Faculty showed steady growth compared to the previous year. The number of subsidy units earned from the Department of Higher Education and Training for publications in 2010 increased from 82.00 in the previous year to 83.74, translating to a total research budget of R1 385 000 for 2011.

Noteworthy is the fact that all the Faculty's publications were, as in the past, in the higher category of ISI-accredited journals. According to the Faculty's policy, 50% of the subsidy is allocated to the authors of each publication, 30% to the responsible department and 20% to the Faculty Research Fund, which is used by the Research Committee to support young or new researchers who have not yet established themselves for external funding purposes. A total amount of R277 000 was therefore available for this purpose. There was also a substantial increase in the funding available for postgraduate bursaries to R522 000, which sufficed for the allocation of 10 PhD and 17 MSc bursaries.

The number of NRF-rated staff members increased from 19 in 2010 to 23 in 2011. The increase over the past three years amounts to 53%, illustrating a gratifying growth in both research capacity and in awareness of the importance of peer evaluation. Excellence in research performance was again recognised by the identification of the Faculty's Top 10 researchers and the allocation of the following research awards:

Outstanding Scientific Achiever;

- Prof IG Horak

Researcher of the Year (shared)

- Prof PN Thompson
- Dr MC Oosthuizen

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

- Prof V Naidoo
- Prof BL Penzhorn
- Prof MN Saulez
- Prof CJ Botha
- Prof AL Leisewitz
- Prof M van Vuuren
- Prof M-CN Madekurozwa
- Prof AL Michel

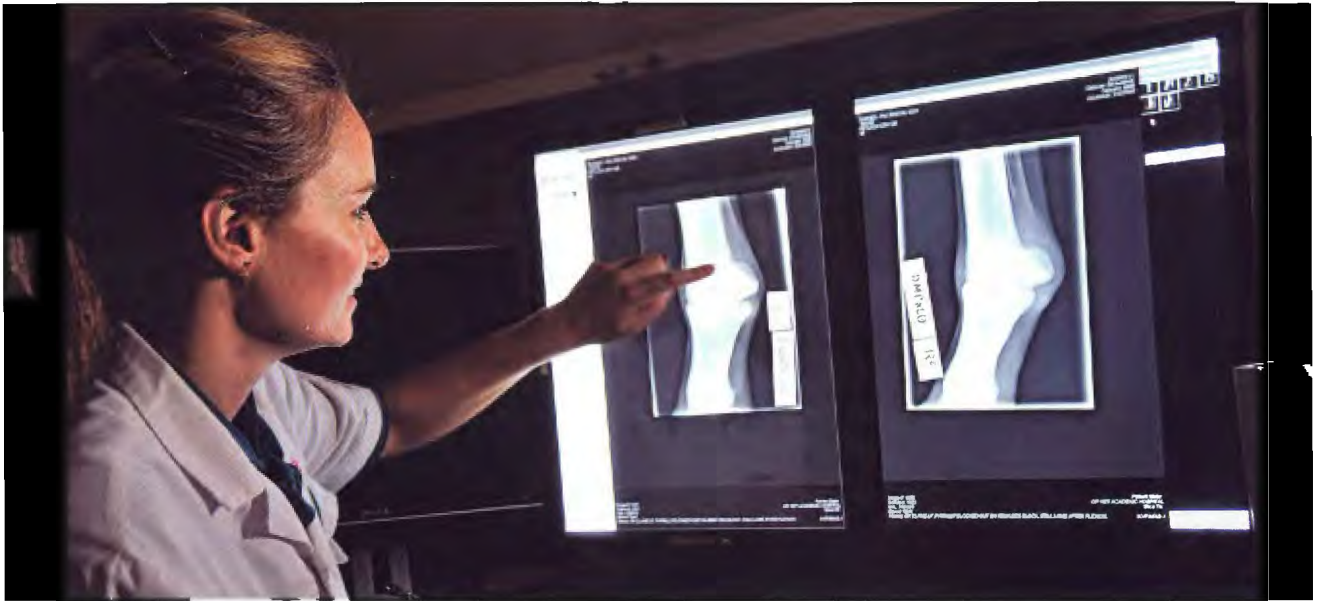
Young Researcher of the Year

- Dr MR Crole
- Runner up: Dr TB Dzikiti



Research Summary: 2011 (continued)

Individual research highlights



A few individual research highlights can be mentioned.

The establishment of a rhino DNA database by the Veterinary Genetics Laboratory under the guidance of Dr Cindy Harper attracted considerable public interest and fortunately also substantial financial support from the private sector. The project consists of the collection of blood samples from as many of the rhinos in southern Africa as possible, followed by DNA sequence determination, which enable the researchers to establish a genetic profile of each animal. These profiles allow them to identify any rhino horn, seized by the police, in terms of its origin and the poaching incident involved. This information is essential for the successful prosecution of poachers and the fight against organised smuggling syndicates. It is expected that the existence of the rhino DNA profile database will play an invaluable role in solving the problem of rhino poaching.

External funding was obtained by Prof Nöthling for another highly relevant project in terms of public interest, namely the potential effects of water polluted by mines on the reproductive processes of wildlife in the Krugersdorp Game Reserve. It is based on the well-known fact that the female ovary is uniquely suited to identify past exposure to pollutants and the male testis to ongoing and current exposure. It is therefore potentially an extremely useful approach to addressing the problem of protecting the environment and wildlife by identifying the hazards caused by exposure to pollutants.

The Phytomedicine Programme has been working on the quality control of herbal medicines for many years. This has resulted in the group obtaining funding of more than R1.8million from several European Union agencies to manage a project on the quality control of the most important African herbal medicines and the establishment of the Association for African Medicinal Plants Standards. Under-representation of African herbal medicines used in Europe, is probably due to the oral transfer of knowledge in the African system compared to the written communication in the Indian and Chinese systems.

This project, managed by Prof Kobus Eloff, culminated in the publication of the *African Herbal Pharmacopoeia* with monographs containing all the current knowledge about the 51 most important African herbal medicines. The programme also celebrated its 100th publication under the auspices of the Veterinary Faculty during 2011.

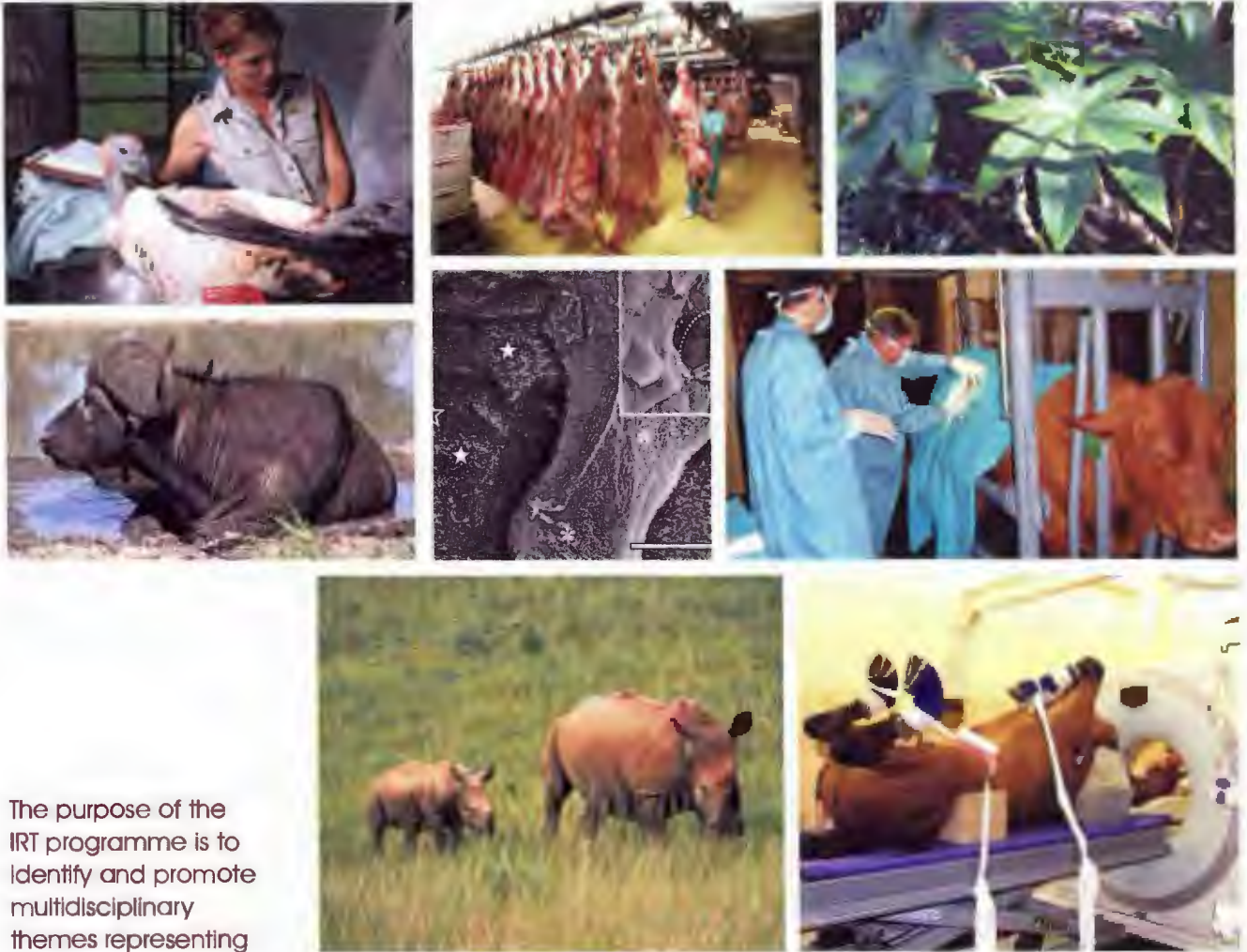
Lumpy skin disease is a viral disease of considerable importance in cattle. The mechanism of its transmission is still largely unknown despite various studies aimed at solving the mystery. Contact and respiratory infection, similar to the situation with other members of the pox virus family, was shown to be inefficient and transmission by flying insects could not be demonstrated. Researchers from the Department of Veterinary Tropical Diseases, in collaboration with the Institute for Animal Health in Pirbright, UK, demonstrated for the first time that ticks in the Ixodes group can transmit the disease. A viraemia in infected animals could be demonstrated by virus isolation and by PCR. The latter technique also detected trans-stadial and trans-ovarial transmission of the virus in the ticks.

The African buffalo plays an important role in the epidemiology of several important livestock diseases including Corridor disease, foot-and-mouth disease, bovine brucellosis and bovine tuberculosis in South Africa and the region. Recent molecular studies in partnership with staff at the Institute of Tropical Medicine, Antwerp, Belgium showed that there is significant genetic diversity in the *Theileria* species that may complicate the diagnosis of *Theileria parva* infection in buffalo and cattle in South Africa and the region. These studies also contributed towards the development of improved molecular diagnostic assays for the detection of *T. parva* and other *Theileria* species in livestock and wildlife.

Considerable progress was made in the field of developing diagnostics tools for tuberculosis in wildlife such as the interferon gamma assay for rhinoceroses. A lion-specific interferon gamma assay was also developed in close collaboration with researchers at Utrecht University, The Netherlands.

Research Summary: 2011 (continued)

Institutional Research Themes (IRTs)



The purpose of the IRT programme is to identify and promote multidisciplinary themes representing areas of expertise existing at UP

which could be developed into internationally recognised niche areas of research. An important event during 2011 was the approval of second-round IRT proposals by UP. The Faculty of Veterinary Science is involved in three of these:

1. Biotechnology and the control of animal and zoonotic diseases
2. Genomics
3. Food and Nutritional Wellbeing

The IRT *Biotechnology and the management of animal and zoonotic diseases*, in which the Faculty will play a pivotal role together with Health Sciences and Natural and Agricultural Sciences, will combine and coordinate the expertise built up over decades in these faculties in applying molecular technologies to combat animal diseases, with an emphasis on those transmissible to man. Specifically relevant to the Faculty's contribution will be molecular studies utilising biotechnology for the development of improved diagnostic techniques and vaccines for animal diseases and for the study of their pathogenesis.

The development of a *Genomics* IRT with a strong core capacity for genomics, bioinformatics and computational biology and recognised as a Centre of Excellence could make an important contribution to Veterinary Science, Health Sciences, and other Life Sciences in South Africa, Africa and the rest of the world. At present this faculty's main contribution will be the establishment of a genetic profile database of our rhino population. Population genetic studies on other animals are also in the pipeline

A new Institute for Food, Nutrition and Wellbeing was recently established at the University with Prof Sheryl Hendriks as the new Director in support of the Institutional Research Theme (IRT) *Food, Nutrition and Wellbeing*. It seeks to address the emerging societal challenges related to food insecurity, nutrition deficiencies and hunger, especially in Africa and to bring together all the University's postgraduate teaching and transdisciplinary research expertise and activities in this field in order to strategically position UP as an internationally recognised Centre of Excellence. The Faculty of Veterinary Science will contribute to research on sustainable animal food production and on food safety.

Research Programme: Oral Presentations

Detection of lumpy skin disease virus in saliva of potentially infected *Amblyomma hebraeum* and *Rhipicephalus appendiculatus* ticks

JC Lubinga¹, E Tuppurainen², WH Stoltz¹, JAW Coetzer¹, EH Venter¹

¹Department of Veterinary Tropical Diseases, Faculty of Veterinary Science, University of Pretoria, Onderstepoort, South Africa; ²Institute of Animal Health, Pirbright, Surrey, UK; jimlubinga@yahoo.com

Lumpy skin disease (LSD) is an economically important disease of cattle occurring across Africa and the Middle East. It is caused by LSD virus (LSDV), a member of the genus *Capripoxvirus*, within the family *Poxviridae*. Transmission of the virus has been associated with blood-feeding flies such as mosquitoes (*Aedes aegypti*) and stable flies (*Stomoxys calcitrans*). Recent studies by the authors have assessed the vector potential of *Ixodid* (hard) ticks in the transmission of LSDV and have demonstrated mechanical and transstadial transmission by *Rhipicephalus appendiculatus* as well as mechanical, transstadial and transovarial transmission by *Amblyomma hebraeum* in cattle. This study demonstrates the presence of LSDV in saliva of adult *R. appendiculatus* and *A. hebraeum* ticks that were fed, as nymphs or adults, on artificially infected donor cattle.

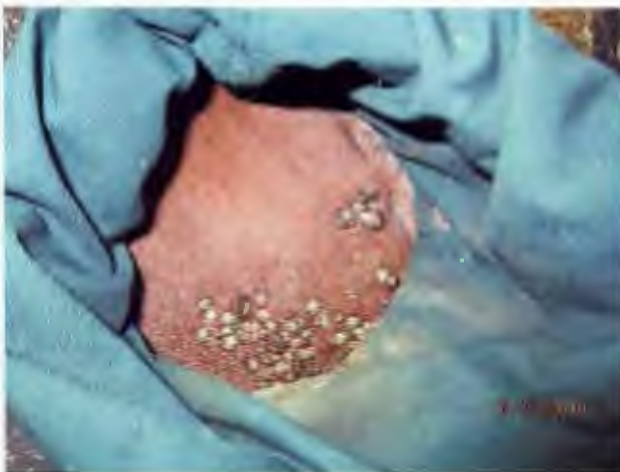
Fourteen *A. hebraeum* and 14 *R. appendiculatus* adult ticks that were partially fed on LSDV-infected donor cattle, were induced to salivate by adding 5 µl pilocarpine hydrochloride to their scutum or conscutum and the saliva was collected. Similarly, saliva was collected from 60 *A. hebraeum* and 85 *R. appendiculatus* adults that were potentially infected by feeding on donor animals as nymphs. The presence of LSDV in the saliva samples was detected by real-time PCR or observation of cytopathic effect (CPE) on cell cultures. Cell culture supernatants were further tested by real-time PCR to confirm that LSDV was responsible for the CPE.

The virus was detected in all groups of saliva samples by real-time PCR. Typical CPE was seen after the third cell culture passage. Real-time PCR of supernatants confirmed that CPE was due to LSDV. Mechanical or intrastadial and transstadial passage of LSDV in *A. hebraeum* and *R. appendiculatus* ticks were demonstrated.

This is the first demonstration of LSDV in tick saliva. These findings confirm that ticks can transmit the virus along with saliva by parenteral inoculation into the host, during the process of obtaining a blood meal from a host.

The demonstration of transstadial passage of the virus in ticks suggests the possible persistence of the virus in ticks between seasons, especially over winter, indicating the potential role of ticks as reservoir or maintenance hosts of LSDV between outbreaks. Mechanical or intrastadial transmission of LSDV by ticks, following interrupted feeding, may also contribute to the spread of infection during an outbreak. The presence of LSDV in tick saliva also indicates the possibility of saliva assisted transmission of LSDV to clean co-feeding ticks. In addition, successful tick transmission of LSDV to cattle may cause a viraemia that may subsequently be mechanically transmitted by other haematophagous insects.

"This is the first demonstration of LSDV in tick saliva. These findings confirm that ticks can transmit the virus..."

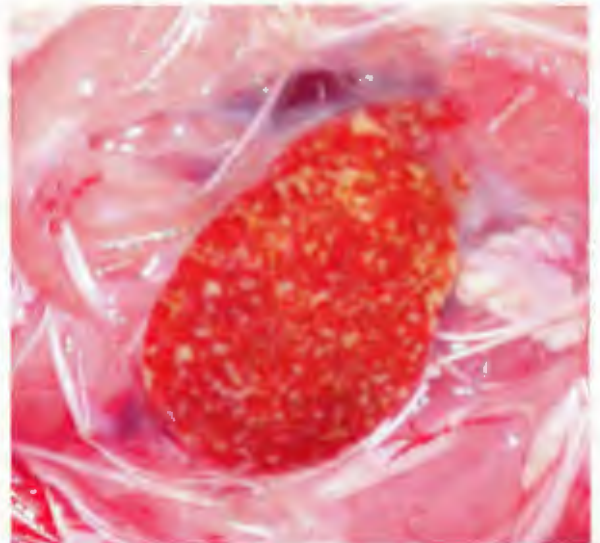


Molecular characterisation of the fowl adenovirus isolates involved in an outbreak of inclusion body hepatitis in South Africa

HW Joubert¹, H Aitchison², EH Venter³, LH Maartens¹

¹Research and Development Department, Deltamune, Centurion, South Africa; ²Consulting Veterinary Services, Avimune, Centurion, South Africa;

³Department of Veterinary Tropical Diseases, Faculty of Veterinary Science, University of Pretoria, Onderstepoort, South Africa; hilda@Deltamune.co.za



Except for a few incidental isolations of avirulent fowl adenovirus (FAdV) serotype 1, also known as chicken embryo lethal orphan (CELO) virus, no previous reports of virulent FAdV in production birds have been made in South Africa.

A sudden increase in mortality rate (20%) in a young (2-7 week old), local meat-producing breeder broiler flock, characteristic of FAdV associated inclusion body hepatitis (IBH), necessitated further investigation. An acute hepatitis with extensive necrosis and prominent intra-nuclear viral inclusions in hepatocytes was observed. This study was undertaken to characterise the agents implicated in the mortality associated with the acute hepatitis.

Liver samples collected during autopsy from broilers showing increased mortality during the period 13 to 25 days of age showed clear macroscopic lesions suggestive of IBH, such as hepatomegaly, hepatic necrosis with or without haemorrhage, an irregular surface and friable consistency of the liver. Histological examination of liver, spleen and kidney tissues from the embryos used for virus isolation yielded valuable information on the aetiology of the isolates. The large, basophilic, intra-nuclear inclusions in hepatocytes and renal tubular epithelium that were observed in a number of affected embryos were highly characteristic of FAdV infection.

Fowl adenovirus serotypes were successfully isolated in embryonated chicken eggs inoculated by the intravascular

route. PCR and sequencing results confirmed the presence of FAdV in livers of embryos that were enlarged and mottled with small, alternating, dull grey, yellow-brown and reddish-brown foci (hepatic necrosis). Some livers showed a diffuse greenish discoloration (bile stasis) with scattered, irregular, grey foci that coalesced in some areas. In embryos that died autolysed livers were small and yellow with faint, white stippling barely visible.

Restriction fragment length polymorphism (RFLP) of the PCR product, obtained from the L1 hexon loop using primer set hexon A/B clearly distinguished two serotypes, D2 and E8, previously described in literature. Sequencing of the PCR products confirmed the results obtained with RFLP.

Phylogenetic analysis was performed on the 300 amino acids, encompassing the L1 loop of the hexon protein which corresponded to residues 49 to 297 of the hexon protein of the reference strain CELO. For phylogenetic analysis the L1 loop of the hexon proteins of all FAdV serotype reference strains were included.

Restriction fragment length polymorphism and phylogenetic distance determination for FAdV showed that the two serotypes, D2 and E8, involved in the outbreak were probably introduced into the country as the isolates are closely related to, if not the same as, the European and USA strains.

"A sudden increase in mortality rate (20%) in a young (2-7 week old), local meat-producing breeder broiler flock, characteristic of FAdV associated inclusion body hepatitis (IBH), necessitated further investigation."

An African horse sickness virus serotype 4 recombinant canarypox virus vaccine elicits specific cell-mediated immune responses in horses

H El Garch¹, JE Crafford⁵, P Amouyal¹, PY Durand¹, C Edlund Toulemonde², L Lemaître², V Cozette³, AJ Guthrie⁴, JM Minke¹

¹Merial SAS, R&D, Lyon, France; ²Merial SAS, CRSV, Lyon, France; ³Merial SAS, Biostatistics Dept, Lyon, France; ⁴Equine Research Centre, and ⁵Department of Veterinary Tropical Diseases, Faculty of Veterinary Science, University of Pretoria, Onderstepoort, South Africa; jannie.crafford@up.ac.za

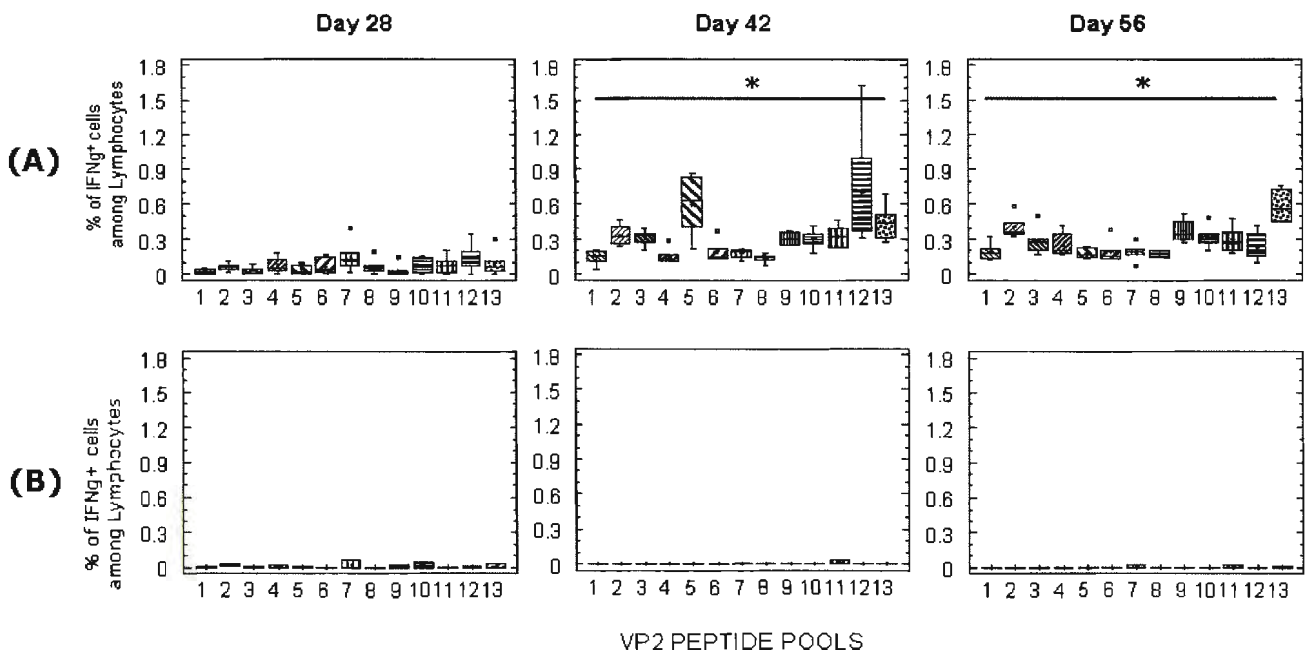
A recombinant canarypox virus vectored vaccine co-expressing synthetic genes encoding outer capsid proteins, VP2 and VP5, of African horse sickness virus (AHSV) serotype 4 (ALVAC®-AHSV4) has been demonstrated to fully protect horses against homologous challenge with virulent field virus. Guthrie et al.¹ detected weak and variable titres of neutralizing antibody (ranging from <10 to 40) 8 weeks after vaccination leading us to hypothesize that there could be a participation of cell mediated immunity (CMI) in protection against AHSV4. This study aimed at characterizing the CMI induced by the experimental ALVAC®-AHSV4 vaccine. Six horses received two vaccine injections 28 days apart and three horses remained unvaccinated. Horses were vaccinated on day 0 and day 28. On days 28, 42 and 56, freshly isolated PBMC were ex-vivo stimulated by 13 overlapping VP2 peptide pools over 12 hours. Brefeldin A was added after 2-hour stimulation in order to block cytokine secretion. The detection of VP2/VP5 specific IFN- γ responses was assessed by enzyme linked immune spot (ELISpot) assay



“...vaccinated horses developed significant IFN- γ production compared to unvaccinated horses.”

and clearly demonstrated that all ALVAC®-AHSV4 vaccinated horses developed significant IFN- γ production compared to unvaccinated horses. More detailed immune responses obtained by flow cytometry demonstrated that ALVAC®-AHSV4 vaccinations induced immune cells, mainly CD8⁺ T cells, able to recognize multiple T-epitopes through all VP2 and only the N-terminus sequence of VP5. Neither VP2 nor VP5 specific IFN- γ responses were detected in unvaccinated horses. Overall, our data demonstrated that an experimental recombinant canarypox based vaccine induced significant CMI specific for both VP2 and VP5 proteins of AHSV4.

¹ Guthrie AJ, Quan, M, Lourens, CW, Audonnet, JC, Minke, JM, Yao, J, He, L, Nordgren, R, Gardner, IA, Maclachlan, NJ 2009 Protective immunization of horses with a recombinant canarypox virus vectored vaccine co-expressing genes encoding the outer capsid proteins of African horse sickness virus. *Vaccine* 27: 4434-4438.



Box plots depicting specific CD8 α ⁺ IFN- γ ⁺ (A) and CD8 α ⁻ IFN- γ ⁺ (B) secreting cells among lymphocytes of six ALVAC®-AHSV4 vaccinated horses. Top and bottom borders of the box represent the 90th and 10th percentiles respectively; the solid bar indicates the median and outliers are illustrated as dots.

Efficacy of alpha-cypermethrin treated mesh against *Culicoides* biting midges

PC Page¹, K Labuschagne², GJ Venter², JP Schoeman¹, AJ Guthrie³

¹Department of Companion Animal Clinical Studies, Faculty of Veterinary Science, University of Pretoria, Onderstepoort, South Africa; ²Agricultural Research Council - Onderstepoort Veterinary Institute, Onderstepoort, South Africa; ³Equine Research Centre, Faculty of Veterinary Science, University of Pretoria, Onderstepoort, South Africa; patrick.page@up.ac.za

Culicoides biting midges (Diptera: Ceratopogonidae) have been implicated as vectors of African horse sickness virus and equine encephalosis virus, and play an important role in equine insect bite hypersensitivity. Recommended control measures for African horse sickness include vaccination, stabling at night, screening of stables with mesh and the use of repellents or insecticides. The efficacy of repellent impregnated mesh applied to light traps against *Culicoides* as well as the insecticidal effect of hair from horses treated with cypermethrin has been reported. The objectives of this study were to determine the field efficacy of alpha-cypermethrin treated high density polyethylene (HDPE) mesh applied to light traps against *Culicoides* and the insecticidal efficacy of the mesh against *Culicoides imicola*.

Two randomized, controlled, observer-blinded experiments were conducted. In Experiment 1 the efficacy of HDPE mesh (Alnet, South Africa) treated with alpha-cypermethrin (Fendona[®]6, BASF, Switzerland), untreated HDPE mesh, and control polyester mesh (standard Onderstepoort light trap mesh, ARC-OVI, South Africa) was compared. The meshes were applied to three Onderstepoort UV light traps operated for 12 nights in a Latin square design. The total number of *Culicoides* and *C. imicola* collected per night was analysed.

In Experiment 2 the *in vitro* insecticidal efficacy of meshes against field collected, nulliparous female *C. imicola* was assessed. Midges were exposed in petri dishes to alpha-cypermethrin treated HDPE mesh for a period of 1 or 3

minutes, or untreated control HDPE mesh for a period of 3 minutes. Mortalities in the three groups were assessed at 5, 10, 30, 60 minutes and 24 hours post exposure. Thirty replicates of each exposure period were conducted. Statistical analyses were done using SigmaPlot 12.0 (Systat Software Inc, USA). $P < 0.05$ was considered significant.

The mean (\pm SE) number of *C. imicola* collected with the control light trap ($2,156 \pm 743$) was significantly ($P < 0.05$) higher than the untreated HDPE mesh (523 ± 214) and the alpha-cypermethrin treated HDPE mesh (303 ± 99).

"Alpha-cypermethrin treated HDPE mesh applied to stables or jet stalls could be used to effectively protect horses against *Culicoides*."

