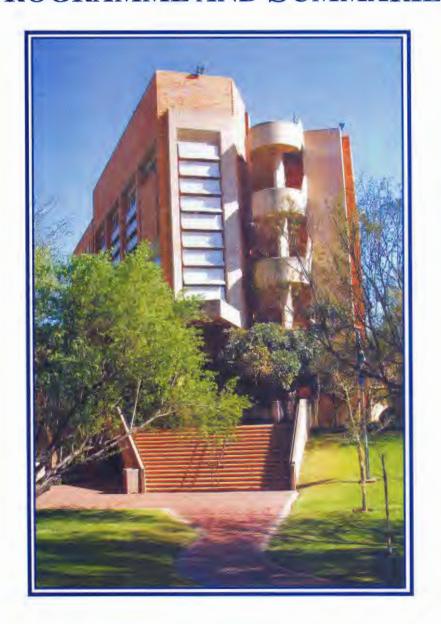


UNIVERSITY OF PRETORIA

FACULTY OF VETERINARY SCIENCE

FACULTY DAY SEPTEMBER 28, 2006

PROGRAMME AND SUMMARIES



FACULTY DAY

FACULTY OF VETERINARY SCIENCE

UNIVERSITY OF PRETORIA

28 SEPTEMBER 2006



Printing of programme sponsored by:



Animal Health

Front cover designed by Marté Smit

SPONSORSHIPS

The Faculty of Veterinary Science wishes to express sincere thanks to the following sponsors for their very generous contribution towards the support of the 2006 Faculty Day programme:



Bayer (Pty) Ltd: Animal Health Division



Elanco Animal Health, a Division of Eli Lilly SA (Pty) Ltd



Hill's Pet Nutrition



Instavet Import & Export (Pty) Ltd



Merial SA (Pty) Ltd



Onderstepoort Biological Products



Animal Health
Pfizer Animal Health
(Pty) Ltd



South African Veterinary Association



Nestlé Purina Petcare



Royal Canin SA (Ptv) Ltd



Virbac South Africa

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 advertising 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 nineteen eighties, the staff, and later students, were involved in the activities of the "Academic Day" which 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 5th 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.

CONTENTS

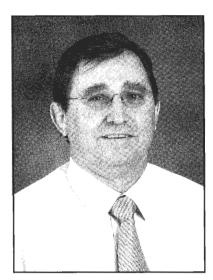
MESSAGE FROM THE DEAN – Prof G E Swan	
CURRICULUM VITAE – Dr B D Perry	8
PROGRAMME	9
SIR ARNOLD THEILER MEMORIAL LECTURE "The global poverty reduction agenda: What are the implications for animal health research and development?" Dr B D Perry	14
RESEARCH PROGRAMME – ORAL PRESENTATIONS	
Endocrine predictors of mortality in canine babesiosis J P Schoeman, M E Herrtage	15
The correlations of clinical and historical data with venous <i>Babesia canis rossi</i> parasitaemias and outcome of infection <i>M Böhm, A L Leisewitz, P N Thompson, J P Schoeman</i>	16
Oxyglobin® and packed red cell transfusions provide similar blood gas, acid base, haemodynamic and subjective benefits in a canine <i>Babesia canis rossi</i> model of anaemia <i>A B Zambelli, A L Leisewitz, J P Schoeman</i>	117
The haematological kinetics of canine babesiosis E Scheepers, A L Leisewitz, M M Christopher, P Thompson	18
Low serum thyroxine concentrations correlate with mortality in canine critical illness J P Schoeman, A Goddard, ME Herrtage	19
Epidemiological studies on gammaherpesviruses in goats and sheep causing malignant catarrhal fever	20
M Stokstad, A M Bosman, M van Vuuren	
Molecular phylogenetic analysis and vector identification of a <i>Hepatozoon</i> organism infecting Nile crocodiles	21
D P Gomersall, N J Smit, M C Oosthuizen, B L Penzhorn	
The incidence and economic significance of persistently infected feedlot cattle with bovine viral diarrhoea virus	22
T Meiring, L Prozesky, E de Preez, S J Clift	
An outbreak of Dermatosparaxis in a commercial Drakensberger cattle herd in South Africa DE Holm, E van Wilpe, C Harper	23