Culicoides imicola mortality at the 5, 10, 30, 60 minute and 24 hour time points in the 1 minute and 3 minute alpha-cypermethrin treated HDPE mesh exposure groups was 63.1, 87.7, 94.9, 99.5, 100% and 78.1, 90.2, 99.0, 99.6 and 99.6%, respectively. *Culicoides imicola* mortality was significantly ($P < 0.001$) higher in the two groups exposed to the alpha-cypermethrin treated HDPE mesh compared to the untreated control HDPE mesh at all time points. Mortality was only significantly higher ($P = 0.017$) in the 3 minute alpha-cypermethrin exposure group compared to the 1 minute exposure group at the 5 minute time point.

Alpha-cypermethrin treated HDPE mesh applied to stables or jet stalls could be used to effectively protect horses against *Culicoides*. Further studies on the field efficacy of alpha-cypermethrin treated HDPE mesh applied to stalls housing horses as well as the effect of the mesh on stall ventilation are required.



What prevents ratites from choking?

MR Crole, JT Soley

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

The avian glottis channels air from the oropharynx to the trachea and is situated on an elevated structure, the laryngeal mound. It is imperative that the glottis be protected and closed during swallowing, which in mammals is achieved by covering the glottis with the epiglottis, as well as by adduction of the arytenoid cartilages. An epiglottis, however, is reportedly absent in birds. Ratites such as the ostrich (*Struthio camelus*), emu (*Dromaius novaehollandiae*) and greater rhea (*Rhea americana*) possess a very wide glottis in comparison to other birds. The question therefore arises as to how these large birds avoid inhalation of ingesta through a wide glottis, with apparently little protection, particularly as their feeding method involves throwing the food over the glottis to land in the proximal oesophagus. Despite the recognition of unique lingual features in ratites (for example, the ostrich tongue pocket and emu tongue root), little functional significance has been attributed to these structures, particularly with regard to their possible role in deglutition.

The tongues with attached laryngeal mounds were removed from the heads of five adult ostrich and five adult emu of either sex that had been slaughtered at a commercial abattoir, and from the heads of one sub-adult greater rhea and four greater rhea chicks that had died on a farm. Appropriate manipulations were performed on the fresh specimens aimed at mimicking the postulated movements that occur during swallowing. These manipulations are not possible in formalin-fixed specimens as fixation does not allow for free movement of the structures

involved. Adduction (closure) of the glottis was achieved by using forceps (Figs. 1b, c) or fingers (Fig. 1a) to apply pressure at the base of the arytenoid cartilages. The tongue body was moved caudally by digital manipulation (Fig. 1a) or by using forceps (Fig. 1c).

In the ostrich, when the glottis was adducted and the tongue body retracted, the smooth tongue root became highly folded and the rostral portion of the laryngeal mound was encased by the pocket in the base of the Ω -shaped tongue body (Fig. 1a). In this position the lingual papillae also hooked over the most rostral laryngeal projections (Fig. 1a). However, in the emu, retraction of the tongue body over the closed glottis resulted in the prominent, triangular tongue root sliding over the rostral portion of the laryngeal mound (Fig. 1b). In the greater rhea it was the tongue base which enclosed a small rostral portion of the adducted glottis and laryngeal mound (Fig. 1c). In all three species these actions resulted in the rostral portion of the laryngeal mound (which represented the weakest point of the adducted glottis) being enclosed and stabilised (Fig. 1).

Only after conducting a comparative study between the three ratite species using fresh specimens did it become clear how specific morphological peculiarities were perfectly specialised to assist in the closure and protection of the wide glottis. These results demonstrate that a unique anatomical mechanism, which functionally replaces an epiglottis, exists in ratites: the linguo-laryngeal apparatus.

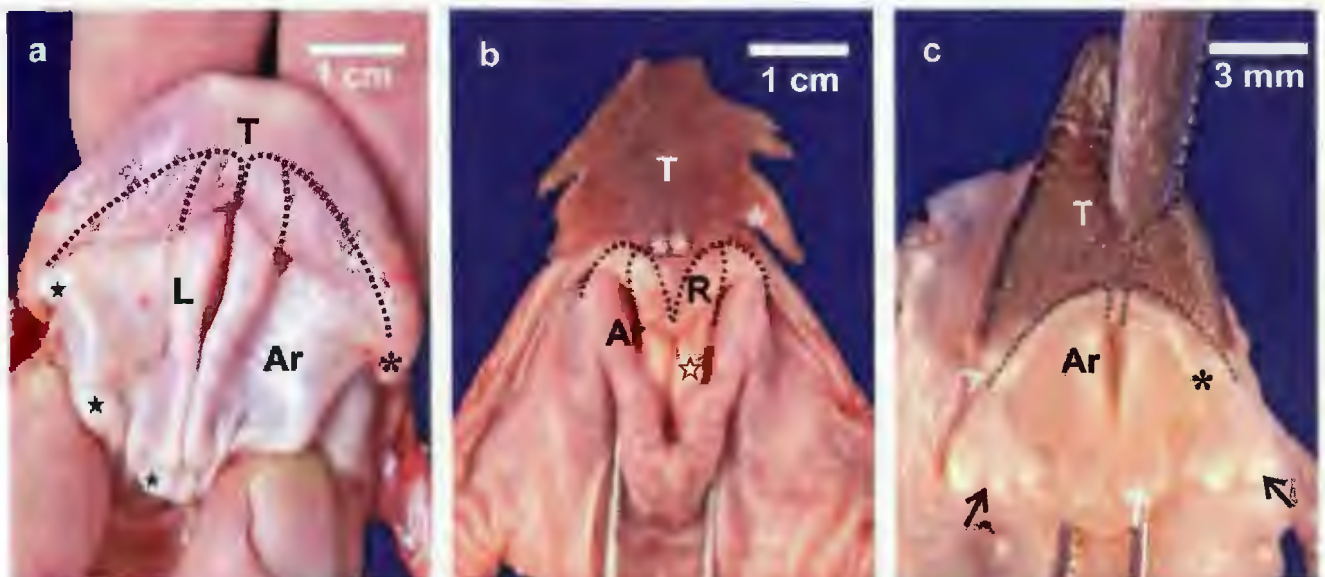


Figure 1. The linguo-laryngeal apparatus of three ratite species with the tongue (T) retracted and the glottis adducted. Arytenoid cartilage (Ar) covered by the mucosa of the laryngeal mound. a) Ostrich. The retracted tongue encases the rostral portion of the laryngeal mound and lips (L) of the glottis (dotted outline). Note how the lingual papillae (*) hook over the first laryngeal projection (black star). b) Emu. The retracted tongue and the tongue root (R) cover the rostral portion of the laryngeal mound and lips (white star) of the glottis (dotted outline). Lateral (*) and caudal (**) lingual papillae. c) Greater rhea. The base of the retracted tongue encloses the rostral portion of the adducted glottis and laryngeal mound. The dorso-medial (*) and ventro-lateral (white dotted arrow) lingual papillae rest on the laryngeal mound. Lateral (black arrow) and medial (white arrow) pharyngeal papillae.

A novel structure with phylogenetic implications found in ratite spermatids

L du Plessis¹, J Soley², J Woodward³, T Sewell³

¹Electron Microscope Unit, and ²Department Anatomy and Physiology, Faculty of Veterinary Science, University of Pretoria, Onderstepoort, South Africa;

³Electron Microscope Unit, University of Cape Town, Cape Town, South Africa; lizette.duplessis@up.ac.za

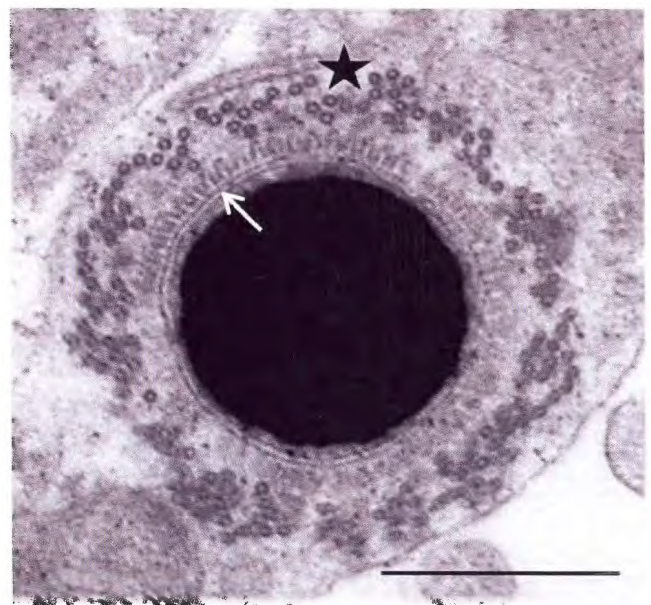
Traditionally, the ratites and tinamous are placed at the base of the avian phylogenetic tree and are considered to be the most primitive living birds. However, the classification of birds and their link to reptiles remains a controversial topic. Whereas molecular and gross morphological studies constitute the accepted methods for constructing phylogenetic trees, the study of spermiogenesis has been acknowledged as an important non-traditional approach to resolving phylogenetic questions. This paper reports the existence of a unique structure that manifests during spermiogenesis in the emu, ostrich and rhea and which appears to support the phylogenetic link between these birds and their sister group, the crocodylians.

The testes of sexually mature and active emus (*Dromaius novaehollandiae*) ($n = 10$) and ostriches (*Struthio camelus*) ($n = 10$) were collected during the breeding season following slaughter at a commercial abattoir. Small blocks of testicular tissue were immediately fixed in 4% phosphate buffered glutaraldehyde and routinely processed for transmission electron microscopy (TEM). Processed, resin embedded testes samples of a rhea (*Rhea americana albisceus*) were kindly supplied by Dr. D.M. Phillips. Standard immunogold labelling for α -, β - and γ -tubulin and actin, as well as electron tomography, was also performed on the emu samples.

TEM of sperm development in all three species revealed the presence of a transient structure not previously described in avian spermatids. This structure displayed a definite uniform symmetry and was most obvious in spermatids at the longitudinal manchette stage of development. The zone of cytoplasm between the manchette and the nuclear membrane was occupied by a continuous array of small, regularly-positioned, finger-like projections which appeared to emanate from the cytoplasmic surface of the nuclear membrane. In transverse sections of spermatids at this stage of spermiogenesis the uniform and symmetrical arrangement of the projections, and their close association with the nuclear membrane, gave the nucleus a cogwheel appearance (Figure). The structure appeared to be present along the whole length of the nucleus, but showed no obvious association with the manchette microtubules at any stage of development. The finger-like projections first became obvious during the early circular manchette stage of spermiogenesis and disappeared abruptly in cells displaying the longitudinal

manchette and dense, homogeneous karyoplasm (late stage of spermiogenesis). Immunogold labelling for α -, β - and γ -tubulin, as well as actin, was negative. Electron tomography revealed that the structure formed a regularly arranged grid with lattice parameters $a = 8$ nm, $b = 11$ nm, $\gamma = 78$ degrees.

A similar structure to that observed in the ratites species studied has been observed in both the Nile crocodile and the caiman, as well as in various lizard species but it appears to be absent in galliform birds. This finding lends support to the basal position of the ratites within the class Aves and supports the broad phylogenetic link between reptiles and birds, particularly the ratites. Although the role of this structure during spermiogenesis has not yet been established, its presence during spermatid differentiation in members of the Crocodylidae, Alligatoridae and Ratidae closely links these families. However, the apparent association between certain lizards and ratites remains obscure. This study also emphasises the value of studying spermiogenesis as an additional source of phylogenetic information.



Cross-section through a late stage emu spermatid. Note the nuclear membrane and associated structure (arrow). Manchette microtubules (star). Bar = 0.5 μ m.

A comparison of stress physiology and behaviour in beef feedlot calves hot-iron branded on the cheek and the upper hind leg

MJ Grobler¹, A Ganswindt^{1,2}, AS Shakespeare¹, PN Thompson¹

¹Department of Production Animal Studies, Faculty of Veterinary Science, University of Pretoria, Onderstepoort, South Africa; ²Mammal Research Institute, Department of Zoology and Entomology, University of Pretoria, Pretoria, South Africa; koba.grobler@up.ac.za



Hot-iron branding in beef feedlot calves is part of the processing procedure at arrival and many feedlots brand cattle on the upper hind limb. However, cheek branding has become a popular method in feedlots due to ease of restraint and better visibility of the brand mark. This study was done to determine whether there are differences in the levels of pain-induced stress experienced between feedlot calves which are branded on the leg, those branded on the cheek and control (sham-branded) animals.

Thirty weaned calves, recently arrived at a feedlot, were habituated to handling in a crush for 7 days and then randomly divided into three groups of 10. Group A was branded on the cheek and Group B on the hind leg. Group C was divided into two groups of five that were sham-branded, one group on the cheek and the other on the leg. Blood was collected at 0, 30, 60, 90 and 120 minutes after branding for cortisol determination with a commercial radioimmunoassay kit (Siemens Coat-A-Count). Faeces were collected on the day after arrival, at 7, 2 and 1 days before branding, on the day of branding and for seven consecutive days thereafter for cortisol faecal metabolite 11,17-DOA determination by enzyme immunoassay. Other outcomes measured included behavioural variables for 7 days after branding, individual average daily weight gain (ADG), average feed intake and feed conversion ratio (FCR) for each group, morbidity and mortality during the feedlot period and histopathological evaluation of the skin after slaughter.

Faecal 11,17-DOA concentrations were higher after transport than after branding ($P < 0.0001$) indicating that the stress of transport and a new environment is greater than that associated with branding. Blood cortisol levels, already elevated at the time of branding, were lower at 60 minutes and increased again at 90 and 120 minutes in all three groups, probably due to extended standing time in the crush. There were no statistically significant differences in blood cortisol or faecal 11,17-DOA between the three groups at any time. Vocalization occurred more frequently at the time of branding in Group A than in Group C ($P < 0.001$) and than in Group B ($P = 0.03$). Subsequently there were no significant differences in behaviour between the groups. Macroscopically, faint brand marks could be seen at the time of slaughter (74 days after branding) on five animals from Group B and no cheek brands were visible. No scarring was seen on histopathological examination. No significant differences were seen in the different production outcomes, and there were no morbidities or mortalities.

In conclusion, there were no significant differences in the levels of pain-induced stress experienced between feedlot calves branded on the cheek, the leg, or sham-branded. Despite habituation, the cortisol levels in our study may be more indicative of other stressors than of pain, or the pain response was too small or too short-lived to increase cortisol levels above the levels associated with handling. We have also shown that the stress of transport and a novel environment is higher than the stress associated with branding.

The effect of blood urea nitrogen on the reproductive performance of beef heifers on different levels of nitrogen supplementation

T Tshuma, DE Holm, GT Fosgate, DC Lourens

Department of Production Animal Studies, Faculty of Veterinary Science, University of Pretoria, Onderstepoort, South Africa; takula.tshuma@up.ac.za



Ruminants have a unique ability to metabolise protein from non-protein nitrogen (NPN) sources, and also to recycle nitrogen back into the rumen, instead of excreting all of it via the urine, faeces or milk. It is well documented that high levels of blood urea nitrogen (BUN) have a negative influence on conception rates. Additionally, a high dietary nitrogen intake poses a challenge to the environment, and this calls for strategies to reduce the environmental impact of livestock production. It is also known that variation exists in the ability to recirculate nitrogen between as well as within cattle breeds. The purpose of this study was to investigate the effects of BUN level on reproductive performance in heifers under different management systems in South Africa.

Serum samples from 374 Bonsmara heifers were taken in November and December 2010 to determine the BUN levels just prior to the onset of the breeding season. These heifers were from five herds with different levels of protein supplementation during the weeks preceding the breeding season. Body mass, age, body condition score (BCS) and reproductive tract score (RTS) were recorded at the time of BUN sampling. Trans-rectal ultrasound and/or palpation was performed between 4 and 8 weeks after the end of a 3-month breeding season to detect pregnancy and to estimate the stage of pregnancy. Days to pregnancy was defined as the number of days from the start of the breeding season until a heifer was successfully mated. Logistic regression and Cox proportional hazards survival analysis were performed to estimate the effect of BUN level on subsequent

pregnancy and days to pregnancy respectively, while stratifying by herd and adjusting for potential confounders. Correlations between BUN, BCS, RTS, age, body mass and reproductive performance within herds were determined using Spearman's rho.

Results indicated that BUN was a significant independent predictor of pregnancy as well as days to pregnancy in heavily and some moderately supplemented herds. As BUN increased, the chance of becoming pregnant decreased, while the days to pregnancy increased.

Herd	n	Age range (months)	Nitrogen supplement level	BUN as a predictor of:					
				Pregnancy outcome			Days to pregnancy		
				Pregnancy proportion (%)	Odds ratio	P-value	Median days to pregnancy	Hazard Ratio	P-value
A	106	22-26	None	77.4	0.833	0.548	43	0.969	0.824
B	34	20-26	Low	100	N.A.	N.A.	24	1.113	0.701
C	36	23-27	Moderate	100	N.A.	N.A.	4	0.997	0.989
D	15	15-26	Moderate	68.2	0.478	0.046	47	0.719	0.033
E	143	12-20	High	47.5	0.656	0.012	43.5	0.736	0.008

It is concluded that Bonsmara heifers with higher BUN, suggesting a better ability to recirculate nitrogen, may be at a disadvantage when the production system includes high levels of NPN supplementation, due to a negative impact on reproductive performance. It is proposed that production systems be adapted to avoid possible selection against animals with improved nitrogen recirculation ability.

Epidemiological investigation of the first reported outbreak of contagious equine metritis in South Africa

CE May¹, ML Schulman¹, AJ Guthrie², D Steckler³

¹Section of Reproduction, Department of Production Animal Studies, and ²Equine Research Centre, Faculty of Veterinary Science, University of Pretoria, Onderstepoort, South Africa; ³Hilton Veterinary Hospital, Hilton, KZN, South Africa; kate.may@up.ac.za



This report describes the epidemiology of the first outbreak of contagious equine metritis (CEM) in South Africa and the validation of real time PCR as a sensitive, specific and practical screening method for the detection of the causative organism, *Taylorella equigenitalis*.

The first outbreak of CEM caused by the bacterium *T. equigenitalis* in South Africa was reported to the OIE on 9 May 2011. The outbreak followed the importation of a Warmblood stallion from Germany and appeared to be confined to one property with a single confirmed case of transmission involving the stallion and one mare. The animals were treated following a standard protocol. They were subsequently retested by swabbing following a standard protocol for bacteriology according to the internationally-accepted practice. In addition, duplicate swabs were obtained for real time PCR (qPCR). An outbreak management protocol was applied. This included categorisation of all in-contact animals as "high risk" or "low-risk" dependent on the sequence of their presence relative to the index cases on the outbreak property. The "high risk" animals were similarly tested and treated. Thereafter "low risk" animals were tested. This resulted in the discovery of an additional eight positive stallions.

"The qPCR test has proved to be a highly specific and sensitive method of screening for CEM."

Following this outbreak, a nationwide qPCR screening program was instituted by the relevant State authority as a prerequisite prior to the authorisation by certification of any stallion to allow breeding, either by natural cover or assisted reproduction techniques. The screening was based on submission for qPCR of two sets of genital swabs (with an interval of at least seven days) proving negative. All swabs were submitted to the laboratory of the Equine Research Centre of the University of Pretoria. A duplex PCR was used. The PCR included controls, being a duplex assay for *T. equigenitalis* and *T. asinigenitalis*, as well as stallion skin microflora detection. Approximately 30 stallions have proved positive to date, and all have been confirmed using bacterial culture.

The qPCR test has proved to be a highly specific and sensitive method of screening for CEM. In addition, the method has significant practical advantages with respect to the associated costs, turn-around times and in-the-field application. Additionally, the qPCR has an inbuilt control method to screen for normal equine skin flora to ensure that the swabs were taken correctly.

Effectiveness of two topical antimicrobial agents used in the treatment of contagious equine metritis in stallions

B Keys¹, ML Schulman², K May², C Joone¹, M Monyai¹, J Marais³, D Mpofo⁴, AJ Guthrie¹

¹Equine Research Centre, ²Section of Theriogenology, Department of Production Animal Studies, and ³Section of Equine Surgery, Department of Companion Animal Studies, Faculty of Veterinary Science, University of Pretoria, Onderstepoort, South Africa; ⁴Gauteng Veterinary Services, Pretoria, South Africa; bronwynkeys@gmail.com

T. equigenitalis, the aetiological agent of contagious equine metritis, is sensitive to a wide range of antimicrobial drugs *in vitro*. Treatment regimes have been used with an antibiotic choice based on sensitivity, along with cleaning of the genitalia and application of a topical antimicrobial agent. The objective of this study was to compare the efficacy of two topical antimicrobial agents and determine duration of treatment required in stallions.

Twenty three out of 32 Lipizzaner stallions tested positive for *T. equigenitalis* on both bacterial culture and qPCR on swabs taken from the external genitalia. All stallions were removed from their usual place of residence and placed in an isolation facility under strict quarantine measures. The centre was kept free of horses for three weeks and all stables and equipment were thoroughly cleaned and disinfected.

The 23 CEM positive stallions were randomly assigned to one of the two topical antimicrobial ointment groups: 0.2% nitrofurazone (Furex[®], Aspen Pharmacare, Woodmead, South Africa) (Group 1: n = 12) or 1% silver sulphadiazine (Silbecor[®], Biotech Laboratories, Midrand, RSA) (Group 2: n = 11). Stallions were sedated to allow effective extrusion of the penis. The treatment protocol consisted of topical

treatment of the external genitalia without inclusion of systemic antimicrobials. The first two consecutive days consisted of topical cleansing of the penis and prepuce with a surfactant solution, using 5% docusate sodium (Docusol[®], Kyron Laboratories, Benrose, RSA) once daily to remove all smegma and external debris. Thereafter, treatment consisted of topical cleansing of the external genitalia with 5% docusate sodium and irrigation with a disinfectant solution using 4% chlorhexidine gluconate (Dismed Bioscrub[®], Dismed Pharma, Halfway House, RSA). The area was then dried and either one of the two topical antimicrobial ointments was applied for a minimum of seven days or until testing negative on qPCR swabs taken daily.

"All stallions were effectively treated and tested negative after three weeks on bacterial culture and qPCR."

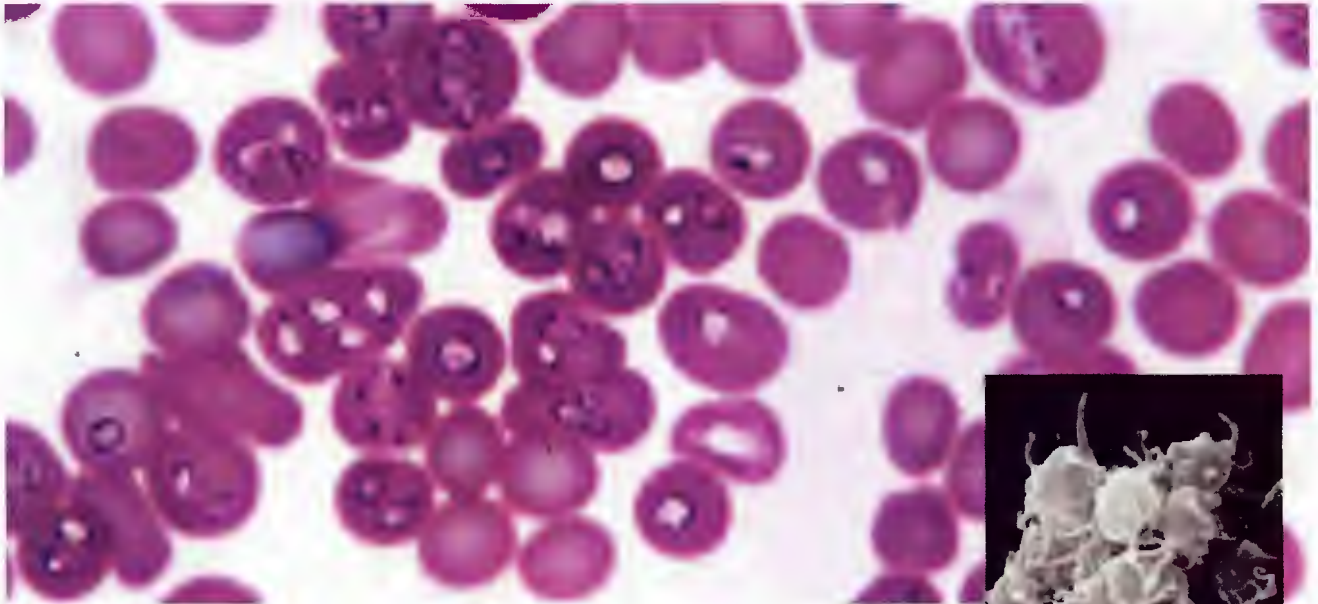
All stallions were effectively treated and tested negative after three weeks on bacterial culture and qPCR. Eleven of 23 stallions (48%) tested negative for the CEM organism at the end of the 9 d treatment protocol regardless of antimicrobial selection. The median treatment duration for 0.2% nitrofurazone was 11 days compared to 12 days for 1% silver sulphadiazine, illustrating that there was no significant difference between the antimicrobial agents. Response to treatment varied greatly between stallions. One stallion required a total of 52 days of treatment. Real time qPCR proved extremely valuable as a tool to monitor the success of treatment.



Platelet indices in virulent canine babesiosis and their association with outcome

A Goddard¹, AT Kristensen², AL Lelsewitz¹, PN Thompson³, JP Schoeman¹

¹Department of Companion Animal Clinical studies and ³Department of Production Animal Studies, Faculty of Veterinary Science, University of Pretoria, Onderstepoort, South Africa; ²Department of Small Animal Clinical Sciences, University of Copenhagen, Copenhagen, Denmark; amelia.goddard@up.ac.za



One of the most common haematological hallmarks of virulent canine babesiosis is severe thrombocytopenia, which is not associated with clinical haemorrhage despite very low platelet counts that would normally cause inability to maintain normal primary haemostatic function. Advances in technology have made it possible to record various platelet indices, some of which are recognised as surrogate markers for platelet activation and are related to platelet size, shape and granularity. The objective of this study was to investigate platelet indices in babesiosis, caused by *Babesia rossi*, and their association with outcome.

At presentation whole blood EDTA samples were collected from 68 *Babesia*-infected and six healthy control dogs. Samples were also collected at 24 and 48 hours in dogs that were admitted to the Onderstepoort Veterinary Academic Hospital (OVAH). *B. rossi* infection was confirmed and co-infection with *Ehrlichia canis* was ruled out using a polymerase chain reaction and reverse line blot. Owner consent was obtained for all the cases for enrolment in this study. Whole blood samples were analysed within 30 minutes of collection on the ADVIA 2120 (Siemens, South Africa). The ADVIA 2120 is an automated haematology analyser that uses 2-dimensional laser scattering to specifically assess platelets. Each analysis produced platelet indices which included: platelet count (PLT), mean platelet volume (MPV), platelet volume distribution width (PDW), thrombocrit (PCT), mean platelet dry mass (MPM), mean platelet component concentration (MPC) and platelet component distribution width (PCDW).

Of the *Babesia*-infected dogs, 30 were treated as outpatients and 38 were admitted to the OVAH, of which 9 died within

24 hours (13%). The following platelet indices were significantly different between the *Babesia*-infected and control dogs:

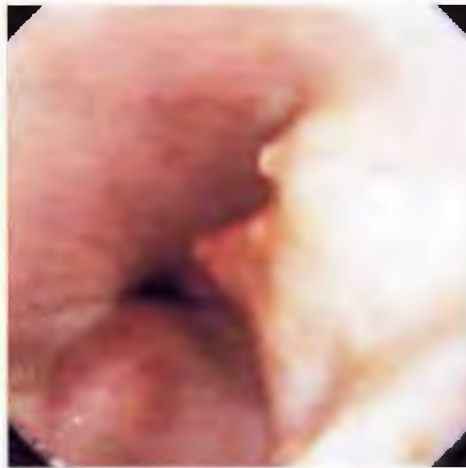
median PLT (32 vs. 259 $\times 10^9/L$; $P < 0.001$); median MPV (15.7 vs. 9.8 fL; $P < 0.0001$); median PDW (60.9 vs. 55.9%; $P = 0.005$); median PCT (0.05 vs. 0.24%; $P < 0.001$); mean MPM (2.3 vs. 1.8 pg; $P = 0.003$) and mean MPC (19.3 vs. 21.6 g/dL; $P < 0.0001$). There were no significant differences in the *Babesia*-infected group between outpatient and admitted dogs, or between dogs that died and dogs that survived. PLT and MPC showed significant increases at 24 hours ($P = 0.012$ and $P = 0.009$, respectively) and 48 hours ($P < 0.0001$ and $P = 0.01$, respectively).

Platelet activation has been implicated in the pathogenesis of a wide range of clinical disorders. The MPC is a measure of platelet granularity and a decrease in MPC can be due to platelet degranulation, indicating activation. MPV is a measure of platelet size, and reflects changes in either the level of platelet stimulation or the rate of platelet production. MPV is known to increase as platelets become activated and change shape. Large platelets are known to have a greater potential for aggregation and secretion, and therefore suggest an increased risk of thrombosis. Therefore, large, degranulated platelets may play a significant procoagulant role in the lack of clinical haemorrhage, despite severe the thrombocytopenia observed in virulent canine babesiosis. Platelet activation, as measured by MPV and MPC, does not appear to be associated with outcome.

Evaluation of haemostatic abnormalities in naturally occurring canine spirocercosis

P Pazzi¹, A Goddard¹, AT Kristensen², E Dvir¹

¹Department of Companion Animal Clinical Studies, Faculty of Veterinary Science, University of Pretoria, Onderstepoort, South Africa; ²Department of Small Animal Clinical Sciences, Faculty of Health and Medical Sciences, University of Copenhagen, Denmark; paolo.pazzi@up.ac.za



Spirocerca lupi is a nematode that infects the oesophagus in dogs, resulting in an inflammatory fibroblastic nodule that progresses to a sarcoma in approximately 25% of cases. This study aimed to determine whether coagulation abnormalities exist in canine spirocercosis, and hypothesised that the severity of abnormalities could be used to differentiate non-neoplastic from neoplastic spirocercosis.

Thirty-nine client-owned *S. lupi*-infected dogs and 15 healthy age- and sex-matched control dogs were included in the study. Blood samples were collected at the time of diagnosis. A complete blood count, prothrombin time (PT), activated partial thromboplastin time (aPTT), fibrinogen concentration, antithrombin (AT) activity, D-dimer concentration and thromboelastography (TEG) analysis (TEG[®] 5000 Thrombelastograph[®] Haemostasis System) were performed. Inflammatory parameters were also determined and included C-reactive protein (CRP) and fibrinogen concentrations. The *S. lupi*-infected dogs were divided into a non-neoplastic group (n = 24) and a neoplastic group (n = 15), based on endoscopic regression of the nodule 6 weeks post treatment or histopathology of endoscopic biopsy samples. Outcomes were compared using the Kruskal-Wallis Test and Dunn's multiple comparisons applied post-hoc. Correlation was determined using Spearman's correlation. Hypercoagulability was based on the maximum amplitude (MA) value derived from TEG. $P \leq 0.05$ was considered significant.

The neoplastic group was significantly more hypercoagulable (MA: 78.9, 61.5-89.9 mm) (median, range) than the non-neoplastic group (66.3, 49.7-79.7 mm) ($P < 0.01$), and the

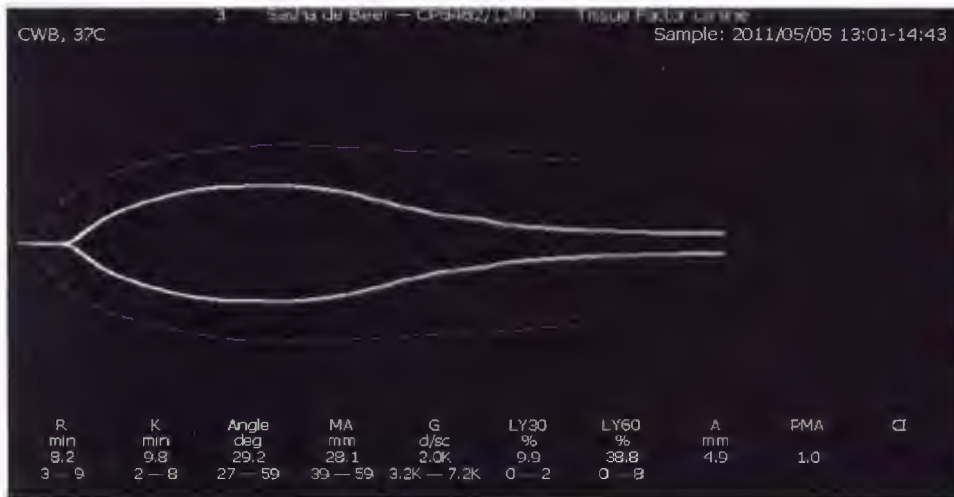
non-neoplastic group was significantly more hypercoagulable than the control group (58.6, 41.9-66.2 mm) ($P < 0.01$). The median fibrinogen concentration was significantly higher in the neoplastic (5.07, 2.87-14.76 g/L) versus the non-neoplastic group (3.27, 1.33-8.86 g/L) ($P < 0.01$), but there was no significant difference between the non-neoplastic and control groups (2.62, 1.69-4.35 g/L). The median CRP concentration was significantly higher in the neoplastic group (85.7, 6.05-151.96 mg/L) compared to the non-neoplastic group (17.46, 5.00-249.85 mg/L) ($P < 0.01$), with no significant difference between the non-neoplastic and control groups (15 mg/l). Compared to the control group (117.1, 107.1-143.9%) the median AT activity was significantly decreased in both the non-neoplastic (90.3, 58-124.1%) and neoplastic (84.3, 58.5-111.6%) groups (both $P < 0.0001$). No significant difference was found between the infected groups. Across the non-neoplastic and neoplastic groups, MA was positively correlated with CRP ($r = 0.73$, $P < 0.0001$) and fibrinogen ($r = 0.82$, $P < 0.0001$). When a MA value of >76 mm was used as the cut-off for the differentiation of neoplastic versus non neoplastic cases, a sensitivity of 73% and specificity of 96% were obtained.

The study showed that spirocercosis is associated with a hypercoagulable state that becomes progressively more hypercoagulable with neoplastic transformation. However, overlap between the two groups makes clinical differentiation of disease difficult. The MA correlated positively with the indicators of inflammation (CRP & fibrinogen) supporting the theory that an inflammatory state induced by the *Spirocerca* nodule is likely partly responsible for the hypercoagulability.

Aflatoxicosis causing a hyperfibrinolytic syndrome in 12 dogs

B Conner, A Goddard

Department of Companion Animal Clinical Studies, Faculty of Veterinary Science, University of Pretoria, Onderstepoort, South Africa; amelia.goddard@up.ac.za



Aflatoxin is an important mycotoxin that has been associated with several commercial pet food-related outbreaks of aflatoxicosis in dogs as well as isolated exposures. Aflatoxicosis is associated with a high rate of mortality in dogs, ranging from 64–100%. Coagulation abnormalities are frequently cited as a significant cause of morbidity and, in particular, disseminated intravascular coagulation (DIC) has been reported as a suspected sequel to aflatoxicosis. The use of thromboelastography (TEG) in dogs with aflatoxicosis has not been described. The objective of this study was to retrospectively evaluate the haemostatic changes, specifically using TEG, in a series of dogs with accidental aflatoxicosis.

Medical records from the Onderstepoort Veterinary Academic Hospital (OVAH) for dogs with confirmed or suspected aflatoxicosis were available for inclusion. Inclusion criteria were: 1) exposure to aflatoxin based on history, confirmation from toxicological testing, post-mortem findings, or confirmed exposure from another dog in the household; and 2) TEG performed at the time of admission, prior to treatment. Medical records were reviewed and clinicopathologic data were evaluated. All TEG analysis were performed on the TEG® 5000 Thrombelastograph® Haemostasis System (Haemoscope, Pro-Gen Diagnostics, South Africa) according to the manufacturer's instructions.

Twelve dogs fulfilled the inclusion criteria. Three dogs survived to hospital discharge. All dogs had evidence of hepatic failure based on severely elevated hepatic enzymes and fasting and/or post-prandial bile acids. TEG results showed that nine of the 12 dogs had evidence of increased fibrinolysis, as measured by the TEG variables LY30 and LY60. These represent clot lysis 30 and 60 minutes after maximal clot formation and are indicators of the clot stability. The mean LY30 was 25.1% (range: 0–60.3%; reference range: < 2%) and mean LY60 was 41.1% (range: 0–72.6%; reference range: 0–8%).

“This may allow for therapy that is tailored to the specific coagulation defect, such as the administration of antifibrinolytic drugs.”

This is the first veterinary report to diagnose increased fibrinolysis in dogs with accidental aflatoxicosis utilizing TEG. This presents an opportunity to better understand the pathophysiology seen with aflatoxin ingestion, and to understand the coagulopathy that is frequently encountered and often fatal. This may allow for therapy that is tailored to the specific coagulation defect, such as the administration of antifibrinolytic drugs. Additionally, this report suggests that TEG may be a viable diagnostic tool for evaluating acquired hyperfibrinolytic disorders in dogs.

Effects on coagulation and colloid osmotic pressure of rapid intravenous fluid administration in healthy horses

E Rioja¹, L Rubio-Martinez¹, M Sanz¹, GT Fosgate², A Goddard¹

¹Department of Companion Animal Clinical Studies, and ²Department of Production Animal Studies, Faculty of Veterinary Science, University of Pretoria, Onderstepoort, South Africa; eva.riojagarcia@up.ac.za



Rapid intravenous infusion of crystalloid solutions has been shown to induce enhanced coagulation in humans *in vitro* and *in vivo*. The objective of this study was to determine the effects of rapid intravenous administration of fluids on coagulation and colloid osmotic pressure (COP) in healthy horses.

The study was designed as a randomized cross-over study including six adult healthy Nooitgedacht horses. Four different intravenous fluid regimes were administered to each horse with a one week wash-out period: plain lactated Ringer's solution (50 mL/kg) (LRS), lactated Ringer's solution with additional 1.5 mmol/L of magnesium (50 mL/kg) (LRS/M1.5), lactated Ringer's solution with additional 3 mmol/L of magnesium (50 mL/kg) (LRS/M3), and a hydroxyethyl starch (130/0.4) bolus (5 mL/kg) followed by plain lactated Ringer's solution (50 mL/kg) (LRS/HES). Fluids were administered rapidly using pressure bags. Thromboelastography (TEG) (reaction time [R], K time, alpha angle, maximum amplitude [MA]) and COP were measured before the treatments

(T0), immediately after the fluids (T1) and one hour after the fluids (T2). Antithrombin activity, total serum protein (TP), electrolytes and physiological parameters were also measured. Fresh non-citrated samples were used for TEG analyses. A linear mixed model and post-hoc Bonferroni adjustments were used for analysis ($P < 0.05$).

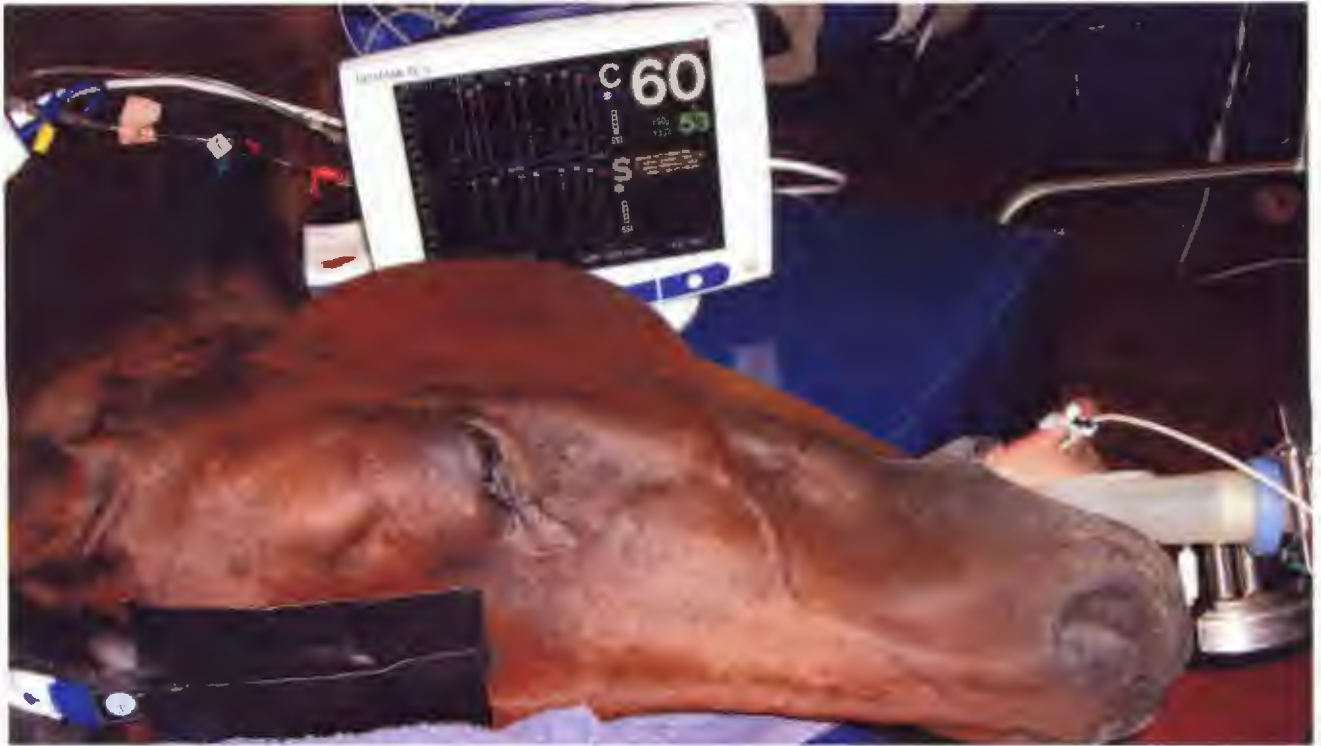
There was a significant change over time for angle and antithrombin with no differences among treatments. There was a significant effect of treatment and time for MA, COP, TP and total magnesium. After treatment LRS/HES, MA was lower at T2 (mean \pm SD, 67.8 ± 2.8 mm) compared to T0 (72.1 ± 4.7 mm) ($P = 0.013$).

In conclusion, rapid intravenous administration of common crystalloid solutions does not affect coagulation in healthy horses, but when a bolus of a hydroxyethyl starch solution is co-administered with the crystalloids, the strength of the clot is reduced, which could be of clinical importance in horses with pre-existing coagulopathies.

Use of near-infrared spectroscopy to identify trends in regional cerebral oxygen saturation in horses

EJ McConnell¹, MN Saulez¹, L Bester¹, GT Fosgate², MG Sanz¹, E Rioja¹, RP Raath³

¹Department of Companion Animal Clinical Studies, and ²Department of Production Animal Studies, Faculty of Veterinary Science University of Pretoria, Onderstepoort, South Africa; ³Jakaranda Pain Clinic, Jacaranda Hospital, Pretoria, South Africa; Emma.McConnell@up.ac.za



Alterations in cerebral haemodynamics may contribute to peri-anaesthetic complications in horses. Currently, neuromonitoring techniques in equine medicine are very limited. Near-infrared spectroscopy (NIRS) is frequently used intra-operatively in humans and provides real-time, continuous information regarding cerebral perfusion in a non-invasive manner. The objectives of this study were to determine whether NIRS could be used to: 1) assess trends in regional cerebral oxygen saturation (rSO_2) in clinically normal horses; 2) detect changes in rSO_2 during periods of induced hypo- or hypercapnoea; and 3) determine whether a correlation exists between rSO_2 and venous oxygen tensions (PvO_2).

Six clinically-healthy adult Nootgedacht mares were used in a prospective pilot study. A disposable cerebral oximeter probe placed over the parietal bone recorded rSO_2 from the *sinus sagittalis dorsalis*. rSO_2 , PaO_2 , PvO_2 , $PaCO_2$, $PvCO_2$, mean arterial pressure (MAP) and lactate (LAC) values were recorded in unsedated, sedated and anaesthetised horses, and during recovery. Following sedation with romifidine (50 $\mu\text{g}/\text{kg}$ IV), horses were anaesthetised using diazepam (10 mg IV) and ketamine (2.2 mg/kg IV), and maintained with isoflurane in oxygen (1.3%). Horses were positioned in right lateral recumbency and mechanically ventilated to achieve states of normo-, hyper- and hypocapnoea. Data

were evaluated descriptively and analysed using linear mixed effects models and Pearson's correlation coefficient. Significance was set at $P < 0.05$.

Overall mean \pm SE values for rSO_2 , PaO_2 , PvO_2 , $PaCO_2$ and MAP varied significantly by recording period ($P < 0.05$). No significant differences in rSO_2 values recorded during periods of normo-, hyper- or hypocapnoea were reported. Significant correlations were identified between rSO_2 and PaO_2 ($r = 0.460$, $P < 0.01$) and rSO_2 and PvO_2 ($r = 0.512$, $P < 0.01$). No significant correlation was detected between rSO_2 and $PaCO_2$ ($r = 0.146$, $P = 0.081$); rSO_2 and $PvCO_2$ ($r = -0.012$, $P = 0.884$); or rSO_2 and plasma [Lac] ($r = -0.257$; $P = 0.082$).

The highest mean rSO_2 value was recorded during the period of induced hypercapnoea ($60.0 \pm 3.3\%$), whereas the lowest mean rSO_2 value was recorded immediately post-recovery ($40.7 \pm 3.3\%$). Significant differences in rSO_2 were identified between values recorded in the normal standing horse and those recorded during the post-anaesthetic periods. To date, this is the first study demonstrating the use of NIRS to identify trends in regional cerebral oxygen saturation in horses. During equine general anaesthesia, NIRS may therefore be used to detect decreases in rSO_2 values, alerting clinicians to cerebral desaturation that may require intervention.

Effects of regional or general anaesthesia on the clinical and pharmacokinetic parameters of amikacin administered by intravenous regional limb perfusion in horses

AT Mahne¹, LM Rubio-Martinez¹, HJ Marais¹, N Villarino², E Rioja¹

¹Department of Companion Animal Clinical Studies, Faculty of Veterinary Science, University of Pretoria, Onderstepoort, South Africa; ²Department of Biomedical and Diagnostic Sciences, College of Veterinary Medicine, University of Tennessee, Knoxville TN, USA; arnold.mahne@gmail.com



Antimicrobial intravenous regional limb perfusion (IVRLP) is clinically performed on anaesthetised or sedated horses with or without regional anaesthesia. This study examined the effects of regional or general anaesthesia (GA) on the clinical parameters and synovial pharmacokinetics of amikacin administered by IVRLP in horses.

Eight healthy horses received four treatments of amikacin IVRLP in a randomised, blinded, cross-over study: under GA, under standing sedation (SED) without regional anaesthesia (CNT), SED with perineural anaesthesia (PNA) and SED with intravenous regional anaesthesia (IVA). Synovial fluid amikacin concentrations were measured over 24 hours and the regional pharmacokinetic parameters (rPkp) calculated. Heart and respiratory rates, visual analogue scale (VAS) of discomfort, number of times the limb was lifted (LIFT) and number of extra sedations administered were recorded. ANOVA cross-over analysis was applied. Significance was set at $P < 0.05$.

Amikacin concentrations and rPkp did not differ significantly among treatments. Heart and respiratory rates were similar among treatments. Scores of VAS (mean \pm SD) were significantly lower with PNA (18 ± 15) versus IVA (68 ± 33) and CNT (82 ± 13). Significantly lower LIFT (median[range]) occurred with PNA ($12.5[4-64]$) versus CNT ($53[15-85]$). No extra sedation was administered in PNA.

The addition of regional or general anaesthesia to IVRLP with amikacin in horses did not have any effect on the synovial concentrations or regional pharmacokinetics. However, during IVRLP on standing sedated horses, PNA improved comfort of horses.

Bayer HealthCare, Pfizer Animal Health and Roche Products supplied materials for this study.

The prevalence of severe combined immunodeficiency, lavender foal syndrome and cerebellar atrophy in Arabian horses in South Africa

CJ Tarr¹, CK Harper², AJ Guthrie³, PN Thompson¹

¹Department of Production Animal Studies, Faculty of Veterinary Science, University of Pretoria, Onderstepoort, South Africa; ²Veterinary Genetics Laboratory, Faculty of Veterinary Science, University of Pretoria, Onderstepoort, South Africa; ³Equine Research Centre, Faculty of Veterinary Science, University of Pretoria, Onderstepoort, South Africa; Carolynne.Tarr@up.ac.za

The prevalence of carriers of three genetic disorders: severe combined immunodeficiency (SCID), lavender foal syndrome (LFS) and cerebellar atrophy (CA), in registered purebred Arabians in South Africa, was assessed. These genetic disorders are inherited in an autosomal recessive manner. A carrier of the disease-causing allele shows no clinical signs. When breeding two carriers, the offspring have a 25% chance of being genetically homozygous normal, a 50% chance of being a carrier of the disorder, and a 25% chance of developing the disease. When breeding a carrier to a normal individual, the offspring from that mating have a 50% chance of being carriers of the mutation and a 50% chance of not carrying the mutation. Through genetic testing for these disorders, carrier-to-carrier matings can be avoided, effectively preventing the birth of clinically affected foals.

Arabian horse owners and breeders routinely submit hair, blood or tissue samples to the Veterinary Genetics Laboratory (VGL), Onderstepoort, for the purpose of parentage verification. This study made use of DNA extracts as well as tissue, blood and hair samples in storage at the VGL. Genotyping for the three disorders was performed on individuals randomly selected from two populations: Arabians born during the intervals 1 August 2004 to 31 July 2005 and 1 August 2009 to 31 July 2010, in line with physiological breeding seasons. This permitted an estimation of the change in prevalence of each disorder between 2004 and 2009, during which time

a genetic test for severe combined immunodeficiency was available to breeders.

To determine the SCID, LFS and CA genotypes of each individual in the sample sets, polymerase chain reaction (PCR) was performed using primers previously described. Following capillary electrophoresis, fragment sizes produced for each marker were recorded and analysed on the software programme STRand version 2.4.49 (University of California, Davis, USA). Statistical analysis was performed with the aid of the software programme StatCalc, and was based on the hypergeometric distribution, taking into account the finite population size. $P < 0.05$ was considered statistically significant.

"These results will encourage the genetic screening of Arabian horses intended for breeding purposes in order to prevent the birth of clinically affected individuals."

The prevalence of LFS and CA for the 2009 breeding season was found to be 11.7% and 5.1% respectively, with no statistically significant change in the prevalence of these disorders between 2004 and 2009 ($P = 0.29$ and $P = 0.56$ for LFS and CA respectively). The prevalence of SCID was found to have decreased significantly from 6.4% in 2004 to 3.4% in 2009 ($P < 0.001$). These results will encourage the genetic screening of Arabian horses intended for breeding purposes in order to prevent the birth of clinically affected individuals. This study also highlights the usefulness of genetic testing as a tool to decrease the prevalence of specific genetic disorders within animal populations.



A study on bovine tuberculosis and associated risk factors in humans in Swaziland

MEM Dlamini^{1,2}, A Michel¹

¹Department of Veterinary Tropical Diseases, Faculty of Veterinary Science, University of Pretoria, Onderstepoort, South Africa; ²Meat Hygiene Services, Department of Veterinary and Livestock Services, Ministry of Agriculture, Swaziland; mcebodlamini@gmail.com



Bovine tuberculosis (BTB) is a chronic, insidious, contagious disease primarily affecting cattle and a wide range of domestic and wild animals, with a worldwide occurrence. Humans consuming products of infected cattle, such as unpasteurised milk and meat, are at risk of being infected by *Mycobacterium bovis*, the causative agent of BTB.

A study was conducted in which carcasses found to be infected with BTB on *post mortem* inspection at an export abattoir, and later confirmed positive for BTB by laboratory diagnosis, were traced back to their diptanks of origin. Those diptanks were selected for a BTB prevalence study. The distribution of the 15 selected study diptanks was representative of all provinces in the country. Ten percent of the animals in the selected study diptanks, whose populations ranged from 500-1800 cattle, owned by about 10-120 farmers per diptank, were randomly selected and tested for BTB using the comparative intradermal skin test. Each kraal in a diptank was included in the testing. A few BTB positive animals from four kraals were slaughtered and tissue samples were collected for culture. Concurrently with the comparative skin testing, a survey was carried out to determine livestock owners' knowledge of the zoonotic aspects of BTB.

Results of the study indicated a prevalence at diptank level ranging from 2.1% to 27.6%, with an average prevalence per diptank of 6.4%. Gross lesions typical of *M. bovis* infection were observed in 8/25 skin test positive cattle slaughtered

and *M. bovis* was isolated from 7 of them, representing two herds.

Of the 789 farmers interviewed, 88.9% consumed unpasteurized milk obtained from their lactating beef cattle while 92.9% consumed meat that had not been inspected on a regular basis. Of the latter, 100% consumed high risk organs such as the head and visceral organs, without removing any lymph nodes. Furthermore, 94.8% admitted to some form of undercooking of the meat, through cooking the meat over an open fire. Lastly, 68.1% of respondents consumed meat from animals that had died on their own whenever it was available. While 98.4% of the people interviewed were aware that TB was a serious human disease, only 5.7% were aware of BTB as a cattle disease and 2.9% were aware of its zoonotic potential.

Consumption of unpasteurized milk and uninspected meat, including high risk organs, and the widespread practice of undercooking meat, expose consumers to infection with *M. bovis* from BTB infected animals. Farmers lack knowledge about BTB as a cattle disease and a serious zoonosis, making the disease a seriously neglected zoonosis. At best, livestock owners' knowledge of the zoonotic aspects of BTB is insufficient and inadequate, with inferences of BTB drawn from human TB. This study is the first to determine the BTB prevalence in selected diptanks and zoonotic risk factors for humans in Swaziland.

The brucellin skin test: is it of any value in investigating bovine brucellosis in South Africa?

N Nyanhongo¹, M Hansen², A Storm², AL Michel³

¹Vryheid Veterinary Laboratory, Vryheid, South Africa; ²Standerton State Veterinary Office, Standerton, South Africa; ³Department of Veterinary Tropical Diseases, Faculty of Veterinary Science, University of Pretoria, Onderstepoort, South Africa; nyanhongo@yahoo.com



Brucellosis is a disease of socio-economic and zoonotic importance worldwide. In animals it is associated with the ingestion of feed that is contaminated with cyetic material from aborting herd-mates, while in humans it is associated with the consumption of unpasteurised milk and dairy products from infected animals. It may also be acquired from contact with infected material of animal origin by farmers, veterinarians, and abattoir and laboratory workers. Brucellosis was first reported in South Africa in the late nineteenth century. It is still present in the country today, with reported annual losses of at least R300 million, and a national annual incidence of 5000 cases in humans. The global incidence of human brucellosis is about half a million infections annually. As the incidence of human brucellosis is directly associated with prevalence in animals, control of animal brucellosis is emphasised.

“Veterinary control is compromised by the chronic nature and the variable incubation period of the disease, with an estimated up to 15% of cattle in infected herds aborting before seroconversion.”

Veterinary control is compromised by the chronic nature and the variable incubation period of the disease, with an estimated up to 15% of cattle in infected herds aborting before seroconversion. Latency, which involves about 5% of calves born from infected dams, is also problematic as these infected animals often test seronegative, only to seroconvert in the periparturient period, thus allowing opportunity for disease spread within and between herds before diagnosis is made. In addition, the currently used serological tests are at times unable to distinguish brucellosis from cross-reacting antibodies from other infections or brucellosis vaccines.

It was the objective of this study to investigate, under South African conditions, the value of the brucellin skin test (BST) in improving the sensitivity and specificity of the currently used serological tests. It has proved a valuable additional test in diagnosing early and latent infections as well as in differentiating brucellosis from cross-reacting organisms in unvaccinated cattle in Europe.

The study was carried out in Mpumalanga Province, on herds selected to reflect prevailing South African farming conditions. These herds were divided into certified *Brucella abortus*-negative herds (608 head) for the estimation of BST specificity, and confirmed *B. abortus*-infected herds (846 head) for the estimation of BST sensitivity.

The results obtained indicated the BST had a specificity of 99.0%, and a sensitivity of 54.0%. However, 68.2% of BST-positive animals were negative on serology. When the high specificity is considered, together with the experiences of other researchers who found that the skin test became positive earlier than serological tests, these animals may be assumed infected. It is concluded that the BST is a valuable addition to the panel of diagnostic tests currently used to identify infected herds and individuals in South Africa.

Comparative studies on immunogenicity and protective efficacy of a live spore *Bacillus anthracis* vaccine versus recombinant peptide vaccine candidates in goats

OC Ndumnego¹, S Koehler², J Crafford¹, W Beyer², H van Heerden¹

¹Department of Veterinary Tropical Diseases, Faculty of Veterinary Science, University of Pretoria, Onderstepoort, South Africa; ²Institute of Environmental and Animal Hygiene, University of Hohenheim, Stuttgart, Germany; okeyndumnego@yahoo.com

The use of the current anthrax live spore vaccine in goats and horses has not been without caution due to vaccine-related adverse reactions and/or deaths in these species. More so, there is paucity of data on the immunogenicity of the Sterne vaccine in herbivores. Current research on the development of second generation anthrax vaccines has shown need for the presence of anti-toxic and anti-bacterial antibodies in vaccinates. These vaccines comprise components of the anthrax tripartite toxin, the protective antigen (PA) and the lethal factor (LF) and spore-specific antigens such as the *Bacillus collagen-like antigen* (BclA).

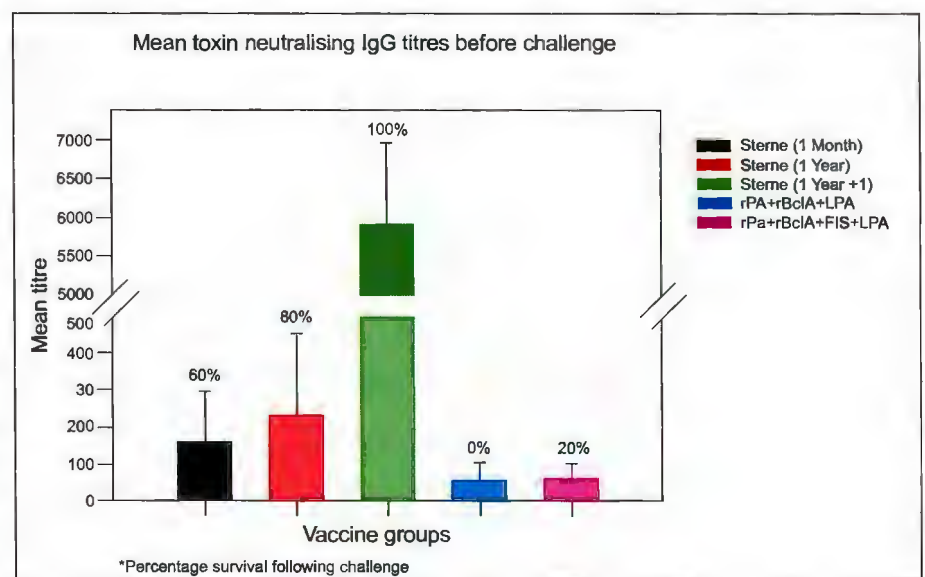
This study compared the immunogenicity and protective efficacy of the live spore vaccine against multicomponent recombinant protein (rPA & rBclA) vaccines supplemented with a lipopeptide adjuvant, with or without formaldehyde inactivated spores (FIS). The acellular vaccines were administered three times at 3 week intervals in goats. Various immunization regimens were explored and compared using current immunological diagnostic tools to determine correlates of protection against anthrax infection in vaccinated goats.