Active neosporosis with intestinal presence of suspected unsporulated oocysts in an 11-month- old Labrador Retriever bitch despite prolonged treatment on 3 different recommended	
antibacterials J H Williams, E Van Dyk, M Böhm, E Van Wilpe, S Prinsloo	24
Exercise-induced pulmonary haemorrhage in South African Thoroughbred racehorses MN Saulez, A J Guthrie, K W Hinchcliff, P S Morley, D Macdonald	25
Ultrasonographic determination of the relative kidney size in the dog W M Wagner	26
An analysis of clinicopathological data in the prediction of mortality in an equine neonatal intensive care unit (NICU) M N Saulez, B Gummow, N M Slovis, T D Byars, M Frazer, K MacGillivray, F T Bain	27
Urinary steroid analysis in the Nile crocodile (Crocodylus niloticus) L C Bekker, J G Myburgh, J H Spies, C J Botha, L J Guillette, G E Swan	28
Nutritional management of small-scale dairy farming systems in Central North West Province P J Sebei, C M E McCrindle, L Prozesky, P Manzana	29
Cellular tropism of Equine Encephalosis Virus A D Pardini, S J Clift, M M E Smit, E van Wilpe, L Prozesky, A J Guthrie	30
Habitat preferences and suppression of the tsetse flies, <i>Glossina austeni</i> and <i>G. brevipalpis</i> , in South Africa	3.1
J R Esterhuizen, K Kappmeier Green, E Nevill, P Van den Bossche	
Genetic diversity of South African <i>Theileria parva</i> isolates K. P. Sibeko, N. Collins, M. Oosthuizen, D. Geysen	32
Sero-prevalence of toxoplasmosis in sheep in South Africa N Abu Samra, C M E McCrindle, B L Penzhorn, B Cenci-Goga	33
Software-based decision-support for sustainable management of haemonchosis in small ruminants D P Reynecke, J A van Wyk	34
A pilot study to compare the bioavailability of a long-acting and conventional flunixin meglumine injectable formulation in cattle R J Peter, V Naidoo, L Bekker, G E Swan, C J Botha	35
Diclofenac: A proposed mechanism of toxicity V Naidoo, G E Swan	36

CONTENTS

Comparison of morphine and carprofen administered alone or in combination for post- operative analgesia in dogs undergoing ovariohysterectomy TB Dzikiti, KE Joubert, LJ Venter, LN Dzikiti	37
Isolation of antioxidant compounds from Combretum apiculatum D T Kgatle, M A Aderogba, J N Eloff	38
Erythrophleum sawdust: toxic bedding for pigs L J McGaw, J N Eloff, T W Naudé, J Segalés	39
Activity of invasive weed plants against plant pathogenic fungi M M Meela, L K Mdee, P Masoko, J N Eloff	40
RESEARCH PROGRAMME – POSTERS	
The immunoperoxidase staining technique as a diagnostic tool S J Clift, M S Smit, R M Phaswane	41
Prognostication in canine parvoviral diarrhoea using basal serum cortisol concentrations J P Schoeman, L J Venter, A Goddard, A L Leisewitz, M E Herrtage	42
Morphologic and molecular characterization of coccidia from the African buffalo (Syncerus caffer) D H Lorom, M C Oosthuizen, B L Penzhorn	43
Cardiovascular effects of lumbar epidural anaesthesia in isoflurane-anaesthetised pigs during surgical devascularisation of the liver <i>G F Stegmann</i>	44
Canine bladder trigone diverticulum W M Wagner, B Meyers	45
Distribution and structural features of Pacinian (Herbst) corpuscles in the non-glandular region of the palate of the ostrich (Struthio camelus) C Tivane, J T Soley, H B Groenewald	46
The arterial microvasculature of the distal ductus deferens, receptaculum ductus deferentis and phallus of the ostrich as revealed by India ink injection MZJ Elias, TA Aire, JT Soley	47
Morphological and immunohistochemical characterization of the testicular capsule and peritubular tissue of ratite birds P.C. Ozegbe, T.A. Aire, M-C. Madekurozwa, J.T. Soley	48