Age-matched Boer goats were split into five groups of five animals each and vaccinated with the different vaccine combinations and six goats served as negative controls (total 31 goats). Virulent spore challenge was done not earlier than 3 weeks after the last vaccination and survival was monitored for 14 days. Serum and blood samples were collected at monthly intervals and analyzed for anti-PA and anti-BclA antibodies using an indirect ELISA. Toxin neutralizing antibody assay (TNA) was assessed using an *in vitro* macrophage culture assay.

Findings suggested that the minimum lethal dose (MLD) of the virulent wildtype strain of *B. anthracis* used was <50 spores per animal. Peracute clinical signs developed in naïve goats with no evidence of blood extravasation from body orifices. Bacteraemia was detectable in blood smears not more



than 2-3 hours before death. The TNA titres correlated with the survival data, with higher survival rates seen in groups with higher TNA titres. Anti-PA antibody titre which has been shown to be a protective correlate in different species lacked the same capacity for goats in the present study. Boosting with acellular vaccines elicited high anti-PA titres but did not lead to higher survival. Sterne-vaccinated animals that were challenged after one year had low anti-PA antibody titres but a higher survival rate than the acellular vaccine groups which had higher pre-challenge titres. BclA elicited a poor antibody response while FIS induced moderate titres. This study suggests that goats are protected for more than a year after vaccination with the Sterne live spore vaccine and that the TNA provides the most reliable information on protection in goats.



An investigation of the cause of enteritis in ostrich (*Struthio camelus*) chicks in the Western Cape Province, South Africa

L Keekilwe^{1,2}, A Olivier³, WP Burger⁴, EH Venter¹, D Morar¹

¹Department of Veterinary Tropical Diseases, Faculty of Veterinary Science, University of Pretoria, Onderstepoort, South Africa; ²Botswana National Veterinary Laboratory, Gaborone, Botswana; ³Ostrich Research Laboratory, Oudtshoorn, South Africa; ⁴South African Ostrich Business Chamber, Oudtshoorn, South Africa; lkeekilwe@gmail.com



Ostrich (*Struthio camelus*) chicks less than 3 months of age may experience a high mortality rate of 30-40% that is often associated with enteritis. In the event of a disease outbreak, mortality can reach 80-100%. Morbidity in those individuals that survive an episode of enteritis, leads to poor growth rate. In some cases, cloacal prolapse has been seen to occur in conjunction with enteritis, which is a multifactorial syndrome that is seen in different animal species. Various infectious agents have been implicated in the syndrome but no clear cause of the condition has yet been identified. *Clostridium perfringens* is recognised as a cause of necrotic enteritis, especially in broiler chickens. *Salmonella* spp. and *Escherichia coli* are recognised factors of enteritis in chickens and turkeys, where damage to the intestinal mucosa occurs as a result of a co-existing viral infection. This study was undertaken to investigate different infectious agents implicated in enteritis in ostrich chicks.

Necropsy was performed on 139 ostrich chicks aged from 1 day to 3 months of age. Small and large intestines were kept on ice or at 4°C for bacterial culture and collected in 10% formalin for

histopathological examination. Fresh intestinal contents were collected for electron microscopy and rectal faeces collected for faecal flotation for helminth or coccidia eggs and staining by

Safranin counterstained with methylene blue to detect *Cryptosporidium* oocysts.

"The findings suggest that viruses and parasites do not play a significant role in the occurrence of enteritis in ostrich chicks."

Necrotic enteritis and ulcerative lesions of the intestines were identified. *E. coli*, *C. perfringens* and *Salmonella* spp. were isolated in association with these lesions. Two enteroviruses and one birnavirus were identified from 73 samples. No helminths, coccidia or *Cryptosporidium* spp. were identified from the samples.

The findings suggest that viruses and parasites do not play a significant role in the occurrence of enteritis in ostrich chicks. The cases of enteritis observed in this study occurred independently of any clinical signs of cloacal prolapse. The isolation of the bacteria *E. coli*, *C. perfringens* and *Salmonella* spp. identifies them as possible factors responsible for the onset of disease in ostrich chicks. *E. coli* and *C. perfringens* may, however, inhabit the intestines as commensals and further typing of these bacteria is required.

Characterisation of the metabolic acid-base disturbances in canine parvoviral enteritis using the strong ion approach

RK Burchell¹, JP Schoeman¹, HA Demorais², AL Leisewitz¹

¹Department of Companion Animal Clinical Studies, Faculty of Veterinary Science, University of Pretoria, Onderstepoort, South Africa; ²Department of Veterinary Internal Medicine, Oregon State University; Richard.Burchell@up.ac.za

The acid-base disturbances in canine parvoviral enteritis (CPV) are not well described. In addition, the pathogenesis of the acid-base changes has not been fully elucidated. Previous studies have shown mild pH changes with mild decreases in HCO_3^- levels. The hypothesis of this study was that within the metabolic compartment, profound acid-base disturbances would be present which are not appreciated by the bicarbonate centred approach. It was conjectured that according to the strong ion model (SIM) a significant strong ion acidosis would be present in CPV which would partially be negated by severe hypoalbuminaemia, resulting in a mild metabolic acidosis.

The study retrospectively analysed data collected from 42 puppies with CPV and 12 healthy controls. The CPV group had been allocated a clinical score to allow classification of the data according to clinical severity. A modified approach was used to assess changes within the metabolic component according to the SIM acid-base principles. In this model the effect of changes in chloride, free water, lactate, albumin and phosphate were summated providing an indication of the contribution of each variable to the overall metabolic acid-base status. A Student's *t*-test was used to compare means between the CPV and control group and Pearson's correlation and linear regression analysis were used to identify significant relationships between variables.

“...the acid-base disturbances in CPV are multifactorial and complex and the SIM provides more information regarding the origin of these changes.”

According to the SIM, 20 patients in the CPV group had a metabolic acidosis, 10 had a metabolic alkalosis and in 12 patients the overall effect was neutralizing. Twenty-eight of the 42 puppies had a strong ion acidosis and within this group 19 had a significant hypoalbuminaemic alkalosis. The most important contributor to the metabolic acid-base changes according to the SIM was chloride ($P < 0.001$). Furthermore, there was a significant difference in the chloride changes between the mild and the severe



group according to the clinical score ($P = 0.002$), where mildly affected patients tended to have a hyperchloraemic acidosis compared to a hypochloraemic alkalosis in the severe group. These findings are consistent with a previous study in which a mild metabolic acidosis was the most consistent finding according to the Henderson-Hasselbalch (HH) model. However, the SIM was able to provide quantitative mechanistic information not appreciated by the HH model. In conclusion, the acid-base disturbances in CPV are multifactorial and complex and the SIM provides more information regarding the origin of these changes.

A comparison of transthoracic echocardiographic measurements to allometric scaling derived values in clinically normal adult dachshunds

CK Lim¹, RM Kirberger¹, GT Fosgate²

¹Department of Companion Animal Clinical Studies, and ²Department of Production Animal Studies, Faculty of Veterinary Science, University of Pretoria, Onderstepoort, South Africa; cheekin.lim@up.ac.za

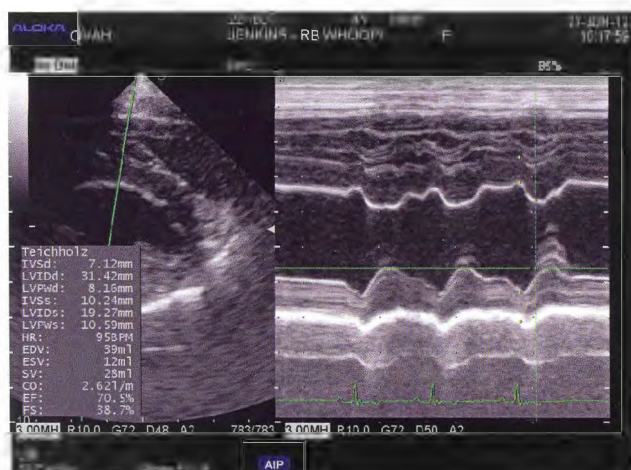
Mitral valve prolapse (MVP) in dachshunds is associated with progressive myxomatous mitral valvular degeneration. Allometric scaling is currently used to predict normal M-mode cardiac measurements in adult dogs. Allometric scaling reference ranges are determined by logarithmic transformation of the allometric equation and changing the bodyweight scaling exponent to approximately 1/3. However, many studies have reported that breed and body conformation influences canine echocardiographic measurements. The aim of this study was to compare transthoracic echocardiographic measurements to allometric scaling derived values in clinically normal adult dachshunds.

Forty-three standard dachshunds, aged 1-7 y, weighing ≥ 5 kg underwent physical examination, cursory echocardiography (for MVP and valvular defects), Doppler blood pressure measurements, electrocardiography assessment, complete blood count and thoracic radiographic examination. Echocardiographic measurements of 34 clinically healthy dachshunds were included. Adequacy of allometric scaling was evaluated by calculating the proportion of measured values that fell within the estimated ranges and its 95% confidence interval (CI). Average allometric scaling values were also compared to true values using Pearson's correlation coefficients (r), paired t -tests and Bland-Altman plots.

Variable	Mean $n = 34$	Measured values range		Allometric scaling range	Proportion of measured values within allometric scaling range (%)	CI (%)
		Min (Mean - 2SD)	Max (Mean + 2SD)	(Mean body weight = 8.3 kg)		
LA (mm)	20.4	15.2	25.6	12.2-20.1	50.0	33-66
Ao (mm)	14.5	11.7	17.3	13.0-19.8	100.0	92-100
IVSd (mm)	6.4	4.8	8.0	4.8-9.8	100.0	92-100
IVSs (mm)	8.7	6.9	10.5	7.2-13.1	97.1	86-100
LVIDd (mm)	27.5	21.0	34.1	23.7-34.5	94.1	82-99
LVIDs (mm)	16.2	10.3	22.1	13.8-25.4	82.4	67-93
LVPWd (mm)	6.5	4.9	8.1	4.7-9.8	100.0	92-100
LVPWs (mm)	9.8	7.3	12.3	7.7-13.9	100.0	92-100

(LA – left atrium, Ao –Aorta, IVS – interventricular septum, LVID – left ventricular internal diameter, LVPW – left ventricular posterior wall, d – diastole, s – systole)

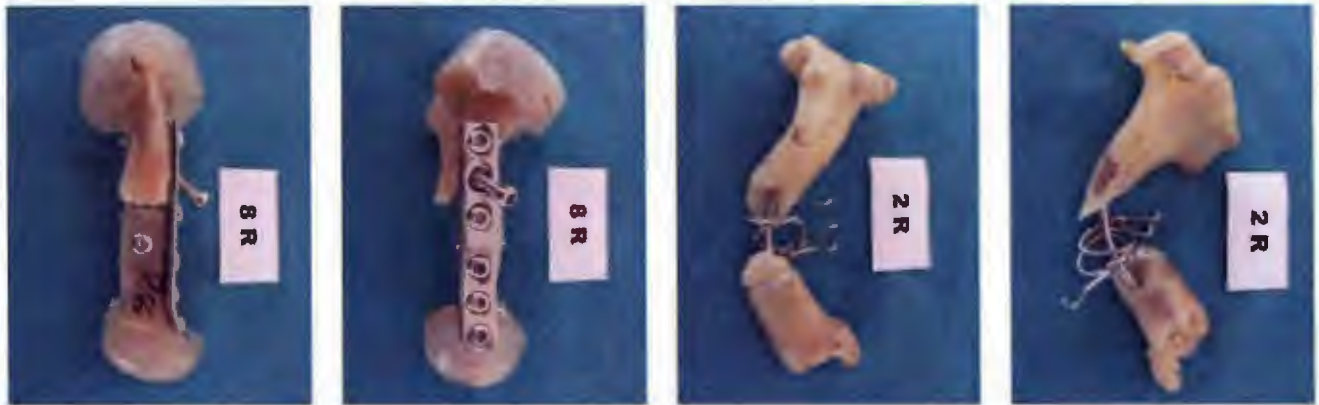
More than 90% of six variables were within the allometric scaling range due to the latter's wide prediction interval. Allometric scaling values may be inappropriate for LVIDs but sample size could be a limiting factor. Acquired LA values did not fit the allometric scaling values because the LA measurements were obtained using 2-dimensional right parasternal short axis view compared to the M-mode technique in allometric scaling. Breed-specific echocardiographic references should be used whenever available.



An *in vitro* biomechanical comparison between intramedullary pinning and the use of plates in the dachshund tibia

F Malan¹, GL Coetzee¹, NDL Burger², J Grimbeek³, PN Thompson⁴, A Carstens¹

¹Department of Companion Animal Clinical Studies, Faculty of Veterinary Science, University of Pretoria, Onderstepoort, South Africa; ²CMTI Consulting (Pty) Ltd, Pretoria, South Africa; ³Department of Statistics, Faculty of Natural and Agricultural Sciences, University of Pretoria, South Africa; ⁴Department of Production Animal Studies, Faculty of Veterinary Science, University of Pretoria, Onderstepoort, South Africa; fredmalan@iantic.net



The dachshund, a chondrodystrophic dog breed, presents a unique challenge in the treatment of tibial fractures by having short and curvaceous tibiae, leading to high risk of implant failure. In this study, intramedullary pins with full cerclage wires (IMPW) as an option in the treatment of oblique diaphyseal tibial fractures was studied *in vitro*. This fixation technique was biomechanically compared with the current gold standard in internal stabilization, bone plates and screws (BPS).

Twenty tibiae recovered from adult dachshund cadavers were randomly allocated into two groups of ten bones each. Oblique mid-diaphyseal fractures, running in a proximocranial-distocaudal direction in the middle third of the tibial diaphysis, simulated by osteotomy, were repaired by either IMPW or BPS. Tibiae were then subjected to a two point single cycle axial compression test, by applying an increasing compression load up to failure. Stress-strain graphs were drawn for each test specimen and stress (applied load) and strain (deformation) at yield, ultimate strength and failure were determined. Radiographs and digital photographs were made pre-osteotomy, post-osteotomy, post-repair and post-test, and modes of failure noted. Mean stress and strain values were compared between groups using ANCOVA.

In the IMPW group, 50% of specimens failed due to unravelling or slippage with displacement of the cerclage wires, 30% due to bone fracture at a cerclage wire, and 20% due to bone fracture elsewhere. In the BPS group, 80% failed due to bone

fracture at one or more screw holes, whereas 20% failed due to bone fracture not directly associated with the implants. No bone plate or screw underwent plastic (permanent) deformation, whereas 80% of the IM pins and 30% of the cerclage wires did. Mean stress at the yield point were 0.323 MPa and 0.403 MPa respectively, at the point of ultimate strength 0.383 MPa and 0.431 MPa respectively, and at the failure point 0.345 MPa and 0.403 MPa respectively, for the IMPW and BPS groups. Mean strain at the yield point were 0.30% and 0.36% respectively, at the point of ultimate strength 0.41% and 0.47% respectively, and at the failure point 0.71% and 0.84% respectively, for the IMPW and BPS groups.

Subjectively, there was an indication that the BPS group was more resistant to deformation by the loads applied than the IMPW group. However, statistically, there were no significant differences in stress at yield ($P = 0.299$), ultimate strength ($P = 0.275$), or failure ($P = 0.137$) between the two groups. Similarly, there were no significant differences in strain at yield ($P = 0.684$), ultimate strength ($P = 0.778$), or failure ($P = 0.505$) between the two groups.

In conclusion, this study did not show enough evidence for a difference between the two methods of fixation. Therefore, it is suggested that intramedullary pins and full cerclage wires may be used as an acceptable alternative to bone plates and screws in the treatment of oblique mid-diaphyseal tibial fractures in chondrodystrophic dog breeds.

Analgesic effects of epidural magnesium sulphate alone and in combination with morphine in dogs

A Bahrenberg¹, E Rioja¹, TB Dzikiti¹, GF Stegmann¹, GT Fosgate²

¹Department of Companion Animal Clinical Studies, and ²Department of Production Animal Studies, Faculty of Veterinary Science, University of Pretoria, Onderstepoort, South Africa; A.Bahrenberg@gmx.de



Epidural administration of drugs is commonly used in veterinary medicine. Magnesium sulphate (MgSO_4) is a physiological antagonist of the N-methyl-D-aspartate (NMDA) receptor. It has been reported to induce analgesia at the level of the spinal cord and may have synergistic effects when administered in combination with opioids. The objective of this study was to evaluate the analgesic effects of MgSO_4 administered epidurally alone and in combination with morphine.

The study was designed as a randomized, blinded cross-over study with six healthy adult Beagle dogs. Treatments included MgSO_4 (2.5 mg/kg) alone (Mg), or combined with morphine (0.1 mg/kg) (MM), morphine alone (0.1 mg/kg) (Mo) or sterile water (0.115 mL/kg) (C), injected into the lumbosacral epidural space. Antinociception was measured using the von Frey mechanical threshold device applied to the carpal pads, thorax and metatarsi. Measurements were recorded over 24 hours. Sedation, behaviour and motor effects were also

measured and data analysed using a linear mixed model with significance set as $P < 0.05$.

There were significant effects of treatment and time in all regions. Overall threshold values in grams (median [interquartile range]) when regions were combined were significantly higher in Mg (164 [135-200]), MM (158 [131-192]) and Mo (156 [129-195]) compared to C (145 [120-179]). Thresholds were significantly higher compared to C in Mg, MM and Mo in the metatarsi and thorax, but only in Mg and Mo in the carpal pads. Onset and duration of analgesia could not be determined. No motor deficits or adverse effects due to drug administration were observed at any time point.

In conclusion, MgSO_4 injected epidurally produced analgesia without motor deficits but did not enhance morphine analgesia in this study. The potential clinical application of epidural of MgSO_4 in animals with central sensitization remains to be studied.

Anaesthetic, analgesic and cardiorespiratory effects of intramuscular medetomidine and ketamine alone or in combination with morphine or tramadol for orchietomy in cats

GE Zeller¹, E Rloja¹, GF Stegmann¹, GT Fosgate², FJ Venter¹, TB Dzikiti¹

¹Department of Companion Animal Clinical Studies, and ²Department of Production Animal Studies, Faculty of Veterinary Science, University of Pretoria, Onderstepoort, South Africa; gareth.zeiler@up.ac.za

Intramuscular administration of general anaesthetic agents is common in feline clinical practice. The objective of this study was to compare the anaesthetic, analgesic and cardiorespiratory effects of intramuscular medetomidine and ketamine administered alone or combined with morphine or tramadol for orchietomy in cats using a randomized, clinical study of 30 cats.

Cats received a combination of medetomidine (60 µg/kg) and ketamine (10 mg/kg) alone (MedK) or combined with morphine (0.2 mg/kg) (MedKM) or tramadol (2 mg/kg) (MedKT) intramuscularly. Induction and anaesthetic recovery times and quality were recorded by a blinded researcher. Pre-surgery arterial blood gases were measured. Heart rate, respiration rate, Doppler arterial blood pressure (DABP), haemoglobin saturation (SpO₂) and end-tidal carbon dioxide (ETCO₂) were recorded every 5 minutes during general anaesthesia and at each surgical stage. Analgesia was evaluated with a visual analogue scale, a composite scoring system and the von Frey mechanical threshold device every hour from 3 to 8 hours post-injection. Data were analysed with a linear mixed model, Kruskal Wallis or chi-square tests ($P < 0.05$).

“Surgery had a significant effect on DABP ($P < 0.001$), SpO₂ ($P < 0.001$), respiratory ($P = 0.003$) and heart rates ($P = 0.002$), which increased, and ETCO₂ ($P = 0.003$), which decreased, with all combinations.”

Median (range) induction and recovery times (minutes) were not significantly different among the combinations: 5.6 (2.7, 8.0), 7.4 (5.1, 9.6) and 8 (5.8, 14.9) for induction and 128 (95, 143), 166 (123, 210) and 143 (123, 180) for recovery, with MedK, MedKT and MedKM, respectively. All three combinations caused low PaO₂ (mean ± SD: 66.2 ± 1.7 mmHg). Surgery had a significant effect on DABP ($P < 0.001$), SpO₂ ($P < 0.001$), respiratory ($P = 0.003$) and heart rates ($P = 0.002$), which increased, and ETCO₂ ($P = 0.003$), which decreased, with all combinations. Non-significant differences were found in pain scores and von Frey results among treatments; however, the von Frey changes over time did vary by treatment ($P < 0.001$) with the MedK group returning to baseline values more rapidly than MedKM and MedKT. None of the cats required rescue analgesics.

All three protocols provide adequate anaesthesia and analgesia for orchietomy in cats. Oxygen should be supplemented.



The value of the Onderstepoort Feedlot and AI challenges as educational tools

DE Holm¹, L Strydom², M van Aarde³, CA Poggenpoel³, SG Beukes³, D Bester³

¹Department of Production Animal Studies, Faculty of Veterinary Science, University of Pretoria, Onderstepoort, South Africa; ²Current address: Unit 3, Orchard Terrace, Alkmaar, Nelpruit; ³Onderstepoort Production Animal Group; dietmar.holm@up.ac.za



A perception exists that the future of veterinary services in the countryside is under threat due to a decrease in young veterinarians entering this career. Veterinary career choice can be influenced by several factors from student selection to first employment. The Onderstepoort Feedlot and AI Challenges were introduced into the veterinary curriculum in 2007 to address the above problem, amongst other objectives. A retrospective interview survey was done by email and telephonic communication in 2011 amongst students and young veterinarians that had completed the Feedlot or AI Challenges to determine whether some of the initial objectives had been met. The questionnaire was divided into two parts, the first being questions classifying each respondent into various categories, and the second part consisted of questions aimed at subjective opinions of the respondent regarding the value of the Feedlot and AI Challenges as educational tools, and the effect they had on career choice. Descriptive statistics were performed, and ratios were compared using the Fisher's exact test in Epicalc 2000.

Male respondents were 1.6 times more likely to consider a career in production animal practice than female respondents (27/38 vs. 44/97, $P < 0.01$). However females contributed 1.6 times more to the total number of young veterinarians interested in production animal practice (44 vs. 27). Similarly, although respondents from a farming background were 1.9 times more likely to consider a career in production animal practice than those from an urban background (24/30 vs. 45/105, $P < 0.01$), the total contribution to a production animal career was higher from urban background respondents.

When interviewed on the educational value of the Challenges, 117 (87%, 95% CI: 80 - 92%) of the respondents were of the opinion that it played a positive role in their studies. Of the recently qualified respondents 22/35 (63%) indicated that the Feedlot Challenge helped them in their professional career in general. Of the respondents who did not consider a career in production animals before the Challenges, 19/63 (30%) indicated that the Challenges motivated them to consider a career switch. Eighteen (95%) and 17 (89%) of these were from an urban background ($P = 0.07$), and female ($P = 0.097$), respectively. Ethnic group did not affect career choice or opinion about the educational value of the Feedlot/AI Challenge.

It is concluded that farming background and gender of respondent were indeed important determinants of veterinary career choice. However, due to the high proportion of female veterinary students and those from an urban background, these two categories contributed more to the total number of young veterinarians interested in entering rural veterinary practice than male respondents and those from a farming background respectively.

It is further concluded that the Feedlot/AI Challenges can influence career choice during the formative years, in particular for female students and those from an urban background, and it is a valuable educational tool for a general veterinary career. An increase in the number of female veterinarians can be expected in future production animal veterinary practice.

The role of the centriolar complex in the formation of sperm defects in the emu (*Dromaius novaehollandiae*)

L du Plessis¹, JT Soley²

¹Electron Microscope Unit, and ²Department of Anatomy and Physiology, Faculty of Veterinary Science, University of Pretoria, Onderstepoort, South Africa; lizette.duplessis@up.ac.za

The head base / neck region of sperm, and in particular that of avian sperm, has been identified as potentially vulnerable and prone to a variety of defects. During spermiogenesis in vertebrates the centriolar complex is responsible for initiating tail formation and for attachment of the flagellum to the base of the nucleus. In mammals, incorrect alignment of the centriolar complex has been implicated in a number of serious sperm defects and studies of defective sperm in the emu appear to indicate a similar aetiology.

Semen samples were collected from the distal *ductus deferens* of 15 sexually mature emus, *Dromaius novaehollandiae*, slaughtered commercially during the mid-breeding season. Samples were immediately fixed in 2.5% 0.13 M Millonig's phosphate-buffered glutaraldehyde. Smears for light microscopy (LM) were prepared from the fixed semen samples, stained with Wright's stain and examined microscopically using phase contrast illumination. Semen samples and testicular material were also collected for transmission electron microscopy (TEM). The semen samples were fixed in the same manner as for LM, while the blocks of testicular material were fixed in a similarly buffered 4% glutaraldehyde solution. Samples were processed for TEM using standard techniques and viewed with a Philips CM10 transmission electron microscope.

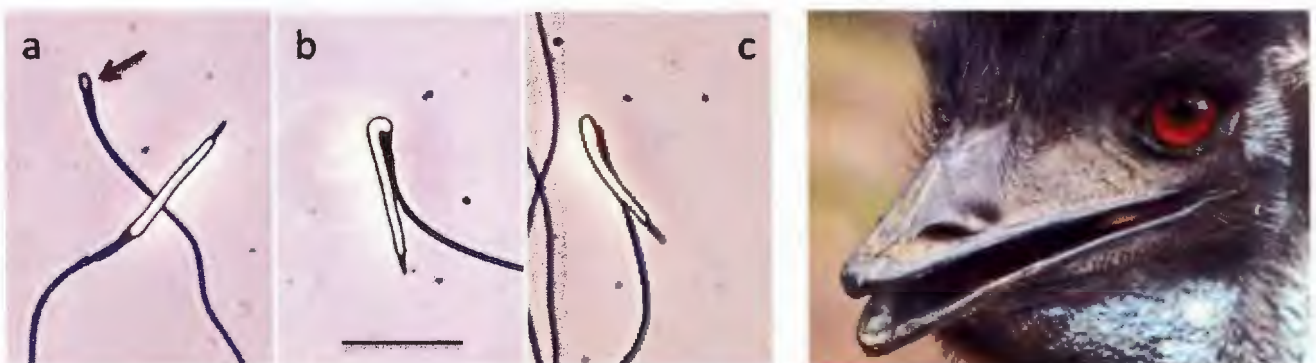
Both semen and testicular samples revealed a range of defects implicating malfunction of the centriolar complex in their formation. These anomalies, albeit in low numbers,

manifested as multiple tails, abaxial tail implantation, bending of the head base as well as disjointed and acephalic sperm (Figure). Multiple flagella resulted from the presence of supernumerary centriolar complexes whereas the other defects originated from the incorrect alignment of the centriolar complex with respect to the nucleus during the early stages of spermiogenesis. Attachment of the centriolar complex to the nucleus at an oblique angle caused head base bending. In both disjointed sperm (in which the midpiece and proximal part of the principal piece lie parallel to the nucleus) and acephalic sperm (where tails are correctly aligned), there was no contact between the nucleus and the centriolar complex. Abaxial tail formation resulted from an as yet unobserved misalignment of the centriolar complex and the nuclear base.

Although the various defects were readily observed by light microscopy, the underlying cause of the anomalies was only revealed by electron microscopy. TEM of testicular samples

revealed that head base bending, multiple-tailed sperm, disjointed sperm and abaxial sperm tails originated during the early stages of spermiogenesis. However, no indication was given of the origin of acephalic sperm, possibly due to the scarcity of the defect and the low probability of sectioning acephalic cells. These findings suggest that various degrees of incorrect orientation of the centriolar complex during early stages of spermiogenesis results in the manifestation of a variety of sperm head-base/neck defects in the emu. The propensity for such defects is exacerbated by the length of the centriolar complex in ratite sperm.

“Both semen and testicular samples revealed a range of defects implicating malfunction of the centriolar complex in their formation.”



Wright's stained smears of emu spermatozoa. (a) Normal sperm head and an acephalic sperm (arrow); (b) Sperm bent at the head base; (c) Disjointed sperm. Bar = 10 μ m.

Steroid profiling in crocodilian urine by gas-chromatography/mass spectrometry

LC Bekker¹, JG Myburgh¹, LG Gullette², CJ Botha¹

¹Section of Pharmacology and Toxicology, Department of Paraclinical Sciences, Faculty of Veterinary Science, University of Pretoria, Onderstepoort, South Africa; ²Department of Zoology, University of Florida, Gainesville, Florida, U.S.A.; lizette.bekker@gmail.com



Aquatic pollution is an important global concern and crocodilians, as top predators in aquatic ecosystems, are considered to be excellent sentinels of ecosystem health. Many aquatic pollutants, e.g. pesticides and pharmaceuticals, are bio-transformed by the liver and excreted in the urine, making urine a very useful diagnostic sample for scientists. Likewise, the evaluation of a urine sample, from individual human or animal patients, is a well-established diagnostic approach to obtain clinical data for evaluation of health and confirmation of specific diseases, e.g. endocrine system abnormalities.

The focus of this investigation was primarily on endogenous steroid hormones and their metabolites. Steroid profiling has been employed for extensive studies of urinary steroid metabolites in humans. The investigation of urinary hormones could provide valuable information on the endocrine axis of the crocodilians, and aid in the evaluation of the significance of this method and matrix (urine) as an indicator of endocrine disruption (ECD).

Urine samples from 22 American alligators (*Alligator mississippiensis*), collected and stored by the Department of Zoology, University of Florida, 7 from males, showing elevated plasma testosterone levels, 7 from females with

high plasma progesterone, and 8 from females with abnormal plasma oestradiol levels, were selected for analyses. The sample preparation and analyses were performed at the College of Medicine, University of Florida.

Preparation of urine samples for gas-chromatographic/mass spectrometric analysis was achieved with a five-step discipline, involving solid phase extraction (SPE), before and after enzymatic hydrolysis, and derivatization of the free steroids. Separation and detection was performed by gas chromatography/mass spectrometry (GC/MS).

Qualitative investigation showed the presence of various steroid hormones and their metabolites. Analytes that were identified included andosterone, etiocholanolone, 11-hydroxyandosterone (HAN), 11-hydroxyetiocholanolone (Het), 11-deoxytetrahydrocortisol (THS), and corticosterone.

In addition to identification, quantitative analysis of steroids and their metabolites in crocodilian urine may serve as a valuable diagnostic tool in the investigation of ECD effects. Establishing reference values for healthy animals from non-polluted environments will also aid in future monitoring of pollution.

Non-invasive monitoring of stress in Nile crocodiles: validation of a faecal glucocorticoid assay

SB Ganswindt¹, A Ganswindt^{2,3}, EZ Cameron^{3,4}, JG Myburgh¹

¹Department of Paraclinical Sciences and ²Department of Production Animal Studies, Faculty of Veterinary Science, University of Pretoria, Onderstepoort, South Africa; ³Mammal Research Institute, Department of Zoology and Entomology, University of Pretoria, Pretoria, South Africa; ⁴School of Zoology, University of Tasmania, Hobart, TAS, Australia; Stefanie.Ganswindt@up.ac.za

Nile crocodiles (*Crocodylus niloticus*) in the Olifants River, South Africa, are currently threatened by a pathological condition characterised by hardening of all their fat depots owing to inflammation (pansteatitis), subsequently causing immobility and a slow death. Owing to the practical difficulties when assessing a crocodile's state of health in the wild, non-invasively collected data on stress responses by crocodiles could help to identify afflicted areas. Such monitoring could also be applied to captive crocodiles because stress is a big concern for crocodile farmers if it negatively affects health and productivity. However, assays for non-invasive hormone monitoring need to be carefully validated to ensure biologically meaningful results. Thus far, no non-invasive method for determining stress-related physiological responses has been established for any crocodylian species.

Our aim was to identify a suitable enzyme immunoassay (EIA) to monitor adrenocortical endocrine function using faeces collected from captive Nile crocodiles as hormone matrix.

We performed an ACTH challenge on ten adult crocodiles (total body length 1.8 - 2.2 m) at Le Croc crocodile farm, South Africa. Intramuscular injections of synthetic ACTH (5 µg/kg) yielded serum corticosterone levels of up to ~1200% above pre-injection levels 1 - 5 hours post-injection. An additional eight individuals were exposed to electric immobilisation and handling only by injecting them with a physiological saline solution. In these animals, serum

corticosterone concentrations increased ~20 - 2700% above pre-injection levels, indicating that handling was already a sufficient stressor during this experiment. Of the 18 animals, six (four ACTH, two saline treated) were singly housed. The remaining animals (six of each treatment group) were either kept as pairs or in groups of four individuals.

All 18 animals were monitored for 17 days pre- and 18 days post-injection. Faecal samples pre- and post-injection could be obtained from only three singly housed crocodiles (two ACTH, one saline treated) and from all ponds housing more than one animal. Average defecation rates varied from 0.6 to 2.6 samples per week. A corticosterone, cortisol and two group-specific EIAs, against 5-reduced cortisol metabolites measuring 11,17-dioxoandrostanes and metabolites with a 5β-3α-ol-11-one structure (3α,11oxo-CM), respectively, were evaluated for faecal glucocorticoid metabolite (FGM) analysis, with the 3α,11oxo-CM EIA performing best. FGM levels in the three singly housed animals reached peaks of 380% (ACTH), 157% (ACTH), and 136% (saline) above pre-injection levels at about 7 to 15 days following treatment, respectively. Grouped FGM levels (interval of 5 days) in pair- and group-housed animals showed an elevation of 91% (ACTH) and 73% (saline) above baseline 7 to 10 days post-injection, respectively.

In conclusion, non-invasive hormone monitoring can be used for assessing adrenocortical function in captive Nile crocodiles based on FGM analysis.



Urban habitat moderates seasonality in the stress physiology, movement ecology and foraging behaviour of free-ranging banded mongooses

P Laver¹, A Ganswindt^{2,3}, SB Ganswindt³, M Williams⁴, K Alexander¹

¹Department of Fish and Wildlife Conservation, Virginia Tech, Blacksburg VA; ²Mammal Research Institute, Department of Zoology and Entomology, University of Pretoria, South Africa; ³Department of Production Animal Studies, and ⁴Department of Paraclinical Sciences, Faculty of Veterinary Science, University of Pretoria, Onderstepoort, South Africa; aganswindt@zoology.up.ac.za

With increasing pressure on pristine wildlife habitat and growing impact of reconciliation ecology, many species are colonizing urban environments. While some work has been done on indirect effects of urban life on wildlife physiology and fitness, these effects, particularly on stress physiology have not been characterized for most species.

We investigated the effect urban life, particularly dietary augmentation, has on stress physiology, movement ecology and foraging behaviour of a social carnivore, the banded mongoose (*Mungos mungo*). We used non-invasive faecal glucocorticoid monitoring, and home range utilization to investigate these effects on fourteen troops of free-ranging mongooses in habitats ranging from near-pristine to urban, in a seasonal environment.

Glucocorticoid metabolite concentrations from 1900 faecal samples strongly supported a candidate mixed effects model with troop, a troop by season interaction and faecal organic content as effects. This suggests that glucocorticoid

metabolite levels are best predicted by dietary constraints and troop/habitat modified by season. Seasonality in glucocorticoid production, expected to vary with food availability, was absent in urban troops while rural troops had elevated glucocorticoids in the food-limiting dry season. Home range utilization was focused on food augmentation sites for urban troops, but was diffuse for rural troops. Troops without access to anthropogenic food had larger home range sizes. Further effects were seen in troop demographics whereby troops with food augmentation were double the size of those without. Chronic elevation of glucocorticoid metabolites has been implicated in lowered skin immunity and lowered mycobacterial immunity. This population has regular outbreaks of a novel pathogen, *Mycobacterium mungi*, with a putative percutaneous portal of entry, which suggests a possible link between banded mongoose disease ecology, stress physiology and anthropogenic habitat modification. These indirect effects and implications for fitness in urban wildlife will increasingly be important considerations in conservation as we continue to expand urban environments.



Facing the challenge: rewilding South China tigers (*Panthera tigris amoyensis*) in South Africa

MC Fàbregas¹, HJ Bertschinger¹, G Koehler²

¹Department of Production Animal Studies, Faculty of Veterinary Science, University of Pretoria, Onderstepoort, South Africa; ²Carnivore Ecology Wildlife Research Unit, Washington Department of Fish & Wildlife, USA; maria.fabregas@uch.ceu.es



The South China tiger (*Panthera tigris amoyensis*) is the most endangered of the remaining six tiger subspecies; there are probably no more than 20 individuals in the wild while the captive population, mainly in Chinese zoos, numbers about 100. Given these numbers, conservation of this species can only be achieved by captive breeding and subsequent reintroduction to the wild. Save China's Tigers (SCT) is a charitable foundation that breeds and rewilds South China tigers for later reintroduction in restored protected areas in China.

The tiger rewilding programme, located on Laohu Valley Reserve, South Africa, has increased their tiger population from four animals in 2004 to 14 tigers in 2011. SCT has developed a "rewilding" methodology where captive-born tigers are provided with self-taught opportunities to learn to hunt prey animals independently. To date, all first and second generation tigers over 2 years of age ($n = 8$) have been successfully rewilded.

To assess their hunting proficiency, five adult rewilded tigers (four males and one female) were placed in groups of two or

three tigers in 40 ha and 100 ha fenced camps together, where a population of free-ranging blesbuck (*Damaliscus dorcas*) occurred at densities ranging from 2.5 to 55 blesbuck/km² in the 40 ha camp, and 15 to 60 animals/km² in the 100 ha camp. Data collection took place from January 2010 to June 2012, with a total of 868 days of behavioural data collection.

Data revealed differences in the mean kill rate between the 40 and the 100 ha camps, where the former reached values of 0.22 kills/tiger/day, while the later did not exceed 0.1 kills/tiger/day, which is the same figure as for free-ranging tigers. Time between kills was inversely related to blesbuck density in the 40 ha camp but not in the 100 ha camp.

"These preliminary results indicate that rewilded tigers are able to kill frequently enough to ensure an adequate food intake compared to wild, free-ranging tigers."

These preliminary results indicate that rewilded tigers are able to kill frequently enough to ensure an adequate food intake compared to wild, free-ranging tigers. Therefore, these animals can be free-released into reserves where they can hunt free-ranging, natural prey. However, additional data are needed to be able to generalize the success of the rewilding process for this species.

The postpartum endometrial inflammatory response: a normal physiological event with potential implications for bovine fertility

A Chapwanya^{1,5}, KG Meade², C Foley^{1,2}, F Narciandj¹, ACO Evans³, ML Doherty⁴, JJ Callanan⁴, C O'Farrelly¹

¹Comparative Immunology Group, School of Biochemistry and Immunology, Trinity College, Dublin, Ireland; ²Animal & Bioscience Research Department, Animal & Grassland Research and Innovation Centre, Teagasc, Co. Meath, Ireland; ³UCD School of Agriculture and Food Science, Belfield, Dublin, Ireland; ⁴UCD School of Veterinary Medicine, Belfield, Dublin, Ireland; ⁵Department of Production Animal Studies, Faculty of Veterinary Science, University of Pretoria, Onderstepoort, South Africa; kieran.meade@teagasc.ie

The uterus is a unique organ with an ability to respond to excessive physiological and pathological insults through a series of complex cellular and molecular interactions. Successful breeding requires timely uterine involution, resolution of the local uterine inflammatory process postpartum and resumption of ovulatory oestrous cycles. The multiple roles of the uterus include facilitating fertilisation, pregnancy and parturition, all of which are necessary for successful reproduction. After calving, the bovine endometrium undergoes marked morphological and functional changes that are necessary for subsequent re-breeding. Regulation and integration of these key events are largely uncharacterised.

Here, first-parity mixed-breed postpartum suckled beef cows ($n = 13$) were studied. The cows included Charolais ($n = 9$), Simmental ($n = 2$), Belgian blue ($n = 1$) and Holstein-Friesian ($n = 1$) cross-breeds. Endometrial swabs and biopsies were taken at 15, 30 and 60 days postpartum (DPP) from the healthy primiparous cows, ten of which subsequently conceived, with a view to characterising innate and inflammatory gene expression profiles. The samples were analysed histologically, by microbial culture and by qRT-PCR for innate immune gene expression.



Endometrial biopsies exhibited severe inflammation (>75 leukocytes per high-power field) at 15 DPP, which had begun to resolve by 30 DPP and had completely resolved by 60 DPP. The severe inflammation at 15 DPP coincided with uterine infection in all cows and a significant increase ($P < 0.05$) in the expression of all of 16 genes investigated, including CD45, IL8, IL6, IL1, TNF, TAP, SAA3 and HP at 15 DPP, relative to 60 DPP. All of these parameters had begun to return to normal physiological levels at 30 DPP. Systemically, serum protein concentrations of IL-8

were elevated at 15 DPP compared with 60 DPP (78 pg/mL vs 48 pg/mL; $P = 0.02$).

The capacity of the endometrium to mount an effective immune response after calving cannot be understated and clearly depends on the ability to recognise microbial ligands, recruitment of leukocytes and secretion of effector molecules.

“Successful breeding requires timely uterine involution, resolution of the local uterine inflammatory process postpartum and resumption of ovulatory oestrous cycles.”

These results suggest that in cattle, postpartum endometrial inflammation and leukocyte infiltration underpinned by increased expression of pro-inflammatory AMP and APP genes are normal responses beneficial for

bacterial clearance, uterine involution and restoration of a healthy endometrium for conception in cattle. Dysregulation of this inflammatory process may determine susceptibility to uterine disease and sub-fertility in cattle.

Endometrial epithelial cells: sentinels of innate immunity and potent producers of tumour necrosis factor, tracheal antimicrobial peptide and serum amyloid A3 gene expression in response to *E. coli* stimulation

A Chapwanya^{1,4}, KG Meade², ML Doherty³, JJ Callanan³, C O'Farrelly¹

¹Comparative Immunology Group, School of Biochemistry and Immunology, Trinity College, Dublin, Ireland; ²Animal & Grassland Research and Innovation Centre, Teagasc, Co. Meath, Ireland; ³UCD School of Agriculture, Food Science and Veterinary Medicine, Dublin, Ireland; ⁴Department of Production Animal Studies, Faculty of Veterinary Science, University of Pretoria, Onderstepoort, South Africa; cliona.ofarrelly@tcd.ie

Endometrial epithelial cells play a critical role in mediating inflammatory mechanisms that are key to bacterial clearance and tissue re-modelling postpartum. Infection of the uterus is an important cause of infertility in dairy cows and dysregulated inflammatory responses to contaminating bacteria underlie endometritis and sub-fertility. Gram negative coliform bacteria induce acute inflammation of the endometrium, and *Escherichia coli* is the predominant pathogen isolated from animals affected with uterine disease where epithelial cells play a key role in local innate immunity.



This study characterised innate immune gene expression by bovine endometrial epithelial cells from three animals in response to *E. coli*, a common cause of bovine uterine disease. Uteri from three non-pregnant cows were collected at a local abattoir immediately after slaughter and kept on ice until further processing in the laboratory. In the laboratory, the endometrium was dissected from the horn, cut into strips and placed in Hanks Balanced Salt Solution to harvest epithelial cells which were then cultured. Pure endometrial epithelial cells were then co-cultured with heat killed *E. coli*. Expression of key innate immune genes, encoding toll-like receptor 4 (TLR4), the transcription factor NFκB1, the chemokine interleukin 8 (IL8), inflammatory cytokines (interleukins IL1β, IL6; tumour necrosis factor, TNF), β-defensins (lingual antimicrobial peptides LAP, tracheal antimicrobial peptide TAP) and acute phase proteins (haptoglobin, HP; serum amyloid A, SAA3) was examined in endometrial epithelial cells stimulated with *E. coli* for 6 and 24 hours using qRT-PCR. After RNA extraction fold changes for qRT-PCR were calculated using the 2^{-ΔΔCt} method as the expression of the gene of interest in stimulated endometrial cells, compared

to expression of the normaliser gene (*GAPDH*), and shown relative to the mean in non-stimulated cells.

Expression of all genes was increased significantly ($P < 0.05$) 6 hours post-stimulation. Expression of IL1b, TNF and SAA3 genes was increased by 121-, 357- and 721-fold respectively ($P < 0.05$). Twenty four hours post-stimulation, IL1b, IL6, IL8, TNF and LAP gene expression was decreased compared to 6 hours, whereas TAP and SAA3 expression was further increased to 209- and 3452-fold ($P < 0.05$). *E. coli* driven expression of immune effector genes demonstrates potent immune, antimicrobial and regulatory capacity of endometrial epithelial cells to respond to this pathogen. Because APPs are proposed as diagnostic markers of uterine infection the present study demonstrates localised endometrial expression of SAA3 is a sensitive diagnostic for uterine and specifically *E. coli* infection in cattle.

“This study characterised innate immune gene expression by bovine endometrial epithelial cells in response to *E. coli*, a common cause of bovine uterine disease.”

Global endometrial transcriptomic profiling during involution: transient immune activation precedes tissue proliferation and repair in postpartum healthy beef cows

C Foley^{1,2}, A Chapwanya^{2,3}, C Creevey¹, F Narciand¹, D Morris⁴, E Kenny⁴, P Cormican⁴, JJ Callanan⁵, C O'Farrelly², KG Meade¹

¹Animal & Bioscience Research Department, Animal & Grassland Research and Innovation Centre, Teagasc, Grange, Co. Meath, Ireland; ²Comparative Immunology Group, School of Biochemistry and Immunology, Trinity College, Dublin, Ireland; ³Department of Production Animal Studies, Faculty of Veterinary Science, University of Pretoria, Onderstepoort, South Africa; ⁴Trinity Genome Sequencing Laboratory, Institute of Molecular Medicine, Trinity College, Dublin, Ireland; ⁵UCD School of Veterinary Medicine, Belfield, Dublin, Ireland; kieran.meade@teagasc.ie

Uterine involution is the time period after parturition, within which the uterus returns to a physiological state capable of nurturing a new conceptus. Completion of involution encompasses physiological and histological processes such as size reduction, contraction, caruncle shedding, necrosis and tissue remodelling. All cows experience endometrial bacterial contamination and tissue remodelling postpartum, instigating local inflammatory activity. However, mechanisms that control inflammation and restore functional endometrium while avoiding pathology are not defined.

This study aimed to identify novel candidate genes indicative of inflammation resolution during involution in healthy beef cows. We generated a genome-wide transcriptomic profile of endometrial biopsies 15 and 30 days postpartum (DPP). DNA was amplified and paired-end sequenced using the Illumina® Genome Analyzer II (GAII) and reads were then mapped to the bovine genome (version Btau_4.0.62) using the genome aligner TopHat. A plot was constructed that displayed the sample relations on multidimensional scaling (MDS). Bovine Ensembl genes were then converted to human Ensembl orthologs.

mRNA-Seq revealed that 1,107 genes were differentially expressed, 73 of which were increased 15 DPP and 1,034 increased at 30 DPP ($P < 0.05$; $n = 3$ at both time-points). GoSeq analysis revealed enrichment of immune response pathways 15 DPP. However, at 30 DPP, expression of the majority of genes was increased with tissue repair and proliferative activity pathways particularly enriched.

Candidate genes from the mRNA-Seq study were independently validated by qRT-PCR in additional cows ($n = 5$) at both time points. SAA2, GATA2, IGF1, SHC2, and SERPINA14 genes remained significantly elevated at 30 DPP in all animals.

These results reveal early activation of inflammatory mechanisms which undergo a temporal functional change toward tissue proliferation and regeneration during endometrial involution in healthy postpartum cows. The molecular changes mirror the physiological evidence of activation and resolution of endometrial inflammation identified by neutrophil infiltration.

The present study has identified novel gene expression patterns associated with involution which may become useful potential markers for unresolved endometrial inflammation in the postpartum cow.

“The molecular changes mirror the physiological evidence of activation and resolution of endometrial inflammation identified by neutrophil infiltration.”



The survival of eight different mastitis pathogens in milk after being frozen at -196°C and -20°C for 18 and 30 weeks respectively

JC Watermeyer, IM Petzer, J Karzis, TJ van der Schans

Section Udder Health, Department of Production Animal Studies, Faculty of Veterinary Science, University of Pretoria, Onderstepoort, South Africa;
corrie.watermeyer@up.ac.za



The aim of this study was to determine the effect of freezing (-20°C) and ultra-freezing (-196°C) on the viability of common mastitogenic bacteria isolated from South African dairy herds. An effective preservation method is necessary for follow-up samples to proactively address udder health through follow up of samples from cows with increased somatic cell counts identified in the South African National Milk Recording Scheme (NMRS). Specifically, the period of time that mastitogenic bacteria could be re-isolated from milk samples was investigated.

Field strains of *Streptococcus agalactiae*, *S. dysgalactiae*, *S. uberis*, *Enterococcus faecalis*, *Escherichia coli*, coagulase negative staphylococci, *Staphylococcus aureus* (phage type, lytic group 3) (STH) and other lytic groups (STA) were diluted in UHT milk equal to a 0.5 McFarland standard and aliquots were placed in Eppendorf tubes and frozen, either in a household freezer at -20°C or in liquid nitrogen at -196°C . Ten samples of each organism were thawed and cultured for each temperature treatment every 2 weeks for 18 weeks, with one additional evaluation at week 30 for samples frozen at -20°C .

Freezing did not prevent isolation nor impair growth of *S. dysgalactiae*, *S. uberis* and *S. aureus* (STH and STA) for both temperature treatments for the full duration of this study. *Enterococcus faecalis* could also be isolated for the study duration from all samples, except at week 8 (at -196°C) when 10% of samples showed no growth. The organism which was most affected by freezing in this study was *S. agalactiae*.

From week 14, *S. agalactiae* could no longer be isolated from 50% of samples frozen at -20°C , but its growth remained strong until week 18 in samples frozen at -196°C . Although coagulase negative staphylococci could be isolated from all samples over the 18 weeks, their growth strength of 3+ and 2+ declined from week 10 at -20°C and week 14 at -196°C . Contrary to previous studies, *E. coli* remained viable for an extensive period of time at both temperature treatments. It could be isolated from all samples frozen at -20°C until week 16, but only from 50% of samples thereafter. In contrast to the other bacteria investigated, the viability of *E. coli* decreased slightly faster when frozen at -196°C than at -20°C . No growth could be detected in 10% of samples frozen at -196°C at weeks 14 and 18.

Results have indicated that producers can freeze milk samples for 12 weeks in their household freezers (-20°C), but thereafter 50% of *S. agalactiae* samples may become false negative on bacterial culture. Most other mastitogenic bacteria investigated could still be isolated for 30 weeks after being frozen at -20°C . Mastitogenic bacteria in milk samples remain viable at -196°C for up to 18 weeks. This can enable producers to sample cows with increased somatic cell counts and freeze these samples at either -20°C or -196°C until shipping to a laboratory. The KOH test used to assist in the identification of Gram-negative organisms took more than double the time to react when samples were frozen for 10 and 12 weeks at -20°C and -196°C , respectively, which may lead to false negative identification in the field.

Antibacterial sensitivities of the primary respiratory bacterial pathogens in the bovine respiratory disease complex in feedlot cattle in South Africa

CAP Carrington¹, GH Rautenbach¹, JA Picard², PN Thompson¹

¹Department of Production Animal Studies, Faculty of Veterinary Science, University of Pretoria, Onderstepoort, South Africa; ²Department of Microbiology and Immunology, School of Veterinary and Biomedical Sciences, Faculty of Medicine, Health and Molecular Sciences, James Cook University, Townsville, Queensland, Australia; chris.carrington@up.ac.za



The decision to use a particular antibiotic to treat bovine respiratory disease (BRD) depends on a range of factors, not least of which include the bacteria isolated and their susceptibility patterns. Transtracheal aspirate samples were collected from 713 clinically affected animals and 33 healthy animals from 37 feedlots throughout South Africa during the period 2000 to 2006 and processed according to standard bacterial culture techniques.

Antibiotics selected for the antibiograms used in the study were determined by the antibiotic groups that were in relatively common usage in the majority of feedlots when the study was started in 2000, as well as those products that have been specifically registered for use in BRD.

Over the survey period, very few multiple resistant organisms were isolated, but there was a trend towards more of these pathogens being identified. This is probably to be expected as the primary thrust in BRD management has up until now been the use of antimicrobials. The few cases of multiple resistant organisms encountered could in the majority of cases also be linked to feedlot operations with poorer management and antibiotic usage protocols.

Mannheimia haemolytica, *Pasteurella multocida* and *Histophilus somni* remained sensitive to the most commonly used antibiotics. *M. haemolytica* (299 isolates) was more than 95% sensitive to amoxycillin, potentiated sulphonamides, ceftiofur and florfenicol, 84% to oxytetracyclines. Enrofloxacin and tilmicosin showed only 75% sensitivity and a definite trend of increasing resistance.

P. multocida (312 isolates) sensitivity patterns were more variable than for *M. haemolytica*. Again, the aminoglycosides (35%) and tilmicosin (55%) showed poor sensitivities, with enrofloxacin showing a trend towards increasing resistance (from 100% to 70%). Sensitivity to amoxycillin, ceftiofur and florfenicol were >90%. *P. multocida* is known to be more resistant to tetracycline and the sensitivity was generally poor (45%).

Only 39 *H. somni* isolates were identified for the period, with >90% sensitivity to ceftiofur, florfenicol and potentiated sulphonamides, 80% to amoxicillin, enrofloxacin and tetracycline, 75% to tilmicosin, 70% to penicillin G and 55% to the aminoglycosides.

Bacterial isolations in bovine respiratory disease complex in feedlot cattle in South Africa

CAP Carrington¹, GH Rautenbach¹, JA Picard², PN Thompson¹

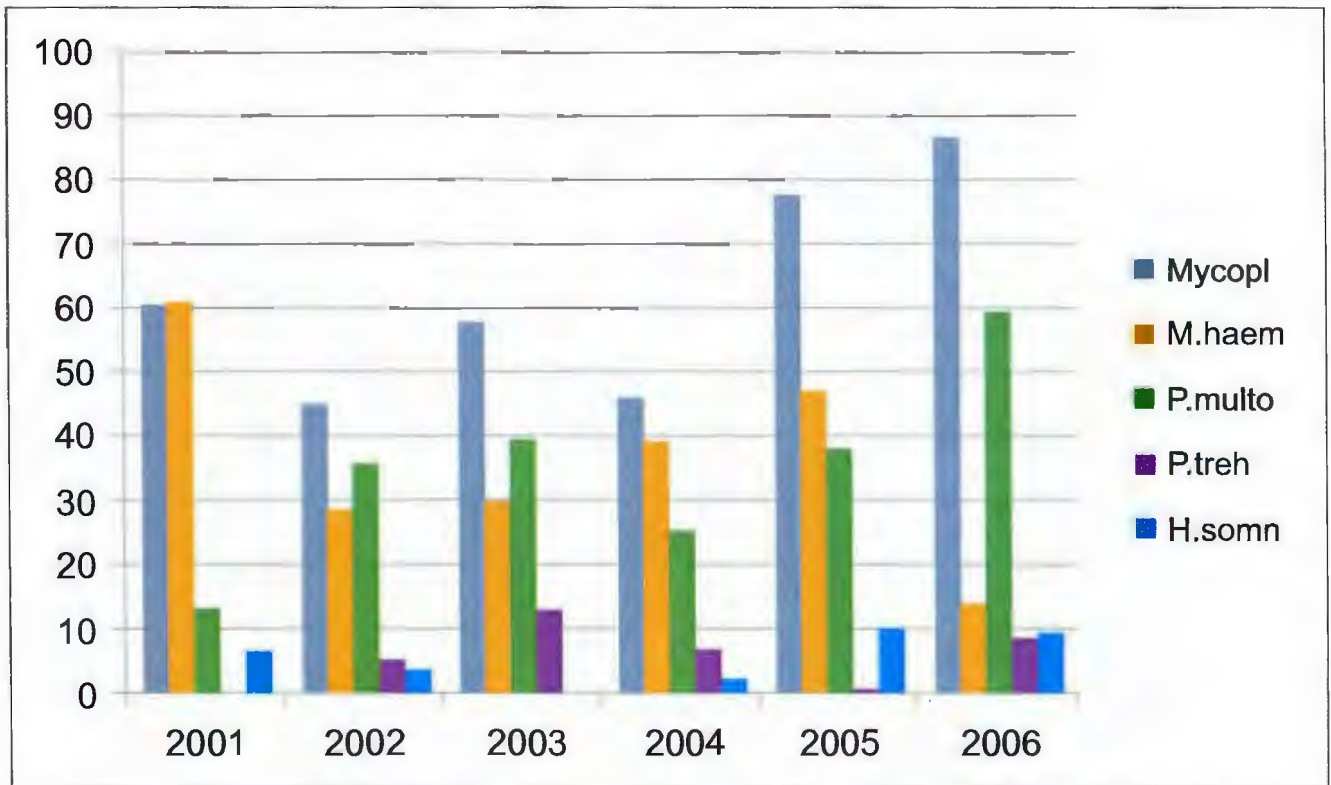
¹Department of Production Animal Studies, Faculty of Veterinary Science, University of Pretoria, Onderstepoort, South Africa; ²Department of Microbiology and Immunology, School of Veterinary and Biomedical Sciences, Faculty of Medicine, Health and Molecular Sciences, James Cook University, Townsville, Queensland, Australia; chris.carrington@up.ac.za

Mannheimia haemolytica type A1 was always considered to be the most important bacterial pathogen isolated from cases of respiratory disease in feedlot cattle in South Africa. However, analysis of transtracheal wash samples collected from acute outbreaks of respiratory disease in South African feedlot cattle for the period 2000 to 2006 showed that *Mycoplasma* spp. and *Pasteurella multocida* were more commonly isolated than *Mannheimia haemolytica* and *Histophilus somni*.

Transtracheal aspirate samples were collected from 713 clinically affected animals and 33 healthy animals from 37 feedlots throughout South Africa during the period 2000 to 2006 and processed according to standard bacterial culture techniques.

The ratio of *M. haemolytica* to *P. multocida* in the study showed an interesting trend, with samples taken in 2000/1 having a *M. haemolytica* isolation rate of 61.0% which was in line with previous findings in South Africa. This isolation rate dropped sharply to 28.8% in 2002 and by the end of the study the isolation rate of *P. multocida* reached 59.6%.

The decrease in 2002 coincided with the availability of a new *M. haemolytica* antileukotoxin vaccine and with the withdrawal of a previously used *P. multocida* bacterin vaccine.



Percentage of mycoplasma and bacterial isolates from infected feedlot cattle.

The role of *Mycoplasma* spp. in bovine respiratory disease complex in feedlot cattle in South Africa

CAP Carrington¹, GH Rautenbach¹, JA Picard², PN Thompson¹

¹Department of Production Animal Studies, Faculty of Veterinary Science, University of Pretoria, Onderstepoort, South Africa; ²Department of Microbiology and Immunology, School of Veterinary and Biomedical Sciences, Faculty of Medicine, Health and Molecular Sciences, James Cook University, Townsville, Queensland, Australia; chris.carrington@up.ac.za



Bovine respiratory disease complex (BRD) is largely a single clinical entity of bronchopneumonia usually associated with the assembly of large numbers of weaner cattle into a feedlot

environment. The presence of *Mycoplasma* spp. has been shown to increase the severity of respiratory disease and the aim of the study was to identify the isolation rates of *Mycoplasma* spp. in feedlot cattle in South Africa.

Transtracheal aspirate samples were collected from 713 clinically affected animals and 33 healthy animals from 37 feedlots throughout South Africa during the period 2000 to 2006.