The effect of rumen lesions caused by subclinical acidosis on growth in feedlot calves <i>P Thompson, A Hentzen, W Schultheiss</i>	49
The public health implications of farming cattle in areas with high background concentrations of vanadium	50
B Gummow, C J Botha, J P T M Noordhuizen, J A P Heesterbeek	



Prof G E Swan

Welcome to Faculty Day 2006. Faculty Day is an important annual event on our calendar. It is a day in which we acknowledge the research contributions of staff and post graduate students. This day serves as a showcase of the scope and quality of the research conducted on our campus. Faculty members are informed of research conducted in other departments and by their fellow colleagues. Young researchers and post graduate students get the opportunity to present their research results for the first time and to gain experience in the skill of scientific presentation. This year's proceedings reflect the continued increase in research output in the Faculty, particularly the contributions by post-graduate students and the shift to more in-depth research, consistent with our strategic focus.

The University of Pretoria's mission is to be an internationally recognized teaching and research University. Faculty Day is used to celebrate the achievements of our staff in this regard by the announcement of the winners of the *Lecturers of the Year* and *Researchers of the Year* awards. These awards have been created to stimulate excellence in teaching and research in the Faculty. Congratulations to the winners in the various award categories. May these awards inspire you to reach further heights!

Steady advance in creating a research ethos in the Faculty has been made over the past few years. This is reflected in the larger number of NRF-rated scientists, growth in our research funding, an increase in the number of Masters and PhD students, larger numbers of post doctoral positions and an improvement in publication outputs. The identification and focus on specific research niche areas has opened the way to direct more resources and to increase our competitive edge in these areas. This has led to the approval by the National Research Foundation of two of the areas, the only ones currently credited to the University of Pretoria. To become truly internationally competitive it is essential that we continue relentlessly on this path to establish ourselves as a research faculty alongside our recognized clinical strength.

My sincerest thanks go to all participants of the scientific programme, as well as to those who have come to support them. I trust that you will have a most enjoyable and enriching day. A great thank you and appreciation also goes to the organizers of the day as well as to our sponsors. Without your contributions and dedication this day would not be possible

GERRY SWAN DEAN



Dr. Brian Perry

Dr Brian Perry

Brian Perry *OBE, BVM&S, DTVM, MSc, DVM&S, FRCVS* is a British citizen and veterinary surgeon by profession. His long and distinguished research career has focused on the resolution of animal health issues affecting developing countries, in particular through integrating quantitative veterinary epidemiology and agricultural economics to inform policy on animal health and poverty reduction. Currently he is Team Leader of the research on Animal Health and Food Safety for Trade at the International Livestock Research Institute (ILRI), Nairobi, Kenya, one of the 15 global research institutes comprising the Consultative Group for International Agricultural Research (CGIAR).

Dr. Perry grew up in a farming family in Norfolk, UK, and graduated as a vet from Edinburgh University in 1969. After a period in general veterinary practice, he specialised in tropical veterinary medicine (gaining a Diploma of Tropical Veterinary Medicine and later a Master of Science, both from Edinburgh University). He then spent 10 years living and working in Ethiopia, Colombia and Zambia on animal disease control projects, defining the major disease constraints to livestock productivity, and better understanding their dynamics, impact and control in different settings.

In 1982 he was recruited as the veterinary epidemiologist to the newly-created Virginia-Maryland Regional College of Veterinary Medicine in Blacksburg, Virginia, USA. There he initiated courses in veterinary epidemiology, protozoology, public health and tropical animal diseases, and conducted research into Potomac horse fever, rabies and improving animal health information systems. In 1985 he was awarded tenure at the University and promoted to Associate Professor. In 1987 he was awarded a Doctorate of Veterinary Medicine and Surgery by his *alma mater*, Edinburgh University. In 1987 he returned to Africa as the veterinary epidemiologist at the International Laboratory for Research on Animal Diseases (ILRAD), Nairobi, Kenya, where he led the newly created Epidemiology and Socioeconomics Programme. His research initially focussed on the dynamics, impact and control of the tick and tsetse-transmitted infections of Africa livestock. ILRAD became ILRI in 1995, and his research expanded to include other diseases and continents. He has specialized in methodologies to determine the impacts, both biophysical and economic, of diseases and of alternative strategies and policies to control them, with particular emphasis on how animal disease control can contribute to development and poverty reduction. He also undertakes strategic planning and facilitation workshops, and uses probing interview techniques ("hard talk" style) as a science communication tool.