According to the literature, mycoplasmas are isolated from 25% to 80% of pneumonic lungs and from 5% to 10% of lungs in healthy feedlot cattle. In this study the average isolation rate in was 62.4% in affected animals and 22.6% in healthy cattle. There was a significant (odds ratio = 4.61, 95% CI: 3.25, 6.54; $P < 0.001$) association between *Mycoplasma* isolation and respiratory disease in feedlot cattle in South Africa.

A fairly consistent finding was that in 11.3% of samples (range 6.9 to 17.5%) *Mycoplasma* spp. were isolated without primary respiratory bacteria being present.

Year	Number of samples	<i>Mycoplasma</i> spp.		<i>M. haemolytica</i>		<i>P. multocida</i>		<i>P. trehalosi</i>		<i>H. somni</i>		No microbial growth		<i>Mycoplasma</i> without primary bacteria	
		n	%	n	%	n	%	n	%	n	%	n	%		
2000/1	99	60	60.6	64	61.0	14	13.3	–	–	7	6.7	18	17.1	11	11.1
2002	111	50	45.0	32	28.8	40	36.0	6	5.4	4	3.6	21	18.9	12	10.8
2003	121	70	57.9	36	29.8	48	39.7	16	13.2	0	0	19	15.7	13	10.7
2004	87	40	46.0	34	39.1	22	25.3	6	6.9	2	2.3	13	14.9	6	6.9
2005	136	106	77.9	64	47.1	52	38.2	1	0.7	14	10.3	14	10.3	15	11.0
2006	114	99	86.8	16	14.0	68	59.6	10	8.8	11	9.6	7	6.1	20	17.5

Summary of mycoplasmal and primary respiratory bacterial pathogens from feedlot cattle with respiratory disease.

Evaluation of medicinal turpentine used for the prevention of bovine babesiosis in southern KwaZulu-Natal and the eastern Free State

LJ Biggs¹, CAP Carrington¹, V Naidoo²

¹Department of Production Animal Studies, Faculty of Veterinary Science, and ²Biomedical Research Centre, University of Pretoria, Onderstepoort, South Africa; drljbiggs@gmail.com

Medicinal turpentine has been used extensively in the eastern Free State and KwaZulu-Natal in the belief that it is able to prevent and treat redwater in cattle, with reportedly excellent results. Redwater is often a fatal disease in cattle and results in losses of large numbers every year in South Africa. In this study redwater is used to refer to disease caused by both *Babesia bigemina* (African redwater) and *Babesia bovis* (Asiatic redwater). Medicinal turpentine is obtained by the distillation of resin obtained from numerous pine tree species as a by-product during the production of chemical wood pulp. This study was initiated in an attempt to investigate the validity in the use of the turpentine as a medicinal agent. For this study the use of turpentine was evaluated in three stages.

The first component of the study involved a survey with ten commercial farmers who were known to be using turpentine. The second part of the study made use of a screening assay of *B. caballi* in a red cell culture which was exposed to various concentrations of turpentine in comparison to diminazene and imidocarb. In the third part a tolerance study was undertaken using 24 replacement heifers. Three different volumes of turpentine were used (1, 3 and 5 times the recommended dose of 2 mL, i.e. 2 mL, 6 mL and 10 mL), and a control group was treated with 2 mL normal saline. All treatments were given by subcutaneous injection in the neck or shoulder

area. Blood was then drawn from these animals at specified intervals and pre-determined parameters were measured. This included obtaining serum for *B. bovis* and *B. bigemina* serology to determine whether the animals used had ever been exposed to either of these parasites. Temperatures, weights, pregnancy status and injection site reactions were also monitored.

The cultures indicated that turpentine may have a delayed static effect on parasite growth or an indirectly cidal effect, as the final parasitaemia was reduced by 30%. The tolerance study revealed that irritation was noted at the injection site at the higher dose. The pregnant animals in the study did not abort or deliver unhealthy calves. There was an indication that turpentine does induce a fever reaction when injected into cattle. The most consistent findings in the blood results, where statistical and biological significance was established, were found in the serum globulin levels; increased globulin concentrations suggested that the product may be acting as an adjuvant and having an immune stimulating effect. In studies of malaria in mice, turpentine clearly demonstrated its ability to decrease schizont development of by up-regulating cytokines. Further studies are necessary to determine the exact effect turpentine has on immune-mediated immunity.



Cytotoxicity and mutagenicity investigation of extracts of common South African ethnoveterinary plants

LJ McGaw¹, EE Elgorash², JN Eloff¹

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

²Toxicology and Ethnoveterinary Medicine, Onderstepoort Veterinary Research Institute, Onderstepoort, South Africa: lyndy.mcgaw@up.ac.za

South African plants used in traditional animal health or ethnoveterinary medicine (EVM) have been shown in various investigations to possess interesting antibacterial, antifungal and antiviral properties, but have rarely been investigated for toxicity. Rural people use a diverse array of plants to treat diseases in animals, especially wounds and infections. These plants need to be tested for toxicity to avoid use of potentially dangerous preparations. Assays to identify cytotoxic and genotoxic effects are a useful precursor to *in vivo* toxicity studies, providing preliminary indications of safety of plant extracts.

Extracts of sixteen plants used popularly in southern African EVM were prepared using acetone, a solvent which extracts compounds with a wide range of polarities. Cytotoxicity was determined using a colorimetric tetrazolium-based (MTT) assay against Vero kidney cells and bovine dermis cells. Mutagenic effects against *Salmonella typhimurium* strains TA98 and TA100 were examined using the Ames test.

Vero cells were generally less sensitive to the extracts than the bovine dermis cells. *Combretum caffrum* was the most

cytotoxic extract against both cell types, with LC₅₀ values less than 50 µg/mL. *Markhamia zanzibarica* was also relatively cytotoxic, but most of the plant extracts showed LC₅₀ values between 0.1 and 1 mg/mL. *Sclerocarya birrea* was not cytotoxic to either cell line up to the highest test concentration of 1 mg/mL. None of the plant extracts showed mutagenicity against the *S. typhimurium* strains tested, indicating lack of frame shift or base pair mutation capabilities without metabolic activation.

Preliminary studies of EVM plant extract toxicity assist in evaluating the potential toxic effects of traditional remedies, particularly as many of the plants in this study are applied topically to treat wounds and skin infections. These studies also inform the selection of plants for laboratory studies concerning isolation of selectively anti-infective compounds in the absence of non-specific toxicity. For confirmation of toxicity, however, *in vivo* studies are necessary.



***Mycobacterium tuberculosis* complex specific antigens for use in serodiagnosis**

BM Modise^{1,2,3}, J Fehrsen¹, AL Michel²

¹Immunology Section, ARC-Onderstepoort Veterinary Institute, Onderstepoort, South Africa; ² Department of Veterinary Tropical Diseases, University of Pretoria, Onderstepoort, South Africa; ³Botswana National Veterinary Laboratory, Department of Veterinary Services, Gaborone, Botswana; ModiseB@arc.agric.za

The effective control of bovine tuberculosis (BTB) in cattle and wildlife is of paramount importance and this can be achieved through the use of accurate and comprehensive diagnostic tests. The current methods used to detect BTB are the skin test and the *in vitro* gamma interferon assay. However, they do not detect anergic animals. Serological tests such as the fluorescence polarization assay (FPA) have shown promise in the diagnosis of tuberculosis. FPA is a simple and rapid test which can be performed in the field or laboratory for high throughput testing. It has the potential to cover the full spectrum of the immune responses during the disease, depending on the design of the tracer.

Hence, our study was aimed at developing a FPA using peptides derived from MPB70. Since no species-specific reagents are required, sera from bovine, buffaloes and lions can be tested.

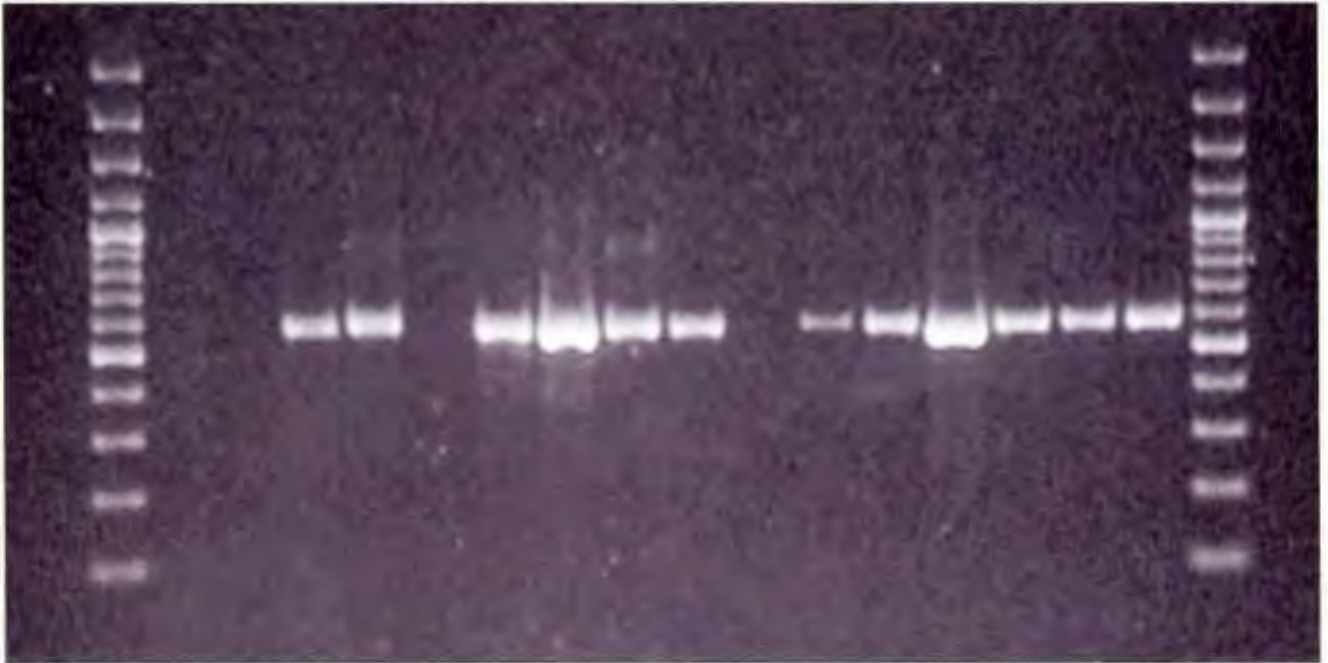
The *mpb70* gene was cloned, expressed as a histidine-tagged recombinant protein (rMPB70), purified and labeled with fluorescein. In addition, the gene was fragmented into three to include predicted epitopes. The gene fragments were also cloned and the resulting proteins were expressed as fusions with the monster green fluorescent protein. Gene fragments 2 and 3 were expressed successfully. In the ELISA, the rMPB70, fragment 2 MGFP fusions reacted with immune sera from BTB infected buffaloes. In the next step this fragment will be analyzed as overlapping peptides to find the optimum target for FPA.



The occurrence of non-tuberculous mycobacteria (NTM) in natural habitats of cattle and African buffalo in South Africa and immunologic reactivity caused in these species by natural NTM exposure (preliminary results)

N Goebe^{1,2}, VPMG Rutten^{2,4}, N Gey van Pittius³, AL Michel²

¹Tuberculosis Laboratory, Agricultural Research Council - Onderstepoort Veterinary Institute, Onderstepoort, South Africa; ²Department of Veterinary Tropical Diseases, Faculty of Veterinary Science, University of Pretoria, Onderstepoort, South Africa; ³Division of Molecular Biology and Human Genetics, Department of Biomedical Sciences, Faculty of Medical Sciences, Stellenbosch University, Tygerberg, South Africa; ⁴Division of Immunology, Department of Infectious Diseases and Immunology, Faculty of Veterinary Medicine, Utrecht University, Utrecht, The Netherlands; gceben@arc.agric.za



Non tuberculous mycobacteria (NTM) are now being recognised as emerging environmental, opportunistic pathogens capable of infecting both humans, especially those with compromised immune systems, and animals. Although most NTM species are known to rarely or never cause any diseases, literature has suggested that exposure of animals to NTM may influence the outcome of immunological assays for tuberculosis (TB) because of cross- reactive responses to antigens common to both members of the *Mycobacterium tuberculosis* complex (MTBC) and NTM. This study aimed to address the occurrence of NTM in cattle and African buffalo and their respective environments in South Africa and the potential of the NTM species to induce an immune response which may influence the diagnosis of TB.

Culture-based isolation methods for mycobacteria from soil and water have been evaluated and the use of NaOH and oxalic acid as decontaminants showed optimum decontamination and was optimised and used in subsequent experiments. We conducted a survey for NTM isolation in all the nine provinces of South Africa. A total of 175 NTM isolates were recovered from 495 samples (swabs, soil and water) originating from bovine and buffalo populations and their respective environments. Speciation of NTM isolates

from a collection of frozen cultures originating from animal lymphnodes, milk and soil samples at the ARC-OVI TB laboratory, as well as from isolates originating from the survey was done by 5'-16s rDNA PCR, followed by sequencing. The sequences were analysed using NCBI Blast searches (www.blast.ncbi.nlm.nih.gov/Blast.cgi) and RIDOM (www.ridom-rdna.de) databases.

A total of 171 NTM isolates was analysed and 31 different NTM species were identified; including unique NTM species closely related to other species in the database as well as potential novel species (NTM species with no close relative in the NCBI or RIDOM databases). Prevalence per animal species and its environment, per sample type as well as per province was defined. The two most prevalent species, *M. nonchromogenicum* and *M. vaccae*, were selected for production of purified protein derivatives (PPD) in order to investigate their potential to induce a cross-reactive immune response. Animal infection experiments to test the PPD are underway. Proteomic analysis of the PPD will be done and compared with proteomes of the commercial bovine (PPD-B) and avian (PPD-A) PPD in order to investigate antigens shared between the four PPD and antigens unique to *M. nonchromogenicum* and *M. vaccae*.

Evidence of TH1/Th17 immune responsiveness and cross reactivity to PPD-F in mice sensitised with atypical mycobacteria

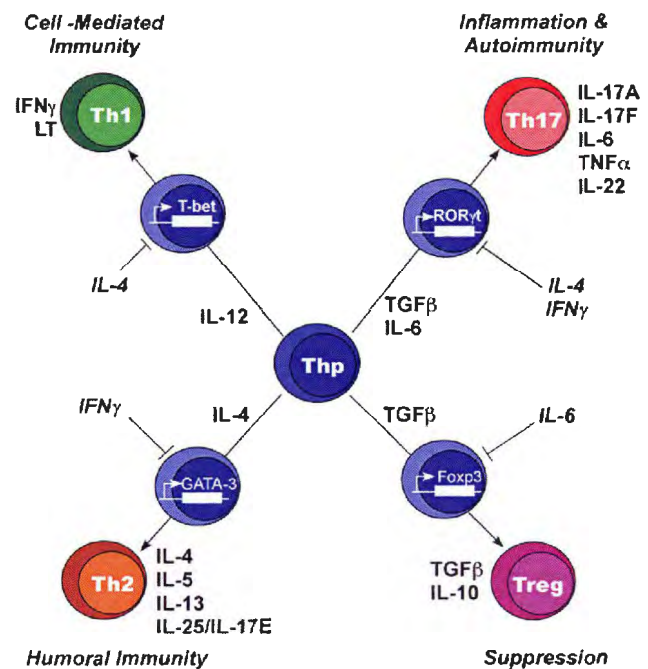
AO Jenkins¹, AL Michel¹, VPMG Rutten^{1,2}

¹Department of Veterinary Tropical Diseases, Faculty of Veterinary Science, University of Pretoria, Onderstepoort, South Africa; ²Infection and Immunity, Faculty of Veterinary Medicine, Utrecht University, The Netherlands; Akin.Jenkins@up.ac.za

Atypical mycobacteria, also known as environmental mycobacteria, have been reported to abound in tropical countries. Variable research findings in experimentally infected animals and naturally exposed animals and humans have raised suggestions with respect to atypical mycobacteria attenuating the effect of the *Mycobacterium bovis* BCG vaccine commonly used in humans. Attempts at using this vaccine in adult cattle, like in adult humans, also resulted in this vaccine providing incomplete protection against pulmonary tuberculosis. This phenomenon was explained to be a result of immunological cross reactivity due to antigens shared between atypical and tuberculous mycobacteria. Immune responses generated prior to BCG vaccination interfered with the multiplication of BCG bacilli, thereby leading to reduced protective efficacy. We investigated the consequences of exposure to atypical mycobacteria prior to BCG vaccination on experimental infection with *M. bovis* in a mouse model.

Immune responsiveness was assessed by determining the kinetics of cytokine production and appearance of clinical signs after exposure to two atypical mycobacteria, i.e. the non-pathogenic *M. fortuitum* and the occasionally pathogenic *M. kansasii*. Mice were pre-sensitized *per os* three times at weeks 1, 3 and 5 with 10^6 CFU/0.2 ml of either of these atypical mycobacteria followed by vaccination with *M. bovis* BCG 10^6 CFU sub-cutaneously at week 9 and then challenged with *M. bovis* by aerosol at week 16. The entire experiment ran a course of 22 weeks and two batches of euthanasia were done, at week 16 prior to *M. bovis* challenge and week 22 after the course of *M. bovis* infection. Th1/Th2 and Th17 cytokines were measured from the supernatant of PPD-F stimulated spleen cells in a multiplex assay using a multiplex bead assay (BD Biosciences).

IL17-A, a co-stimulatory cytokine, was measured in significantly higher quantities in the mice that were vaccinated prior to *M. bovis* infection (BCG+*M. bovis*+ mice) than in mice that were pre-sensitized with either atypical mycobacteria prior to vaccination and challenge. It was also found to be significantly higher in the BCG+*M. bovis*+ mice than mice that received *M. bovis* alone, suggesting that atypical sensitization prior to BCG vaccination is capable of further reducing BCG efficacy, and confirming previous studies in which BCG vaccination resulted in increased Th17 immunity. Although high IL17-A levels is not indicative of full protection, it is known to be secreted in high quantities



especially early after BCG vaccination triggering the secretion of pro-inflammatory cytokines, TNF- α and IL-6, the highest level of TNF- α seen in the *M. fortuitum*+BCG+*M. bovis*+ group and IL-6 seen in the *M. kansasii*+BCG+*M. bovis*+ group respectively. Th1 immune response is known to confer protection in tuberculous infection but was somewhat compromised in this study with the highest IFN- γ responses observed in mice that had *M. kansasii* prior to *M. bovis*. This group of mice however had significantly low IL-17A and generally low levels of IL-6 and TNF- α , suggesting that the pro-inflammatory qualities of the IL-6 and TNF- α were being utilized, and IFN- γ responses suggested a reasonable level of protection as unreasonably high levels of TNF- α and IL-6 normally results in tissue damage and subsequent progression to pathology, i.e. tending towards Th2 immunity.

In conclusion, the balance between Th1 and Th17 cytokines which has been described as essential for protection in tuberculous infection was displayed here and the effect of atypical mycobacteria in altering these dynamics was also shown. This information will be useful for future vaccine and diagnostic interventions.

Occurrence of tick-borne haemoparasites in the African buffalo (*Syncerus caffer*) in northern Botswana

D Eygelaar¹, F Jor², M Mokopasetso³, EM Debella¹, NE Collins¹, I Vorster¹, M Troskle¹, MC Oosthuizen¹

¹Department of Veterinary Tropical Diseases, Faculty of Veterinary Science, University of Pretoria, Onderstepoort, South Africa; ²Mammal Research Institute, Department of Zoology and Entomology, University of Pretoria, South Africa; ³FAO-ECTAD Office for Southern Africa, Gaborone, Botswana; hillfoxx@gmail.com



The African buffalo (*Syncerus caffer*) is host for many pathogens known to cause economically important diseases and is often considered an important wildlife reservoir for livestock diseases. Theileriosis, heartwater, babesiosis and anaplasmosis are considered the most important tick-borne diseases (TBD) of livestock in sub-Saharan Africa, resulting in extensive economic losses to farmers in endemic areas. The most pathogenic and economically significant *Theileria* species in sub-Saharan Africa is *Theileria parva*, which causes east coast fever (ECF), corridor disease (which is a controlled disease in South Africa) and January disease in cattle. *T. parva* appears to have evolved in the African buffalo and, although only causing subclinical infection in buffalo, causes fatal disease in cattle. Bovine babesiosis is a tick-borne disease found worldwide, caused by *Babesia bigemina*, *B. bovis*, *B. divergens* and *B. major*. Of these, *B. bovis* and *B. bigemina* have a significant impact on cattle health and productivity in tropical and subtropical countries. *Ehrlichia ruminantium*, the causative agent of heartwater (cowdriosis) is an intracellular rickettsial bacterium that causes severe disease of domestic ruminants (cattle, sheep and goats) in sub-Saharan Africa. The primary causative agent of bovine anaplasmosis is *Anaplasma marginale*, a Gram-negative obligate intracellular bacterium, causing significant economic losses mainly due to the high morbidity and mortality in susceptible cattle herds.

There are no reports of the presence of *T. parva* in Botswana and information on significant tick-borne haemoparasites, especially in northern Botswana, is scarce. The aim of the study was to screen buffalo samples for the presence of

Theileria, *Babesia*, *Ehrlichia* and *Anaplasma* species using a reverse line blot (RLB) hybridization assay. DNA was extracted from buffalo blood smear samples obtained from two geographically different areas in northern Botswana, the Chobe National park ($n = 64$) and the Okavango Delta ($n = 57$). A fragment of the small subunit rRNA gene was amplified using genus-specific primers and amplicons were identified by hybridization to species-specific probes. Samples were also specifically screened for the presence of *T. parva* using a *T. parva* specific 18S rRNA real-time PCR assay. The RLB

results revealed the presence of *Theileria*, *Babesia*, *Anaplasma* and *Ehrlichia* species, either as single or mixed infections. Of the *Theileria* spp. present, *T. parva* (33%) and *T. mutans* (21%) were the most prevalent. Other species of interest were *Anaplasma centrale* (50%), *A. marginale* (32%), *Babesia occultans* (12%) and *Ehrlichia ruminantium* (11%). Real-time PCR results indicated that

74% of the samples tested positive for the presence of *T. parva*.

This is the first report of *T. parva* in the buffalo population of northern Botswana. Buffalo act as asymptomatic reservoirs for these haemoparasites. When these infected buffalo share the same home ranges with cattle, haemoparasites can be transmitted to cattle through infected tick bites. This information on the circulation of TBD can contribute to raise awareness among veterinary officials and cattle owners so that control measures (prevention of wildlife-cattle contacts, regular dipping) can be implemented to mitigate their economic impact.

“This information on the circulation of TBD can contribute to raise awareness among veterinary officials and cattle owners...”

Molecular phylogeny of novel *T. buffeli*-like and *T. sinensis*-like genotypes of the African buffalo (*Syncerus caffer*) based on their 18S rRNA gene and internal transcribed spacer sequences

ME Chaisi^{1,2}, L He^{3,4}, NE Collins¹, MC Oosthuizen¹

¹Department of Veterinary Tropical Diseases, Faculty of Veterinary Science, University of Pretoria, Onderstepoort, South Africa; ²Department of Biology, National University of Lesotho, Faculty of Science and Technology, Lesotho; ³State Key Laboratory of Agricultural Microbiology, Huazhong Agricultural University, Hubei Wuhan, China; ⁴College of Veterinary Medicine, Huazhong Agricultural University, Hubei Wuhan, China; mechaisi@yahoo.co.uk

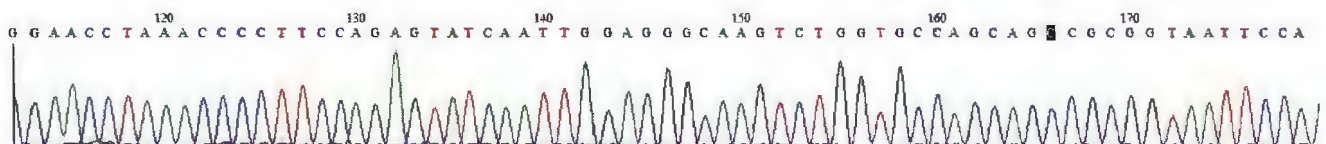
Theileria buffeli/sergenti/orientalis is a group of closely related parasites of cattle and buffalo with a cosmopolitan distribution. Ticks of *Haemaphysalis* spp. act as vectors in Australia, Asia and Europe, but the vectors in Africa and the USA are still unknown. The classification of these benign parasites is still confusing and is complicated by their similar morphology, serology, vector transmission, geographic distribution, difficulties in obtaining pure isolates and incompletely known life-cycles. It is still unclear whether these organisms represent the same species or different species. Another closely related species, *Theileria sinensis*, was recently described in China and is regarded as a cause of bovine theileriosis in that country.



The aims of this study were to characterize the 18S rRNA gene and complete internal transcribed spacer (ITS) region (ITS1-5.8S-ITS2) of the South African *T. buffeli*, to determine the level of genetic variation between the novel and known *T. buffeli*-like genotypes, and to establish their phylogenetic positions based on their 18S rRNA gene and ITS sequences. Ribosomal DNA was amplified from DNA extracted from blood samples originating from buffalo in the Hluhluwe-iMfolozi Game Park (HIP) and the Addo Elephant Game Park (AEGP), cloned, and recombinants were sequenced.

We identified *T. buffeli* from samples originating from buffalo from the two localities. Phylogenetic analyses of the 18S rRNA gene and the ITS regions of *T. buffeli* indicated the presence of 18S rRNA and ITS sequences which are similar

to *T. buffeli*-like sequences from cattle and buffalo in China and India, and the 18S rRNA sequences which are similar to *T. sinensis* 18S rRNA sequences of cattle and yak in China. There was extensive sequence variation between the novel *T. buffeli*-like genotypes of the African buffalo and previously described *T. buffeli* and *T. sinensis* genotypes. The presence of organisms with *T. buffeli*-like and *T. sinensis*-like genotypes in the African buffalo is of significant importance to the cattle industry in South Africa as these animals may act as sources of infection to naïve cattle. Our study provides important information which will assist in the classification of the complex *T. buffeli*/*T. sergenti*/*T. orientalis* group of benign and mildly pathogenic species.



Coagulation abnormalities in dogs undergoing elective and traumatic orthopaedic surgery

E Rioja¹, A Bahrenberg¹, B Conner¹, PN Thompson², A Goddard¹

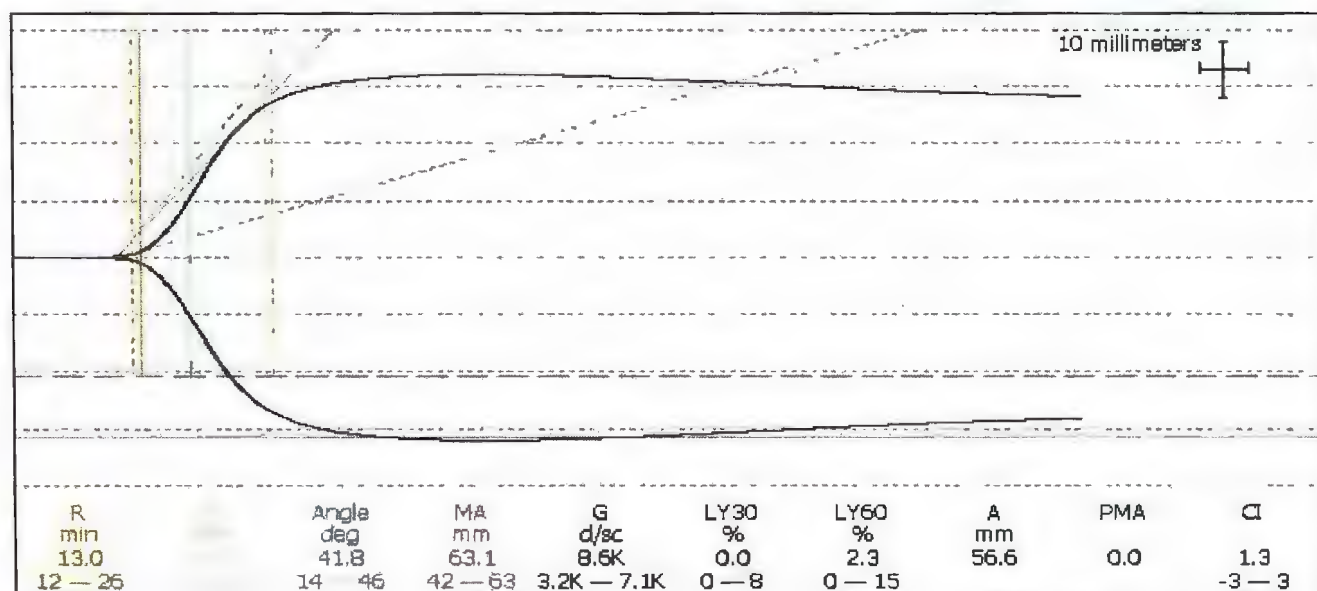
¹Department of Companion Animal Clinical Studies, and ²Department of Production Animal Studies, Faculty of Veterinary Science, University of Pretoria, Onderstepoort, South Africa; eva.riojagarcia@up.ac.za

Coagulation abnormalities secondary to trauma are recognized in humans. Traumatic injury requiring surgery is common in veterinary patients and an understanding of the potential coagulation complications is important for the peri-operative period.

Dogs requiring surgical repair for traumatic and chronic skeletal injuries were prospectively eligible for inclusion. Blood was collected for analysis immediately before surgery, immediately after surgery, and 24 hours after surgery completion. Values measured at each time point included: haematocrit, antithrombin activity, platelet count, total magnesium concentration, and thromboelastography (reaction time [R], K time, alpha angle, maximum amplitude [MA]). Repeated measures ANOVA with post-hoc Bonferroni corrections, Student's *t*-tests or Wilcoxon rank-sum tests were used ($P < 0.05$).