Dr. Perry has published more than 230 scientific articles in refereed journals, books and proceedings. He is a past Chairman of the International Society for Veterinary Epidemiology and Economics (ISVEE), and for 15 years was on the editorial board for *Preventive Veterinary Medicine*, the international flagship journal for veterinary epidemiology and economics. He has consulted to projects in many countries, including Australia, Costa Rica, Guatemala, Nicaragua, Peru, Uruguay, Egypt, Mozambique, Nigeria, Zimbabwe, South Africa, Thailand, Philippines and Laos. He speaks Spanish and Swedish fluently and has a working knowledge of French. He has served on expert committees of the FAO and the World Health Organisation (WHO). He was made a Fellow of the Royal College of Veterinary Surgeons in 1995 for "meritorious contributions to learning in the field of veterinary epidemiology". In 2002 he was appointed Officer of the Order of the British Empire (OBE) in the Queen's New Year Honours for "services to veterinary science in developing countries". In 2004 he won the International Outstanding Scientist Award from the Washington-based CGIAR.

Away from work, he is an active polo player; he has been Chairman of the Nairobi Polo Club for almost 10 years, and until recently was also Chief Steward of the Jockey Club of Kenya.

FACULTY OF VETERINARY SCIENCE, UNIVERSITY OF PRETORIA

FACULTY DAY

THURSDAY 28TH SEPTEMBER 2006

PROGRAMME

07:45-08:15 Registration and Coffee

Master of Ceremonies: Prof N M Duncan

08:15-08:30 Welcome and Opening Address

Dean: Prof G E Swan

08:30-09:30 RESEARCH PROGRAMME: ORAL PRESENTATIONS I

SESSION CHAIRPERSON: Doctor M E de Vries

Endocrine predictors of mortality in canine babesiosis

J P Schoeman, M E Herrtage

The correlations of clinical and historical data with venous *Babesia canis rossi* parasitaemias and outcome of infection

M Böhm, A L Leisewitz, P N Thompson, J P Schoeman

Oxyglobin® and packed red cell transfusions provide similar blood gas, acid base, haemodynamic and subjective benefits in a canine *Babesia canis rossi* model of anaemia *A B Zambelli, A L Leisewitz, J P Schoeman*

The haematological kinetics of canine babesiosis

E Scheepers, A L Leisewitz, M M Christopher, P Thompson

Low serum thyroxine concentrations correlate with mortality in canine critical illness J P Schoeman, A Goddard, M E Herrtage

09:30-10:20 Sir Arnold Theiler Memorial Lecture:

"The global poverty reduction agenda: What are the implications for animal health research and development?"

Dr Brian D Perry

10:20-10:40 Awards Presentation: Lecturer of the Year; Nursing Lecturer of the Year; Researcher of the Year; Young Researcher of the Year

10:40-11:30 TEA and Viewing of Posters, Commercial Exhibits and Photographic Exhibition

11:30-12:30 RESEARCH PROGRAMME: ORAL PRESENTATIONS II SESSION CHAIRPERSON: Doctor V Naidoo

Epidemiological studies on gammaherpesviruses in goats and sheep causing malignant catarrhal fever

M Stokstad, A M Bosman, M van Vuuren

Molecular phylogenetic analysis and vector identification of a *Hepatozoon* organism infecting Nile crocodiles

D P Gomersall, N J Smit, M C Oosthuizen, B L Penzhorn

The incidence and economic significance of persistently infected feedlot cattle with bovine viral diarrhoea virus

T Meiring, L Prozesky, E de Preez, S J Clift

An outbreak of Dermatosparaxis in a commercial Drakensberger cattle herd in South Africa