Nineteen dogs were included in the study: 11 dogs with trauma and 8 dogs scheduled for elective orthopaedic procedures. Dogs with trauma compared to dogs undergoing elective procedures had lower haematocrit (0.40 vs 0.47, $P = 0.02$), shorter K (1.3 vs 2.0 min, $P = 0.02$), greater angle (72.5° vs 61.1°, $P < 0.001$), and larger MA (72.7 vs 61.9 mm, $P < 0.001$), but no difference in antithrombin, platelet count, magnesium, or R before surgery. Dogs in both groups had significantly larger MA 24 hours post-operatively compared with pre-operative values ($P < 0.05$).

Hypercoagulability, identified using thromboelastography, was a common peri-operative finding in dogs with orthopaedic diseases, especially traumatic musculoskeletal injuries. Additionally, hypercoagulability becomes more pronounced after surgical repair. Increased awareness of the diseases and injuries causing hypercoagulability should alert clinicians to the potential complications associated with hypercoagulability and warrants consideration of therapies aimed at reducing thromboembolic complications.



Thromboelastographic evaluation of haemostatic function in dogs with natural envenomation by South African snakes

SS Nagel¹, A Goddard¹, B Willberg², JP Schoeman¹

¹Department of Companion Animal Clinical Studies, Faculty of Veterinary Science, University of Pretoria, Onderstepoort, South Africa; ²Small Animal Hospital, Department of Small Animal Clinical Sciences, Faculty of Life Sciences, University of Copenhagen, Denmark; salome.nagel@up.ac.za

Snakebites are medical emergencies and occur regularly in dogs. Multiple venom components, especially procoagulant toxins, can alter the haemostatic profile of the victim. Inadvertent activation of coagulation may also result due to expression of large amounts of tissue factor from injured tissues at the bite site. The purpose of this study was to investigate haemostatic changes in dogs envenomated by South African snakes using thromboelastography.

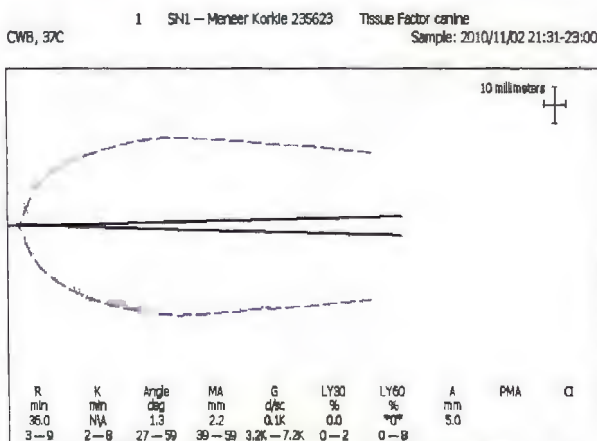


Twenty-three dogs were enrolled in this prospective study. Nine dogs (39%) were envenomated by puffadder (*Bitis arietans*), twelve (52%) by snouted cobras (*Naja annulifera*) and two (8.7%) by Mozambique spitting cobras (*Naja Mossambica*). Blood was collected at admission and 12 hours after admission. A complete blood count was performed on the ADVIA 2120 (Siemens). Prothrombin time (PT), activated partial thromboplastin time (aPTT) and fibrinogen assays were performed on the STart[®] 4 analyser (Diagnostica Stago, Roche). Antithrombin (AT) was measured using an automated spectrophotometric analyzer (Cobas Integra 400, Roche). D-dimer was measured using an immunometric flow-through principle (D-dimer Single test, Nycocard Reader II, Medinor A/S). Thromboelastography (TEG) was performed using the TEG[®] 5000 Thrombelastograph[®] Haemostasis System (Haemoscope, Pro-Gen Diagnostics). The data were analysed using the Mann-Whitney U Test. *P* < 0.05 was considered significant.

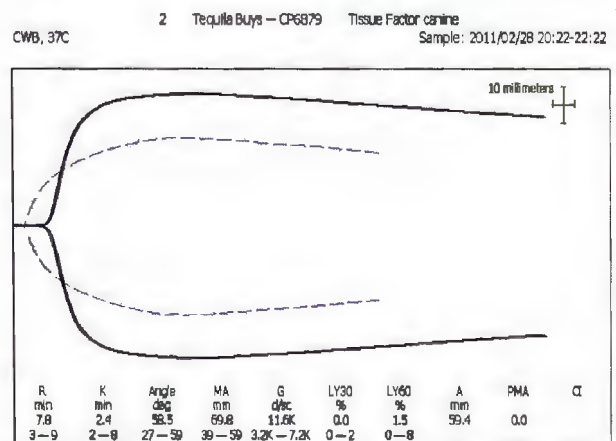
The median platelet count was significantly lower in the puffadder group compared to the combined cobra group at admission; 76.0 vs. 259.0 $\times 10^9/l$ (*P* = 0.027) (Normal: 200-

500). The median R-value was significantly prolonged in the puffadders at admission, 21.9 vs. 7.2 min (*P* = 0.018); and 12 hours, 20.9 vs. 8.2 min (*P* = 0.008) (Normal: 3-9). The median angle was significantly smaller in the puffadders at admission, 4.8° vs. 42.8° (*P* = 0.023) (Normal: 27-59). The median MA-value was significantly smaller in the puffadders at admission, 12.1 vs. 63.75 mm (*P* = 0.038); and 12 hours, 33.2 vs. 64.2 mm (*P* = 0.036) (Normal: 39-59). The median G-value was significantly smaller in the puffadders at admission, 0.7 vs. 8.8 dyn/cm² (*P* = 0.041); and 12 hours, 3.4 vs. 9.0 dyn/cm² (*P* = 0.03) (Normal: 3.2-7.2). None of the other coagulation parameters showed any significant differences between the two groups.

The thromboelastogram in all cobra victims was hypercoagulable, whereas marked hypocoagulability was observed for up to 12 hours in most of the puffadder victims, before a hypercoagulable state resulted. We postulate that the hypocoagulable state seen with puffadder envenomation could be due to abnormal thrombin production, platelet consumption or abnormal platelet function. TEG proved a more sensitive tool in detecting abnormal coagulation status in envenomated victims compared to traditional coagulation assays.



TEG at admission - *Bitis arietans* (Puffadder) - hypocoagulable.



TEG at admission - *Naja annulifera* (Snouted cobra) - hypercoagulable.

The effects of repeated intravenous iohexol administration on renal function in healthy beagles

N Cassel, RM Kirberger, A Goddard, A Carstens

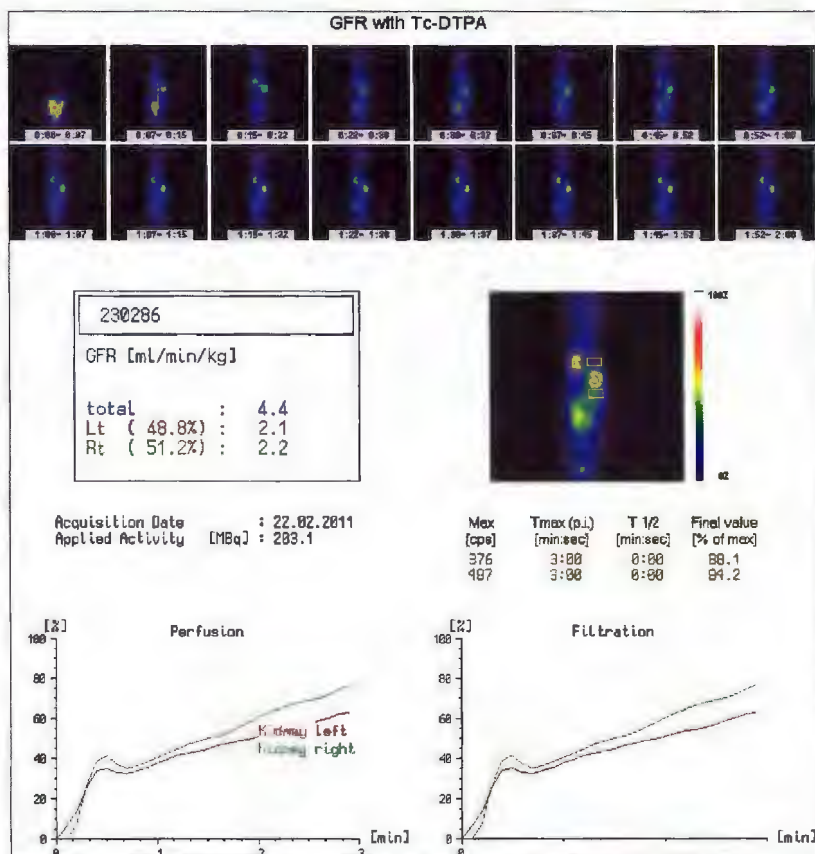
Department of Companion Animal Clinical Studies, Faculty of Veterinary Science, University of Pretoria, Onderstepoort, South Africa; nicky.cassel@up.ac.za

Since the turn of the 21st century there has been an increase in the use of computed tomography (CT) in veterinary science due to increasing accessibility, enhanced applications and its minimal invasiveness. Many of these CT studies include the use of intravascular contrast medium. Contrast-induced nephrotoxicity (CIN) is a well described syndrome in humans undergoing contrast medium examinations and is one of the leading complications of contrast use. To date CIN has received minimal attention in the veterinary literature despite the increasing use of contrast medium examinations in computed tomographic studies.

This prospective study evaluated the effect of 1290 mg/kg iohexol given intravenously to five normal adult purpose-bred beagles in a divided dose at an interval of 6-8 weeks. Renal function was evaluated qualitatively by means of scintigraphically determined glomerular filtration rate (GFR). Specific renal clinical pathological tests that were conducted included urinalysis, urine gamma glutamyl transpeptidase (GGT), urine protein and serum creatinine levels. The GGT: creatinine ratio and urine protein creatinine ratios were also determined. Additionally, a specific serum biochemistry panel (serum urea, creatinine, serum inorganic phosphate, sodium and potassium) was also performed. These tests were conducted 72 hours prior to, 72 hours after and 2 weeks after each dose of intravenous contrast agent.

There was no significant difference between the various groups' combined data for the urine and serum biochemistry panel changes. The only significant changes were between individual data groups for serum creatinine and SIP. The GFR showed a significant decrease (17%) after the second injection but not to a pathologically significant level.

No clinically significant effect of repeated contrast medium administration was determined in this limited study. However in dogs with reduced renal function the risk of CIN is likely to increase after contrast administration.



The effects of hydroxyethyl starch 6% 130/0.4 on thromboelastography in healthy horses

A Viljoen, MN Saulez, PC Page, GT Fosgate

¹Section of Equine Medicine, Department of Companion Animal Clinical Studies, and ²Section of Epidemiology, Department of Production Animal Studies, Faculty of Veterinary Science, University of Pretoria, Onderstepoort, South Africa; adrienne.viljoen@up.ac.za

Hydroxyethyl starch solutions (HES) restore and maintain intravascular blood volume and plasma colloid osmotic pressure (pCOP). The potential benefits of colloid administration have been described in hypoproteinaemic horses and in horses suffering from colic or colitis. Reported safe dosage requirements are extrapolated from human literature. Thromboelastography (TEG) has been validated for horses. The objective of the study was to determine the haemostatic and oncotic effects of HES 6% 130/0.4 (Voluven[®]) administered in healthy horses.

Six clinically healthy Nootgedacht mares were used in a randomized crossover, prospective study. Mares were assigned to three treatment groups and received 10, 20 and 40 mL/kg Voluven[®] infusions with a 2-week washout period. Kaolin-activated TEG and pCOP measurements were performed before (baseline), immediately after each infusion (0 hours), and at 1, 6, 12, 24, 48 and 96 hours. TEG values: reaction time (R), clot formation time (K), maximum amplitude (MA), and angle (α) were evaluated.

Overall mean \pm SD values for 40 vs. 20 and 10 mL/kg groups were:

- R = 18.5 \pm 5.84 min vs. 16.3 \pm 3.41 ($P = 0.492$) and 13.4 \pm 2.91 ($P = 0.041$);
- K = 4.61 \pm 1.85 min vs. 3.65 \pm 0.71 ($P = 0.025$) and 3.13 \pm 0.74 ($P = 0.011$);
- MA = 55.1 \pm 7.43 mm vs. 60.4 \pm 4.94 ($P = 0.158$) and 58.1 \pm 3.99 ($P = 0.353$);
- $\alpha = 39.8 \pm 10.56^\circ$ vs. 46.1 \pm 6.92 ($P = 0.042$) and 50.4 \pm 6.60 ($P = 0.016$).

Overall mean \pm SD pCOP values for 40 vs. 20 and 10 mL/kg were:

24.1 \pm 3.59 mmHg vs. 21.7 \pm 2.01 ($P = 0.001$) and 21.0 \pm 1.78 ($P = 0.001$). When inter-dosage differences were compared at specific points in time, significant differences were seen at the following time points:

- R: 6 hours ($P = 0.005$);
- K: 1 hour ($P = 0.035$) and 6 hours ($P = 0.005$);
- pCOP: 0, 1, 6, 12 and 24 hours ($P = 0.005$).



The most significant inter-dosage differences in measured TEG parameters were seen in the first 12 hours following Voluven[®] administration, while pCOP still differed significantly at 24 hours. Compared to lower dosages, the administration of Voluven[®] at 40 mL/kg is more likely to induce changes consistent with hypocoagulability as measured by TEG. Further analysis is needed to establish the duration and extent of hypocoagulability as well as the significance and duration of the increase in pCOP.

Evaluation of serum amyloid A, haptoglobin, nucleated cell count, total protein and haemolysis in peritoneal fluid for differentiation of medical and surgical colic in horses

E Scheepers¹, T Holberg Pihl², M Sanz¹, P Page¹, A Goddard¹, S Jacobsen²

¹Department of Companion Animal Clinical Studies, Faculty of Veterinary Science, University of Pretoria, Onderstepoort, South Africa; ²Department of Large Animal Sciences, Faculty of Life Sciences, University of Copenhagen, Taastrup, Denmark; elrien.scheepers@up.ac.za



Equine colic involves a large variety of disease processes, each with different degrees of inflammation affecting the gastrointestinal tract. The most severe forms of colic are disease processes with severe tissue damage and inflammation such as strangulating obstructions and acute infections like duodenitis-proximal jejunitis (DPJ) and necrotizing typhlo-colitis. Horses with severe medical colic caused by DPJ, colitis or peritonitis that do not need surgery, can be difficult to differentiate from horses with strangulating obstructions or displacements that do need surgery. Peritoneal fluid (PF) levels of the acute phase proteins (APPs) haptoglobin (Hp) and fibrinogen were shown to be increased in response to gastrointestinal inflammation. The APP serum amyloid A (SAA) has, however, not been evaluated in PF before. This prospective observational multicentre study was undertaken to evaluate the usefulness of SAA and Hp measured in PF to differentiate between horses with mild medical colic, severe medical colic and surgical colic; compared to the classic PF biomarkers: nucleated cell count (NCC), total protein and haemolysis.

Peritoneal fluid was collected by abdominocentesis on admission in EDTA tubes from 138 horses with mild medical colic (group 1), 46 horses with severe medical colic (group 2) and 124 horses with surgical colic (group 3). Pre-admission duration of colic

“The most severe forms of colic are disease processes with severe tissue damage and inflammation such as strangulating obstructions and acute infections...”

(<4 hours, 5-12 hours, 13-24 hours, >24 hours) and haemolysis of PF were noted. SAA was measured by LZ SAA immunoturbidometric assay and Hp by phase range Haptoglobin assay, both on an ADVIA 1800. NCC was measured on an ADVIA 3000 and total protein by refractometry. PF concentrations of SAA, Hp, NCC, total protein and haemolysis were compared between the three groups using ANOVA and chi-squared test. The effect of duration of colic was included in the analysis.

Haemolysis was present in 36% of horses in group 3, 24% in group 2 and 10% in group 1. All biomarkers were significantly higher in groups 2 and 3 when compared to group 1. NCC was the only biomarker with a difference ($P = 0.015$) between group 2 (mean: $5.2 \times 10^9/L$) and group 3 ($1.6 \times 10^9/L$). SAA ($P < 0.001$) and Hp ($P = 0.009$) were increased after 12 hours of colic duration, whereas NCC was only increased ($P = 0.01$) after 24 hours.

Not all horses with haemolysis in PF require surgery. Measuring a panel of traditional and novel inflammatory biomarkers in PF can assist in differentiating between three groups of horses with colic that require fundamentally different treatments. Additionally, when interpreting levels of a measured biomarker, duration of disease should be considered.

Post mortem survey of equine dental disorders

DC Vemming, G Steenkamp, A Carstens, P Page

Department of Companion Animal Clinical Studies, Faculty of Veterinary Science, University of Pretoria, Onderstepoort, South Africa; ditte.vemming@up.ac.za

Dental disorders are commonly found on routine dental examination in equine practice and *post mortem* studies have shown a high prevalence (up to 80%) of clinically significant, undiagnosed dental conditions. No survey has been published on dental disorders in South African horses, with specific reference to oral examination findings.

To investigate dental disorders in different age groups of an abattoir population of South African horses a prospective cross-sectional prevalence survey on heads from horses consigned for routine slaughter was initiated. After euthanasia at the abattoir, estimated age from dental evaluation was recorded. Heads were severed transversely behind the incisors and then bisected sagittally using a band saw. A detailed clinical examination of the oral cavity was performed and lesions recorded on a standardized dental chart using the modified Triadan system. A dental probe was used to explore dental pockets.

Sharp enamel points were the most common abnormality detected, similar to other equine *post mortem* dental studies. Whilst these may be classified as a normal physiological finding due to normal dental wear, they have been associated with buccal and lingual ulceration. Severe pathological findings including fractures, caries, step mouth and diastemata were more prevalent in the oldest age group, whereas abnormalities of wear such as hooks and wave mouth were not confined to any age group. In younger horses, certain findings such as pockets and wave mouth were expected with normal loss of the dental caps.



These preliminary findings indicate that dental disorders are common in an abattoir population of horses and highlight the importance of routine dental examination and prophylactic dental treatment to improve dental health and welfare of horses.

The preliminary oral pathology results of 22 heads investigated are shown in the table below.

	Age group			Total (%)
	2-5 yrs (n=7 heads)	6-14 yrs (n=11 heads)	≥15 yrs (n=4 heads)	
Sharp enamel points	7	9	4	20 (90.9)
Dental pockets	6	7	3	16 (72.7)
Diastemata	3	3	4	10 (45.5)
Wave mouth	3	1	3	7 (31.8)
Fractures	0	3	3	6 (27.2)
Hooks	1	4	1	6 (27.2)
Caries	0	1	4	5 (22.7)
Step mouth	0	0	3	3 (13.6)
Buccal abrasions	0	2	0	2 (9.1)

Overground endoscopy for diagnosis of upper airway abnormalities in thoroughbred racehorses in South Africa

J Mirazo, P Page, L Rublo-Martínez, J Marais, C Lyle

Equine Section, Department of Companion Animal Clinical Studies, Faculty of Veterinary Science, University of Pretoria, Onderstepoort, South Africa; Javier.mirazo@up.ac.za

Upper airway endoscopy has been the diagnostic method of choice for equine upper respiratory tract (URT) pathology since its development in the 1970's. Initially this procedure could only be performed at rest which meant that dynamic abnormalities which were only present at exercise could not be detected. Such abnormalities include palatal instability (PI), dorsal displacement of the soft palate (DDSP), recurrent laryngeal neuropathy (RLN), left corniculate process collapse (LCPC), axial deviation of the aryepiglottic folds (ADAF) and vocal cord collapse (VCC). The subsequent development of high speed treadmill endoscopy (HSTE) improved the sensitivity of upper airway endoscopy by allowing the examiner to observe the horse's pharynx during exercise. However, the level of exertion achieved on the treadmill may not always represent that achieved by the horse during normal exercise as surface, rider, tack and environmental variables are altered. Financial costs, time to train the horse, transport requirements and misconceptions regarding safety are recognized as further disadvantages of HSTE. Recently, the development of overground endoscopy has addressed some of the shortcomings of HSTE. Overground endoscopy allows real time evaluation of the horse's pharynx during normal exercise, via wireless transmission to a handheld console, whilst also recording the examination for observation at a later time. The endoscope is placed via a nostril into the horse's pharynx and is connected to a light source which is mounted on the horse. Several studies have validated overground endoscopy on the basis of safety and reliability.

A retrospective study was undertaken to describe the upper airway abnormalities detected during overground endoscopy in horses presenting with poor performance and/or respiratory noise in South Africa. Patient records of Thoroughbred racehorses undergoing overground endoscopy from

November 2011 to May 2012 were reviewed. Data collected included signalment, primary complaint, distance exercised, maximum speed attained and dynamic airway abnormalities detected.

A total of 37 cases were identified. Fifteen colts (40%), six geldings (16%) and 16 females (44%) with a median age of 3 y (range 2 -7 y) were examined. The primary complaints for horses presenting for overground endoscopy were respiratory noise at exercise (23/37, 62%), poor performance (8/37, 22%) and a combination of both respiratory noise and poor performance (6/37, 16%). The horses were exercised over a mean distance of 1150 m (range 600 - 1600 m) and the mean maximum speed attained was 56.9 km/h (range 43.4 - 64.1 km/h).

The main abnormalities detected included DDSP (10/37, 27%), RLN (10/37, 27%), ADAF (10/37, 27%) and VCC (12/37, 33%). Eleven cases (30%) presented with single abnormalities and 13 cases (35%) presented with multiple abnormalities. No abnormalities were detected in 13 horses (35%). A diagnosis was more likely to be reached in horses presenting with respiratory noise and/or respiratory noise with poor performance (23/29, 79%) than in horses only presenting with poor performance (1/8, 13%).

The use of overground endoscopy is a useful technique to detect URT abnormalities in Thoroughbred racehorses in South Africa. The main abnormalities detected were DDSP, RLN, ADAF and VCC, which is comparable to studies in other populations. Detection of multiple abnormalities in 35% of horses suggests that dynamic endoscopy is also indicated in horses with an obvious abnormality on resting endoscopy, particularly when surgical intervention is being considered.



Scientific peer-refereed articles published in 2011 where a member of the Faculty of Veterinary Science, University of Pretoria, was an author

- Abu Samra, N, Jori, F, Samie, A and Thompson, PN. 2011. The prevalence of *Cryptosporidium* spp. Oocysts in wild mammals in the Kruger National Park, South Africa. *Veterinary Parasitology* 175: 155-159
- Adams, HR, van Vuuren, M, Bosman, A-M, Kanla, S and Kennedy, M. 2011. Detection and genetic analysis of feline immunodeficiency virus (FIV ple) in southern African lions (*Panthera leo*). *South African Journal of Wildlife Research* 41 (2): 173-180
- Ademola, IO and Eloff, JN. 2011. Anthelmintic efficacy of cashew (*Anacardium occidentale*) on *in vitro* susceptibility of the ova and larvae of *Haemonchus contortus*. *African Journal of Biotechnology* 10 (47): 9700-9705
- Ademola, IO and Eloff, JN. 2011. *In vitro* anthelmintic effect of *Anogeissus Leiocarpus* (DC.) Guill. & Perr. Leaf extracts and fractions on developmental stages of *Haemonchus Contortus* *African Journal of Traditional Complementary and Alternative Medicines* 8 (2): 134-139
- Ademola, IO and Eloff, JN. 2011. Ovicidal and larvicidal activity of *Cassa alata* leaf acetone extract and fractions on *Haemonchus contortus*: *In vitro* studies. *Pharmaceutical Biology* 59 (5): 539-544
- Ademola, IO and Eloff, JN. 2011. Anthelmintic activity of acetone extract and fractions of *Vernonia amygdaline* against *Haemonchus contortus* eggs and larvae. *Tropical Animal Health and Production* 43: 521-527
- Aderogba, MA, McGaw, LJ, Bezabih, M and Abegaz, BM. 2011. Isolation and characterisation of novel antioxidant constituents of *Croton zambesicus* leaf extract. *Natural Product Research* 25 (13): 1224-1233
- Adesiyun, AA, Fosgate, GT, Seebarsingh, R, Brown, G, Stoute, S and Stewart-Johnson, A. 2011. Virulence of *Bruceella abortus* isolated from cattle and water buffalo. *Tropical Animal Health and Production* 43: 13-16
- Apanaskevich, Dr, Horak, IG, Matthee, CA and Matthee, S. 2011. A new species of *Ixodes* (Acari: Ixodidae) from South African mammals. *Journal of Parasitology* 97 (3): 389-398
- Amot, LF, Veale, DJH, Steyl, JCA and Myburgh, JG. 2011. Treatment rationale for dogs poisoned with aldicarb (carbamate pesticide). *Journal of the South African Veterinary Association* 82 (4): 232-238
- Bath, GF. 2011. Non-pharmaceutical Control of Endoparasitic Infections in Sheep. *Veterinary Clinics of North America-Food Animal Practice* 27 (1): March 157-162
- Bath, GF, Janse van Rensburg, A, Pettey, KP, van Vuuren, M and Kidanemariam, A. 2011. A literature review and investigation of staphylococcal necrotic dermatitis in sheep. *Journal of the South African Veterinary Association* 84 (4): 227-231
- Bernitz, H, Bernitz, Z, Steenkamp, G, Blumenthal, R and Stols, GH. 2011. The individualisation of a dog bite mark: a case study highlighting the bite mark analysis, with emphasis on differences between dog and human bite marks. *International Journal of Legal Medicine* (10.1007/s00414-011-0575-4) July Online
- Bisi-Johnson, MA, Obi, CL, Hattori, T, Oshima, Y, Li, S, Kambizi, L, Eloff, JN and Vasaikar, SD. 2011. Evaluation of the antibacterial and anticancer activities of some South African medicinal plants. *Bmc Complementary And Alternative Medicine* 11 (14): 1-5
- Blignaut, DJC, Holm, DE, Leask (Anderson), R, Stander, N and Steyl, JCA. 2011. Congenital reflex myoclonus in two Merino cross lambs in South Africa. *Veterinary Record* 169 (26): 684-685
- Booth, KK and Webb, EC. 2011. Effect of Blockage of the Ducts of the Vomeronasal Organ on LH Plasma Levels during the "Whitten Effect" in Does. *Veterinary Medicine International* 2011: 1-8
- Booyse, DG and Dehority, BA. 2011. Rumen protozoa in South African sheep with a summary of the worldwide distribution of sheep protozoa. *Onderstepoort Journal of Veterinary Research* 78 (1): 1-7
- Botha, CJ, Steenkamp, PA, Olivier, A and Bekker, LC. 2011. *Nicotiana glauca* poisoning in ostriches (*Struthio camelus*). *Journal of the South African Veterinary Association* 82 (2): 116-119
- Botha, H, van Hoven, W and Guilette, LJ. 2011. The decline of the Nile crocodile population in Loskop Dam, Olifants River, South Africa. *Water SA* 37 (1): 103-108



Brothers, PS, Collins, NE, Oosthuizen, MC, Bhoora, R, Troskie, M and Penzhorn, BL. 2011. Occurrence of blood-borne tick-transmitted parasites in common tsessebe (*Damaliscus lunatus*) antelope in Northern Cape Province, South Africa. *Veterinary Parasitology* 183: 160-165

Bwala, DG, Fasina, FO, van Wyk, A and Duncan, NM. 2011. Effects of Vaccination with Lentogenic Vaccine and Challenge with Virulent Newcastle Disease Virus (NDV) on Egg Production in Commercial and SPF Chickens. *International Journal of Poultry Science* 10 (2): 98-105

Bwala, DG, Duncan, NM and Bisschop, SPR. 2011. Uterine adenocarcinoma with transcoelomic metastases in breeder hens (*Gallus domesticus*). *Journal of the South African Veterinary Association* 82 (1): 53-55

Carpenter, S, Wilson, A, Barber, J, Veronesi, E, Mellor, P, Venter, GJ and Gubbins, S. 2011. Temperature dependence of the extrinsic incubation period of orbiviruses in *Culicoides* biting midges. *Plos One* 6 (11): 1-8

Cenci-Goga, BT, Rossitto, PV, Sechi, P, McCridle, CME and Cullor, JS. 2011. Toxoplasma in animals, food and humans: an old parasite of concern. *Foodborne Pathogens And Disease* 751-762

Chaisi, ME, Sibeko, KP, Collins, NE, Potgieter, FT and Oosthuizen, MC. 2011. Identification of *Theileria parva* and *Theileria* sp. (buffalo) 18S rRNA gene sequence variants in the African Buffalo (*Syncerus caffer*) in southern Africa. *Veterinary Parasitology* 182: 150-162

Christie, J, Schwan, EV, Bodenstern, LE, Sommerville, JEM and van der Merwe, LL. 2011. The sensitivity of direct faecal examination, direct faecal flotation, modified centrifugal faecal flotation and centrifugal sedimentation/flotation in the diagnosis of canine spirocercosis. *Journal of the South African Veterinary Association* 82 (2): 71-75

Conradie Van Wyk, I and Boomker, JDF. 2011. Parasites of South African wildlife. XVIV. The prevalence of helminths in some common antelopes, warthogs and a bushpig in the Limpopo province, South Africa. *Onderstepoort Journal of Veterinary Research* 78 (1): 1-11

Crafford, JE, Guthrie, AJ, van Vuuren, M, Mertens, PPC, Burroughs, JN, Howell, PG, Batten, CA and Hamblin, C. 2011. A competitive ELISA for the detection of group-specific antibody to equine encephalosis virus. *Journal of Virological Methods* 174: 60-64

Crole, MR and Soley, JT. 2011. Distribution and structure of glandular tissue in the oropharynx and proximal oesophagus of the emu (*Dromaius novaehollandiae*). *Acta Zoologica* 92: July 206-215