D E Holm, E van Wilpe, C Harper

Active neosporosis with intestinal presence of suspected unsporulated oocysts in an 11-month-old Labrador Retriever bitch despite prolonged treatment on 3 different recommended antibacterials

J H Williams, E Van Dyk, M Böhm, E Van Wilpe, S Prinsloo

12:30-13:00 RESEARCH PROGRAMME: PRESENTATION OF POSTERS SESSION CHAIRPERSON: *Professor M van Vuuren*

- P1. The immunoperoxidase staining technique as a diagnostic tool S J Clift, M S Smit, R M Phaswane
- P2. Prognostication in canine parvoviral diarrhoea using basal serum cortisol concentrations

J P Schoeman, L J Venter, A Goddard, A L Leisewitz M E Herrtage

P3. Morphologic and molecular characterization of coccidia from the African buffalo (Syncerus caffer)

D H Lorom, M C Oosthuizen, B L Penzhorn

P4. Cardiovascular effects of lumbar epidural anaesthesia in isoflurane-anaesthetised pigs during surgical devascularisation of the liver *G F Stegmann*

P5. Canine bladder trigone diverticulum

W M Wagner, B Meyers

P6. Distribution and structural features of Pacinian (Herbst) corpuscles in the non glandular region of the palate of the ostrich (Struthio camelus)

C Tivane, J T Soley, H B Groenewald

P7. The arterial microvasculature of the distal ductus deferens, receptaculum ductus deferentis and phallus of the ostrich as revealed by India ink injection MZJ Elias, TA Aire, JT Soley

P8. Morphological and immunohistochemical characterization of the testicular capsule and peritubular tissue of ratite birds

P C Ozegbe, T A Aire, M-C Madekurozwa, J T Soley

- P9. The effect of rumen lesions caused by subclinical acidosis on growth in feedlot calves P Thompson, A Hentzen, W Schultheiss
- P10. The public health implications of farming cattle in areas with high background concentrations of vanadium

B Gummow, C J Botha, J P T M Noordhuizen, J A P Heesterbeek

13:00-13:45 Light LUNCH in Cafeteria

13:45-15:00 RESEARCH PROGRAMME: ORAL PRESENTATIONS III SESSION CHAIRPERSON: Doctor C H Annandale

Exercise-induced pulmonary haemorrhage in South African Thoroughbred racehorses M N Saulez, A J Guthrie, K W Hinchcliff, P S Morley, D Macdonald

Ultrasonographic determination of the relative kidney size in the dog W M Wagner

An analysis of clinicopathological data in the prediction of mortality in an equine neonatal intensive care unit (NICU)

M N Saulez, B Gummow, N M Slovis, T D Byars, M Frazer, K MacGillivray, F T Bain

Urinary steroid analysis in the Nile crocodile (Crocodylus niloticus)

L C Bekker, J G Myburgh, J H Spies, C J Botha, L J Guillette, G E Swan

Nutritional management of small-scale dairy farming systems in Central North West Province

P J Sebei, C M E McCrindle, L Prozesky, P Manzana

15:00-16:00 RESEARCH PROGRAMME: ORAL PRESENTATIONS IV

SESSION CHAIRPERSON: Professor R A Mogotlane

Cellular tropism of Equine Encephalosis Virus

A D Pardini, S J Clift, M M E Smit, E van Wilpe, L Prozesky, A J Guthrie

Habitat preferences and suppression of the tsetse flies, Glossina austeni and G brevipalpis, in South Africa

J R Esterhuizen, K Kappmeier Green, E Nevill, P van den Bossche

Genetic diversity of South African Theileria parva isolates

K P Sibeko, N Collins, M Oosthuizen, D Geysen

Sero-prevalence of toxoplasmosis in sheep in South Africa

N Abu Samra, C M E McCrindle, B L Penzhorn, B Cenci-Goga

Software-based decision-support for sustainable management of haemonchosis in small ruminants

D P Reynecke, J A van Wyk

16:00-16:30 TEA and Viewing of Posters, Commercial Exhibits and Photographic Exhibition

16:30-17:45 RESEARCH PROGRAMME: ORAL PRESENTATIONS V

SESSION CHAIRPERSON: Doctor J G Myburgh

A pilot study to compare the bioavailability of a long-acting and conventional flunixin meglumine injectable formulation in cattle

R J Peter, V Naidoo, L Bekker, G E Swan, C J Botha

Diclofenac: A proposed mechanism of toxicity

V Naidoo, G E Swan

Comparison of morphine and carprofen administered alone or in combination for postoperative analgesia in dogs undergoing ovariohysterectomy

T B Dzikiti, K E Joubert, L J Venter, L N Dzikiti

Isolation of antioxidant compounds from Combretum apiculatum

D T Kgatle, M A Aderogba, J N Eloff

Erythrophleum sawdust: toxic bedding for pigs

L J McGaw, J N Eloff, T W Naudé, J Segalés

Activity of invasive weed plants against plant pathogenic fungi

M M Meela, L K Mdee, P Masoko, J N Eloff

17:45- COCKTAIL FUNCTION and PRIZE GIVING

During the cocktail function the following awards will be presented:

Best Scientific Paper; Best Scientific Poster; Photography prizes

THE FOLLOWING EXHIBITIONS ARE ON VIEW IN THE FOYER OF THE SIR ARNOLD THEILER BUILDING THROUGHOUT THE DAY:

1. PHOTOGRAPHIC EXHIBITION

An exhibition of photographs taken by staff and students. The photographs will be judged by Ludwig Jacobs en Naas Rautenbach who have extensive national judging experience.

Organisor: Dr E van Dyk

- 2. EXHIBITS BY SPONSORS
- 3. SCIENTIFIC POSTERS

The global poverty reduction agenda: What are the implications for animal health research and development?

BD Perry (b.perry@cgiar.org)

International Livestock Research Institute (ILRI), P.O. Box 30709, Nairobi 00100, Kenya

It is generally recognised that poverty is the greatest constraint to global harmony and the well being of the peoples of the world. Poverty is a problem of extraordinary proportion, with almost half of the world's 6 billion people living on less than US\$ 2 per day. But this is not a static situation, and during the next 25 years, the human population is predicted to grow by a further 2 billion, 97% of which will be in the countries of the developing world. These are dramatic figures. And it is these figures and trends that were behind the creation of the Millenium Development Goals (MDGs), centred on reducing the proportion of people living in extreme poverty by half between 1990 and 2015. An ambitious target indeed.

So where do livestock and their diseases fit into this picture, and how can improved animal health contribute to our meeting the MDG targets? It has been estimated that livestock form a component of the livelihoods of 70% of the world's poor. Livestock are important in supporting the livelihoods not only of poor farmers, but also of consumers, traders and labourers throughout the developing world, and of the national economies of many developing countries through international trading in their livestock products. Growing markets, both domestic and international, can provide a poverty reduction mechanism, particularly for poor farmers. However animal diseases, or the threat of them, are an every day occurrence to these groups of people.

While exposed to a wide array of risks related to animal disease, many developing countries, and the poor livestock keepers in them, often have a reduced capacity to cope. Those existing close to the survival threshold tend to be more risk averse, and so less likely to "take a chance" on preventive disease technologies. More importantly, low income and few assets mean that the poor have few options available for managing crises, are less resilient to shocks and are slower to recover. Livestock diseases are particularly damaging since they threaten one of the few assets that the poor keep on hand for dealing with other shocks.

This presentation will review our understanding of the distribution and dynamics of poverty, the diverse roles of livestock to poor countries and the poorer sectors of society in them, and the diverse impacts of animal diseases and of interventions to control them. The presentation will discuss the merits of prioritisation of animal health constraints for optimal impact on poverty reduction, and the increasingly complex impact assessment methodologies that contribute to such evaluations. In discussing these issues, the presentation will pay particular attention to the combined roles of veterinary epidemiology and agricultural economics in fostering greater understanding of the impacts of diseases and their control on poverty.

Teasing out priorities is one thing, and this in itself is an evolving and somewhat controversial science, but acting on them is quite another. How well are veterinarians and others in the animal health arena responding to these continental and global challenges, and how do their efforts fit in with the other growing and diversifying challenges and opportunities for the veterinary profession?