Cumming, GS, Caron, A, Abolnik, C, Catolli, G, Bruinzeel, L, Burger, Ce, Cecchetti, K, Chiweshe, N, Mochotloane, B, Mutumi, GL and Ndlovu, M. 2011. The ecology of Influenza A viruses in wild birds in southern Africa. *Ecohealth* 8 (1): 4-13

De Cramer, KGM, Stylianides, E and van Vuuren, M. 2011. Efficacy of vaccination at 4 and 6 weeks in the control of canine parvovirus. *Veterinary Microbiology* 149: 126-132

Dossin, O, Rupassara, SI, Weng, HY, Williams, DA, Garlick, PJ and Schoeman, JP. 2011. Effect of Parvoviral Enteritis on Plasma Citrulline Concentration in Dogs. *Journal of Veterinary Internal Medicine* 25: 215-221

Du Plessis, EC, Prozesky, L and Botha, CJ. 2011. The pathology of acute *Nolletia gariepina* poisoning in cattle. *Journal of the South African Veterinary Association* 82 (3): 144-149

Du Plessis, L and Soley, JT. 2011. Head-base bending and disjunct spermatozoa in the emu (*Dromaius novaehollandiae*): A morphological comparison of two closely related defects. *Theriogenology* 76: 1275-1283

Du Plessis, L and Soley, JT. 2011. Incidence, structure and morphological classification of abnormal sperm in the emu (*Dromaius novaehollandiae*). *Theriogenology* 75: 589-601

Dvir, E, Schoeman, JP, Cliff, SJ, McNeilly, TN and Mellanby, RJ. 2011. Immunohistochemical characterization of lymphocyte and myeloid cell infiltrates in spirocercosis-induced oesophageal nodules. *Parasite Immunology* 33: 545-553



- Dzikiti, TB, Stegmann, GF, Cromarty, AD, Dzikiti, LN and Hellebrekers, LJ. 2011. Effects of propofol on isoflurane minimum alveolar concentration and cardiovascular function in mechanical ventilation goats. *Veterinary Anaesthesia and Analgesia* 38 (-): 44-53
- Dzikiti, TB, Stegmann, GF, Dzikiti, LN and Hellebrekers, LJ. 2011. Effects of midazolam on isoflurane minimum alveolar concentration in goats. *Small Ruminant Research* 97: 104-109
- Dzikiti, TB, Stegmann, GF, Dzikiti, LN and Hellebrekers, LJ. 2011. Effects of fentanyl on isoflurane minimum alveolar concentration and cardiovascular function in mechanically ventilated goats. *Veterinary Record* – (168): April Online
- Eloff, JN, Ntloedibe, T and van Brummelen, R. 2011. A simplified but effective method for the quality control of medicinal plants by Planar Chromatography. *African Journal of Traditional Complementary and Alternative Medicines* 8 (S): 1-12
- Fasina, FO, Rivas, AL, Bisschop, SPR, Stegeman, AJ and Hernandez, J. 2011. Identification of risk factors associated with highly pathogenic avian influenza H5N1 virus infection in poultry farms, in Nigeria during the epidemic of 2006-2007. *Preventive Veterinary Medicine* 98: 204-208
- Fosgate, GT, Diptee, MD, Ramnanan, A and Adesiyun, AA. 2011. Brucellosis in domestic water buffalo (*Bubalus bubalis*) of Trinidad and Tobago with comparative epidemiology to cattle. *Tropical Animal Health and Production* 43: 1479-1486
- Furniss, C, Carstens, A and van den Berg, SS. 2011. Radiographic changes in Thoroughbred yearlings in South Africa. *Journal of the South African Veterinary Association* 82 (4): 194-204
- Gallivan, GJ, Spickett, A, Heyne, H, Spickett, AM and Horak, IG. 2011. The dynamics of questing ticks collected for 164 consecutive months off the vegetation of two landscape zones in the Kruger National Park (1988-2002). Part III. The less commonly collected species. *Onderstepoort Journal of Veterinary Research* 78 (1): 1-9
- Goddard, A, Schoeman, JP, Leisewitz, AL, Nagel, S and Aroch, I. 2011. Clinicopathologic abnormalities associated with snake envenomation in domestic animals. *Veterinary Clinical Pathology* 40 (3): 282-292
- Govender, D, Oosthuizen, MC and Penzhorn, BL. 2011. Piroplasm parasites of white rhinoceroses (*Ceratotherium simum*) in the Kruger National Park, and their relation to anaemia. *Journal of the South African Veterinary Association* 82 (1): 36-40
- Harrison, A, Bown, KJ and Horak, IG. 2011. Detection of *Anaplasma bovis* in an undescribed tick species collected from the eastern rock sengi *Elephantulus myurus*. *Journal of Parasitology* 97 (6): 1012-1016
- Heise, A, Thompson, PN and Gerber, D. 2011. Influence of seminal plasma on fresh and post-thaw parameters of stallion epididymal spermatozoa. *Animal Reproduction Science* 123: 192-201
- Henton, M, Eager, H, Swan, GE and van Vuuren, M. 2011. Part VI. Antibiotic management and resistance in livestock production. *South African Medical Journal (SAMJ)* 101 (8): 583-587
- Hill, JM, Leisewitz, AL and Goddard, A. 2011. The utility of uric acid assay in dogs as an indicator of functional hepatic mass. *Journal of the South African Veterinary Association* 82 (2): 86-93
- Hlokwe, MT, Jenkins, AO, Streicher, EM, Venter, EH, Cooper, D, Godfroid, JXL and Michel, AL. 2011. Molecular characterisation of *Mycobacterium bovis* isolated from African buffaloes (*Syncerus caffer*) in Hluhluwe-iMfolozi park in KwaZulu-Natal, South Africa. *Onderstepoort Journal of Veterinary Research* 78 (1): 1-6
- Horak, IG, Gallivan, GJ and Spickett, AM. 2011. The dynamics of questing ticks collected for 164 consecutive months off the vegetation of two landscape zones in the Kruger National Park (1988-2002). Part I. Total ticks, *Amblyomma hebraeum* and *Rhipicephalus decoloratus*. *Onderstepoort Journal of Veterinary Research* 78 (1): 1-9
- Horak, IG, Welman, S, Hallam, SL, Lutermiann, H and Mzilikazi, N. 2011. Ticks of four-toed elephant shrews and Southern African hedgehogs. *Onderstepoort Journal of Veterinary Research* 78 (1): 1-3
- Huchzermeyer, KDA, Govender, D, Pienaar, DJ and Deacon, AR. 2011. Steatitis in wild sharptooth catfish, *Clarias gariepinus* (Burcell), in the Olifants and Lower Letaba rivers in the Kruger National Park, South Africa. *Journal of Fish Diseases* 34: 489-498
- Jenkins, AO, Cadmus, SIB, Venter, EH, Pourcel, C, Hauk, Y, Vergnaud, G, Godfroid, JXL. 2011. Molecular epidemiology of human and animal tuberculosis in Ibadan, Southwestern Nigeria. *Veterinary Microbiology* 151: 139-147
- Jongejan, F, Fourie, JJ, Chester, ST, Manavella, C, Mallouk, Y, Pollmeier, MG and Baggott, D. 2011. The prevention of transmission of *Babesia canis canis* by *Dermacentor reticulatus* ticks to dogs using a novel combination of fipronil, amitraz and (S)-methoprene. *Veterinary Parasitology* 179: 343-350
- Jori, F, Brahmabhatt, D, Fosgate, GT, Thompson, PN, Budke, C, Ward, A, Ferguson, K and Gummow, B. 2011. A questionnaire-based evaluation of the veterinary cordon fence separating wildlife and livestock along the boundary of the Kruger National Park, South Africa. *Preventive Veterinary Medicine* 100: 210-220
- Joubert, KE, Serfontein, T, Scantlebury, M, Manjerovic, ME, Bateman, PW, Bennett, NC and Waterman, J. 2011. Determination of an optimal dose of medetomidine-ketamine-buprenorphine for anaesthesia in the Cape ground squirrel (*Xerus inauris*). *Journal of the South African Veterinary Association* 82 (2): 94-96
- Kaikabo, AA and Eloff, JN. 2011. Antibacterial activity of two biflavonoids from *Garcinia livingstonei* leaves against *Mycobacterium smegmatis*. *Journal of Ethnopharmacology* 138: 253-255
- Khan, F, Vorster, JH, van Vuuren, M and Mapham, P. 2011. Evaluation of the effects of long-term storage of bovine ear notch samples on the ability of 2 diagnostic assays to identify calves persistently infected with bovine viral diarrhoea virus. *Journal of the South African Veterinary Association* 82 (1): 18-23
- Kik, M, Nijhof, AM, Balk, JA and Jongejan, F. 2011. *Babesia* sp. EU1 infection in a forest reindeer, the Netherlands. *Emerging Infectious Diseases* 17 (5): 936-938
- Kirberger, RM, Schulman, ML and Hartman, MJ. 2011. Ultrasonographic and laparoscopic evaluation of the reproductive tract of the captive female African lion (*Panthera leo*). *Theriogenology* 76: 810-818
- Kitshoff, AM, McClure, V, Lim, CK and Kirberger, RM. 2011. Bilateral multiple cystic kidney disease and renal cortical abscess in a Boerboel. *Journal of the South African Veterinary Association* 82 (2): 120-124
- Kohn, TA, Burroughs, R, Hartman, MJ and Noakes, TD. 2011. Fiber type and metabolic characteristics of lion (*Panthera leo*), caracal (*Caracal caracal*) and human skeletal muscle. *Comparative Biochemistry and Physiology A-Molecular & Integrative Physiology* 159: 125-133

- Kruger, K, Stegmann, GF and Becker, PJ. 2011. Preliminary investigation of concurrent administration of phenylbutazone and romifidine in healthy horses. *Veterinary Anaesthesia and Analgesia* 38: 505-509
- Lane, EP, Miller, S, Lobetti, RG, Caldwell, P, Bertschinger, HJ, Burroughs, REJ, Kotze, A and van Dyk, A. 2011. Effect of diet on the incidence of and mortality owing to gastritis and renal disease in captive cheetahs. *Zoo Biology* 30: 1-14
- Lé Roux-Pullen, L and Lessing, D. 2011. Should veterinarians consider acrylamide that potentially occurs in starch-rich foodstuffs as a neurotoxin in dogs? *Journal of the South African Veterinary Association* 82 (2): 129-130
- Lebopa, KC, Boomker, EA, Chimonyo, M and Mokoboki, HK. 2011. *In sacco* dry matter and crude protein degradation of woody plant species in Tswana and Boer goats. *Life Science Journal-Acta Zhengzhou University Overseas Edition* 8 (S2): 81-90
- Lebopa, KC, Boomker, EA, Chimonyo, M, Mulugeta, SD. 2011. Factors affecting the feeding behaviour of free ranging Tswana and Boer goats in the False Thornveld of the Eastern Cape, South Africa. *Life Science Journal-Acta Zhengzhou University Overseas Edition* 8 (S2): 70-80
- Maartens, L, Erasmus, BJ and Clift, SJ. 2011. Tissue Tropism of African Horseshickness Virus in the Chicken Embryo Demonstrated With the Avidin-Biotin Complex Immunoperoxidase Method. *Veterinary Pathology* 48 (6): 1085-1093
- Magano, SR, Nchu, F and Eloff, JN. 2011. *In vitro* investigation of the repellent effects of the essential oil of *lippia javanica* on adults of *Hyalomma marginatum rufipes*. *African Journal of Biotechnology* 10 (44): 8970-8975
- Mans, BJ, de Klerk, D, Pienaar, R and Latif, AA. 2011. *Nuttalliella namaqua*: A living fossil and closest relative to the ancestral tick lineage: Implications for the evolution of blood-feeding in ticks. *Plos One* 6 (8): 1-11
- Mans, BJ, Pienaar, R, Latif, AA and Potgieter, FT. 2011. Diversity in the 18S SSU rRNA V4 hyper-variable region of *Theileria* spp in Cape buffalo (*Syncerus caffer*) and cattle from southern Africa. *Parasitology* 138: 766-779
- Marais, HJ and Page, PC. 2011. Treatment of Equine sarcoid in seven Cape Mountain Zebra (Equus Zebra Zebra). *Journal of Wildlife Diseases* 47 (4): 917-924
- Maree, FF, Bliognaut, B, Esterhuysen, JJ, de Beer, TAP, Theron, J, O'Neill, HG and Rieder, E. 2011. Predicting antigenic sites on the foot and mouth disease virus capsid of the South African Territories types using virus neutralization data. *Journal of General Virology* 92: 2297-2309
- Matos, CA, Siteo, C, Afonso, S, Banze, J, Baptista, J, Dias, G, Rodrigues, F, Atanasio, A, Nhamusso, A, Penrith, MI and Willingham III, AL. 2011. A pilot study of common health problems in smallholder pigs in Angonia and Boane districts, Mozambique. *Journal of the South African Veterinary Association* 82 (3): 166-169
- Meiring, T, Prozesky, L, du Preez, ER and Verwoerd, DJ. 2011. The diagnosis and prevalence of persistent infection with bovine viral diarrhoea virus in South African feedlot cattle. *Onderstepoort Journal of Veterinary Research* 78 (1): 1-8
- Mellanby, RJ, Handel, IG, Clements, DN, d Bronsvort, Bm, Lengeling, A and Schoeman, JP. 2011. Breed and Sex Risk Factors for Canine Babesiosis in South Africa. *Journal of Veterinary Internal Medicine* 779: 1-4
- Michel, AL, Cooper, D, Jooste, J, de Klerk, LM and Jolles, A. 2011. Approaches towards optimising the gamma interferon assay for diagnosing *Mycobacterium bovis* infection in African buffalo (*Synderus caffer*). *Preventive Veterinary Medicine* 98: 142-151
- Millward, IR, Kirberger, RM and Thompson, PN. 2011. Comparative popliteal and mesenteric computed tomography lymphangiography of the canine thoracic duct. *Veterinary Radiology & Ultrasound* 52 (3): 295-301
- Mitchell, G and Skinner, JD. 2011. Lung volumes in giraffes, *Giraffa camelopardalis*. *Comparative Biochemistry and Physiology B-Biochemistry & Molecular Biology* 158 (Part A): 72-78
- Mogale, MA, Lebelo, SL, Shai, LJ and Eloff, JN. 2011. *Aloe arborescens* aqueous gel extract alters the activities of key hepatic enzymes and blood concentration of triglycerides, glucose and insulin in alloxan-induced diabetic rats. *African Journal of Biotechnology* 10 (20): 4242-4248
- Mouton, A and Gummow, B. 2011. The occurrence of gut associated parasites in the South African abalone, *Haliotis midae* in Western Cape aquaculture facilities. *Aquaculture* 30: 1-6
- Mukorera, V, Dvir, E, van der Merwe, LL and Goddard, A. 2011. Serum C-Reactive Protein Concentration in Benign and Malignant Canine Spirocercosis. *Journal of Veterinary Internal Medicine* 25 (4): 963-971
- Mukorera, V, van der Merwe, LL, Lavy, E, Aroch, I and Dvir, E. 2011. Serum alkaline phosphatase activity is not a marker for neoplastic transformation of esophageal nodules in canine spirocercosis. *Veterinary Clinical Pathology* 40 (3): 389-392
- Naidoo, V, Wolter, K, Espie, I and Kotze, A. 2011. Vulture rescue and rehabilitation in South Africa: An urban perspective. *Journal of the South African Veterinary Association* 82 (1): 24-31
- Naidoo, V, Mompoti, KF, Duncan, NM and Taggart, MA. 2011. The Pied crow (*Corvus albus*) is insensitive to Diclofenac at concentrations present in carrion. *Journal of Wildlife Diseases* 47 (4): 936-944
- Nakao, R, Magona, JW, Zhou, L, Jongejan, F and Sugimoto, C. 2011. Multi-locus sequence typing of *Ehrlichia ruminantium* strains from geographically diverse origins and collected in *Amblyomma variegatum* from Uganda. *Parasites & Vectors* 4 (137): 1-9
- Nana, P, Maniania, NK, Maranga, RO, Boga, HI, Kutima, HL and Eloff, JN. 2011. Compatibility between *Calpurnia aurea* leaf extract, attraction aggregation, and attachment pheromone and entomopathogenic fungus *Metarhizium anisopliae* on viability, growth, and virulence of the pathogen. *Journal of Pest Science* : Online
- Nchu, F, Githiori, J, McGaw, LJ and Eloff, JN. 2011. Anthelmintic and cytotoxic activities of extracts of *Markhamia obtusifolia* Sprague (Bignoniaceae). *Veterinary Parasitology* 183: 184-188
- Ncube, H, Duncan, P, Grange, S, Cameron, EZ, Barnier, F and Ganswindt, A. 2011. Pattern of faecal 20-oxopregnane and oestrogen concentrations during pregnancy in wild plains zebra mares. *General and Comparative Endocrinology* (172): 358-362
- Nel, S, van Heerden, MB, Steenkamp, G, van Heerden, WFP and Boy, SC. 2011. Immunohistochemical profile of Odontogenic Epithelium in developing dog teeth (*Canis familiaris*). *Veterinary Pathology* 48 (1): January 276-282
- Nguyen, TKA, Wieland, W, Santema, W, Hoeboer, J, van Eeden, W, Rutten, VPMG, Koets, AP and van Rhijn, I. 2011. Immune response of cattle immunized with a conjugate of the glycolipid

glucose monomycolate and protein. *Veterinary Immunology and Immunopathology* 142: 265-270

Nyangiwe, N, Goni, S, Hervé-Claude, LP, Ruddat, I and Horak, IG. 2011. Ticks on pastures and on two breeds of cattle in the Eastern Cape province, South Africa. *Onderstepoort Journal of Veterinary Research* 78 (1): 1-9

Oberholster, PJ and Botha, A-M. 2011. Dynamics of phytoplankton and phyto-benthos in Lake Loskop (South Africa) and downstream irrigation canals. *Fundamental And Applied Limnology* 17: 169-178

Oberholster, PJ, Musee, N, Botha, A-M, Chelule, PK, Focke, WW and Ashton, PJ. 2011. Assessment of the Effect of Nanomaterials on Sediment-Dwelling Invertebrate *Chironomus tentans* larvae. *Ecotoxicology and Environmental Safety* 74 (3): January 416-423

Penrith, ML, Vosloo, W and Mather, C. 2011. Classical swine fever (Hog cholera): Review of aspects relevant to control. *Transboundary and Emerging Diseases* 58: 187-196

Penzhorn, BL. 2011. Why is Southern African canine babesiosis so virulent? An evolutionary perspective. *Parasites & Vectors* 4: 51-55

Pfister, S, Oosthuizen, MC, Bosman, A-M, Vorster, I and Penzhorn, BL. 2011. Tick-borne blood parasites in nyala (*Tragelaphus angasii*, Gray 1849) from KwaZulu-Natal, South Africa. *Veterinary Parasitology* 176: 126-131

Pienaar, R, Potgieter, FT, Latif, AA, Thekiso, OMM and Mans, BJ. 2011. Mixed *Theileria* infection in free-ranging buffalo herds: implications for diagnosing *Theileria parva* infections in Cape buffalo (*Syncerus caffer*). *Parasitology* 138: 884-895

Pienaar, R, Potgieter, FT, Latif, AA, Thekiso, OMM and Mans, BJ. 2011. The Hybrid II assay: a sensitive and specific real-time hybridization assay for the diagnosis of *Theileria parva* infections in Cape buffalo (*Syncerus caffer*) and cattle. *Parasitology* 138: 1935-1944

Potgieter, M, Pretorius, E, van der Merwe, CF, Beukes, M, Vieira, WA, Auer, REJ, Auer, M and Meyer, S. 2011. Histological assessment of SJL/J mice treated with the antioxidants coenzyme Q10 and resveratrol. *Micron* 42: 275-282

Pulker, T, Carstens, A and Williams, JH. 2011. Antibacterial chondrodysplasia in New Zealand white rabbits (*Oryctolagus cuniculus*). *Journal of the South African Veterinary Association* 82 (3): 176-178

Pypers, AR, Holm, De and Williams, JH. 2011. Fatal congenital anaplasmosis associated with bovine viral diarrhoea virus (BVDV) infection in crossbred calf. *Journal of the South African Veterinary Association* 82 (3): 179-182

Rakabe, MM, van Wyngaardt, W and Fehrsen, J. 2011. Chicken single-chain antibody fragments directed against recombinant VP7 of bluetongue virus. *Food and Agricultural Immunology*. 283-295

Reynecke, D, van Wyk, JA, Gummow, B, Dorny, P and Boomker, JDF. 2011. A stochastic model accommodating the FAMACHA® system for estimating worm burdens and associated risk factors in sheep naturally infected with *Haemonchus contortus*. *Veterinary Parasitology* 177: 231-241

Reynecke, D, van Wyk, JA, Gummow, B, Dorny, P and Boomker, JDF. 2011. Application of ROC curve analysis to FAMACHA® evaluation of haemonchosis on two sheep farms in South Africa. *Veterinary Parasitology* 177: 224-230

Reynecke, D, van Wyk, JA, Gummow, B, Dorny, P and Boomker, JDF. 2011. Validation of the FAMACHA® eye colour chart6 using sensitivity / specificity analysis on two South African sheep farms. *Veterinary Parasitology* 177: 203-211

Riley, DG and van Wyk, JA. 2011. The effects of penalization of FAMACHA® scores of lambs treated for internal parasites on the estimation of genetic parameters and prediction of breeding values. *Small Ruminant Research* 99: 122-129

Santema, W, van Kooten, P, Hoek, A, Leeflang, M, Overdijk, M, Rutten, VPMG and Koets, AP. 2011. Hsp70 vaccination-induced antibodies recognize B cell epitopes in the cell wall of *Mycobacterium avium* subspecies *paratuberculosis*. *Vaccine* 29: 1364-1373



- Santema, W, Rutten, VPMG and Koets, AP. 2011. Bovine paratuberculosis: recent advances in vaccine development. *Veterinary Quarterly*; 183-191
- Scheepers, E, Leisewitz, AL, Thompson, PN and Christopher, MM. 2011. Serial haematology results in transfused and non-transfused dogs naturally infected with *Babesia rossi*. *Journal of the South African Veterinary Association* 82 (3): 136-143
- Scheffer, EG, Venter, GJ, Joone, C, Osterrieder, N and Guthrie, AJ. 2011. Use of real-time quantitative reverse transcription polymerase chain reaction for the detection of African horse sickness virus replication in *Culicoides imicola*. *Onderstepoort Journal of Veterinary Research* 78 (1): 1-4
- Schlotter, YM, Rutten, VPMG, Riemers, FM, Knol, EF and Willemse, T. 2011. Lesional skin in atopic dogs shows a mixed Type-1 and Type-2 immune responsiveness. *Veterinary Immunology and Immunopathology* 143: 20-26
- Schoeman, JP, Kitshoff, AM, du Plessis, CJ and Thompson, PN. 2011. Serial plasma glucose changes in dogs suffering from severe dog bite wounds. *Journal of the South African Veterinary Association* 82 (1): 41-46
- Sibeko, KP, Collins, NE, Oosthuizen, MC, Troskie, M, Potgieter, FT, Coetzer, JAW and Geysen, D. 2011. Analyses of genes encoding *Theileria parva* p104 and polymorphic immunodominant molecule (PIM) reveal evidence of the presence of cattle-type alleles in the South African *T. parva* population. *Veterinary Parasitology* 181: 120-130
- Sixholo, J, van Wyngaardt, W, Mashau, C, Frischmuth, J, du Plessis, DH and Fehrsen, J. 2011. Improving the characteristics of a mycobacterial 16 kDa-specific chicken scFv. *Biologicals* 39: 110-116
- Solomon, P, Abolnik, C, Joannis, TM and Bisschop, SPR. 2011. Virulent Newcastle disease virus in Nigeria: identification of a new clade of sub-lineage 5f from livebird markets. *Virus Genes* 42 (1): September Online
- Spickett, AM, Gallivan, GJ and Horak, IG. 2011. The dynamics of questing ticks collected for 164 consecutive months off the vegetation of two landscape zones in the Kruger National Park (1988-2002). Part II. Total ticks, *Rhipicephalus appendiculatus* and *Rhipicephalus zambeziensis*. *Onderstepoort Journal of Veterinary Research* 78 (1): 1-9
- Stander, N and Kirberger, RM. 2011. Diagnostic imaging of migrating kebab (sosatje) sticks – a review of 8 cases. *Journal of the South African Veterinary Association* 82 (3): 160-165
- Stansfield, FJ, Picton, HM and Nöthling, JO. 2011. Early primary-rather than primordial follicles constitute the main follicular reserve in the African elephant (*Loxodonta africana*). *Animal Reproduction Science* 123: 112-118
- Stansfield, FJ, Nöthling, JO and Ansari, T. 2011. The distribution of small preantral follicles within the ovaries of prepubertal African elephants (*Loxodonta africana*). *Animal Reproduction Science* 129: 96-103
- Stephenson, JD, Mills, A, Eksteen, JJ, Milewski, AV and Myburgh, JG. 2011. Geochemistry of mineral licks at Loskop Dam Nature Reserve, Mpumalanga, South Africa. *Environmental Geochemistry and Health* 33: 49-53
- Tivane, C, Ridrigues, MN, Soley, JT and Groenewald, HB. 2011. Gross anatomical features of the oropharyngeal cavity of the ostrich (*Struthio camelus*). *Pesquisa Veterinaria Brasileira* 31 (6): Junho 543-550
- Uys, JL, Lourens, DC and Thompson AH. 2011. The effect of unrestricted milk feeding on the growth and health of Jersey calves. *Journal of the South African Veterinary Association* 82 (1): 47-52
- Van Dyk, E, Bosman, A-M, van Wilpe, E, Williams, JH, Bengis, RG, van Heerden, J and Venter, EH. 2011. Detection and characterisation of papillomavirus in skin lesions of giraffe and sable antelope in South Africa. *Journal of the South African Veterinary Association* 82 (2): 80-85
- Van Wyk, JA and Reynecke, D. 2011. Blueprint for an automated specific decision support system for countering anthelmintic resistance in *Haemonchus* spp. at farm level. *Veterinary Parasitology* 177: 212-223
- Vatta, AF, Kandu-Lelo, C, Ademola, IO and Eloff, JN. 2011. Direct anthelmintic effects of *Cereus jamacaru* (Cactaceae) on trichostrongylid nematodes of sheep: *In vivo* studies. *Veterinary Parasitology* 180: 279-286
- Veenhof, EZ, Knol, EF, Schlotter, YM, Vernooij, JC, Rutten, VPMG, Willemse, M. 2011. Characterisation of T cell phenotypes, cytokines and transcription factors in the skin of dogs with cutaneous adverse food reactions. *Veterinary Journal* 187: 320-324
- Venter, EH, Gerdes, T, Wright, I and Terblanche, J. 2011. An investigation into the possibility of bluetongue virus transmission by transfer of infected ovine embryos. *Onderstepoort Journal of Veterinary Research* 78 (17): 1-7
- Venter, GJ, Labuschagne, K, Boikanyo, SNB, Morey, L and Snyman, MG. 2011. The repellent effect of organic fatty acids on *Culicoides* midges as determined with suction light traps in South Africa. *Veterinary Parasitology* 181: 365-369
- Venter, M, Human, S, van Niekerk, S, Williams, JH, van Eeden, C and Freeman, F. 2011. Fatal Neurological Disease and Abortion in Mare Infected with Lineage 1 West Nile Virus, South Africa. *Emerging Infectious Diseases* 17 (8): August 1534-1536
- Williams, JH, van Dyk, E, Nel, PJ, Lane, E, van Wilpe, E, Bengis, RG, de Klerk-Lorist, L-M and van Heerden, J. 2011. Pathology and immunohistochemistry of papillomavirus-associated cutaneous lesions in Cape mountain zebra, giraffe, sable antelope and African buffalo in South Africa. *Journal of the South African Veterinary Association* 82 (2): 97-106
- Williams, JH, Bester, L, Venter, LJ, Pretorius, D and Greyling, F. 2011. Barbiturate ingestion in three adult captive tigers (*Panthera tigris*) and concomitant fatal botulism of one. *Journal of the South African Veterinary Association* 82 (4): 244-249
- Young, BD, Levine, JM, Porter, BF, Chen-Allen, AV, Rossmeis, JH, Platt, SR, Kent, M, Fosgate, GR and Schatzberg, SJ. 2011. Magnetic resonance imaging features of intracranial astrocytomas and oligodendrogliomas in dogs. *Veterinary Radiology & Ultrasound* 52 (1): 132-141
- Zimmermann, D, Anderson, MD, Lane, E, van Wilpe, E, Carulei, O, Douglass, N, Williamson, A-L, Kotze, A. 2011. Avian Poxvirus Epizootic in a Breeding Population of Lesser Flamingos (*Phoenicopterus minor*) at Kamfers Dam, Kimberley, South Africa. *Journal of Wildlife Diseases* 47 (4): 989-993

Faculty Day 2012: Committees



Organising Committee

Dr PEA van Dam (Chairperson)

Sr S Johnson (Exhibitors and sponsors)

Mr C van Blerk (Marketing and publications)

Ms N Tromp (Administration)

Ms R Zeeman (Administration)

Master of Ceremonies

Dr L Le Roux-Pullen

Scientific Committee

Prof PN Thompson

Prof A Carstens

Prof RA Meintjes

Prof MC Oosthuizen

Adjudicators

Oral

Prof HB Groenewald

Dr LJ McGaw

Prof LM Rubio-Martínez

Posters

Dr H van Heerden

Dr R Moerane

Dr PC Page

Parallel Student Programme

Prof K Pettey

Cover sketch

Ms Faye Scheepers

Layout and design

Janine Smit Editorial Services

Editor

Chris van Blerk

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



UNIVERSITEIT VAN PRETORIA
UNIVERSITY OF PRETORIA
YUNIBESITHI YA PRETORIA
Faculty of Veterinary Science