Endocrine predictors of mortality in canine babesiosis

JP Schoeman¹, ME Herrtage² (johanp.schoeman@up.ac.za)

Department of Companion Animal Clinical Studies, Faculty of Veterinary Science, University of Pretoria,
Private Bag X04, Onderstepoort 0110, South Africa
Department of Clinical Veterinary Medicine, University of Cambridge, United Kingdom

Various endocrine predictors of mortality have been studied in humans. Basal cortisol is well-recognised for its positive correlation and thyroxine (TT4) for its negative correlation to mortality in human critical illness. Many factors including; breed, age, Ht, parasitaemia, serum lactate and glucose concentrations as well as cerebral and renal involvement have been shown to correlate with outcome in the virulent form of canine babesiosis found in South Africa. Despite its close resemblance to human falciparum malaria, this disease has never been used as a model to test the hypotheses that basal cortisol and total thyroxine are predictors of mortality in canine illness.

A prospective study was undertaken to determine the serum cortisol and TT4 concentrations of dogs with canine babesiosis at presentation to the Onderstepoort Veterinary Academic Hospital and observed the associated mortality. Ninety five patients were studied. The diagnosis of canine babesiosis was made on peripheral blood smear examination. The babesia subtype was confirmed as *B. canis rossi* and all patients were negative for *E. canis* by PCR and RLB. Serum cortisol and TT4 concentrations were determined by a commercial canine radioimmunoassay kit (Coat-a-count®, USA). Normal reference ranges were taken as 10-160 nmol/l and 15-45 nmol/L for cortisol and TT4 respectively. Data is expressed as median and interquartile range (IQR). The cortisol and TT4 concentrations between the survivors and non-survivors were compared using the Mann Whitney U test for non-parametric data. Correlation coefficients (r_s) between the variables were obtained by using the Spearman's rank order correlation.

Overall mortality was 7.5 % (7/95 dogs). Median serum cortisol and TT4 concentrations were 128 and 9 nmol/L for the whole cohort. Median cortisol concentration was significantly different between the groups, at 115 nmol/L (IQR 65-205), in the dogs that survived (Group 0) and 482 nmol/L (IQR 468-526) in the dogs that died (Group 1) (P<0.001). Median TT4 concentration was significantly different between the groups, at 10 nmol/L (IQR 0.8 - 16) in the dogs that survived (Group 0) and 0.24 nmol/L (IQR 0.005-3.4) in the dogs that died (Group 1) (P<0.05). A significant negative correlation was detected between serum cortisol and TT4 concentration (r_s = -0.493, P<0.01). Mortality was positively correlated with cortisol (r_s = 0.404, P<0.01) and negatively with TT4 (r_s = -0.227, P<0.05).

This study demonstrated a significant correlation between low TT4 and high cortisol and mortality in a defined population of dogs with canine babesiosis caused by *B. canis. rossi*.

The correlations of clinical and historical data with venous Babesia canis rossi parasitaemias and outcome of infection

M Böhm¹, A L Leisewitz², P N Thompson³, <u>J P Schoeman</u>¹ (andrew.leisewitz@up.ac.za)

Departments of Companion Animal Clinical Studies,

Veterinary Tropical Diseases, and

Production Animal Studies, Faculty of Veterinary Science, University of Pretoria, Private Bag X04,

Onderstepoort 0110, South Africa

Previous investigators have postulated that the type of immune response mounted strongly influences outcome of infection with *Babesia canis rossi*. The nature of this immune response could conceivably be affected by patient immaturity and prior exposure to the parasite. Pyrexia and immune-mediated haemolysis could represent clinical manifestations of particular immune responses. This study determined whether historical or simple clinical data was correlated with babesia parasitaemia and / or outcome of infection.

One hundred and seventeen naturally infected dogs were enrolled. Reverse line blot (RLB) confirmed *B. c. rossi* infection. Temperature and in saline agglutination (ISA) status were recorded at presentation. Parasitaemias on venous smears were manually counted and expressed as the percent parasitised red blood cells. Dogs with concurrent infections (10), with incomplete outcome data (5) or whose slides were damaged (2) were excluded, leaving 100 dogs.

The 82 dogs older than 6 months had significantly lower venous parasitaemias (median 0.099%; range 0-14.3%; interquartile range (IQR) 0.046-0.36%) than the 17 younger dogs (median 0.64%; range 0.04-30.6%; IQR 0.14-8.32%; (P=0.006). There was no association between age and outcome (P=0.68). The 72 dogs infected for the first time had significantly higher venous parasitaemias (median 0.19%; range 0.30.58%; IQR 0.048-0.9%) than the 16 dogs with a history of prior infections (median 0.047%; range 0.04-0.98%, IQR 0.04-0.048%) (P<0.001). There was no significant association between survival and prior babesia infection (P=0.34). There was no significant correlation between body temperature and venous parasitaemias (Spearman's r=-0.05; P=0.62). However, the 53 dogs with a rectal temperature above 39.5° C were significantly less likely to die than the 46 dogs that were normo- or hypothermic (P=0.005). Lastly, the venous parasitaemia of the 12 ISA positive dogs (median 0.12%; range 0.04-14.63%; IQR 0.046-4.18%) was not significatly different from that of the 88 ISA negative dogs (median 0.17%; range 0-30.6%; IQR 0.047-0.45%) (P=0.98). In addition, ISA status was not significantly associated with outcome (P=0.1).

We conclude that dogs older than 6 months and those previously infected had significantly lower venous parasitaemias. Thus maturity of the immune system and prior exposure may influence an individual's ability to curb babesia parasite proliferation. Pyrexia on presentation was significantly associated with survival.

Oxyglobin® and packed red cell transfusions provide similar blood gas, acid base, haemodynamic and subjective benefits in a canine *Babesia canis rossi* model of angemia

AB Zambelli¹, AL Leisewitz², JP Schoeman¹ (anthony.zambelli@up.ac.za)

Departments of ¹Companion Animal Clinical Studies, and ²Veterinary Tropical Diseases, University of Pretoria, Private Bag X04, Onderstepoort 0110, South Africa

Babesia canis rossi causes severe anaemia and other complications in canines, and is similar to human falciparum malaria in many respects. Standard therapy for the most common form (severe anaemia) includes packed red blood cell transfusions (pRBCT) to improve oxygen availability and correct acid base disturbances. A polymerised bovine haemoglobin-based oxygen-carrying (HBOC) solution, Oxyglobin® (HB-301, Biopure Corporation, Cambridge, USA) has been licensed for use in canines for various diseases with anaemia, haemorrhage or disturbed perfusion, as an oxygen "bridge", nitric oxide (NO) scavenger (anti-hypotensive) and to improve perfusion and buffering status.

This randomised, non-blinded pilot study compared two treatment groups of naturally infected canines suffering from uncomplicated severe anaemia caused by *Babesia canis rossi*. Patients (6 in each group) were monitored at t=0, 1, 4, 8, 24, 48 and 72 hours via femoral arterial and mixed venous sampling, oscillometric blood pressure measurement, and a subjective rubric assessment of habitus and appetite. Variables assessed included: pO₂, pCO₂, pH, [HCO₃-], blood pressure (mean arterial(MAP), systolic pressure, diastolic pressure) and heart rate. Analysis of covariance was performed to demonstrate equivalence of treatments with 80% power to detect differences of ±0.05 pH, ±8 mmHg pCO2, ±18 mmHg pO2 and ±5 mEq/L HCO3-. Wilcoxin ranked sum analysis of habitus and appetite were also performed. Significance was determined at P<0.05. Both groups were equivalent for all parameters at time t=0 and the only differences in treatment was the administration of either 20 mL/kg pRBCT or Oxyglobin® over 4 hours. 5/6 Oyxglobin®-treated and 6/6 packed red cell-treated dogs recovered. The above results are summarised in the table below.

Parameter	Differences
pH (art.)	At t=4, Oxyglobin pH>pRBCT pH (mean = 7.438 vs 7.392)
pCO ₂ (art.)	At t=4, pRBCT pCO2>Oxyglobin pCO2 (mean = 29.2 vs 23.9 mmHg)
pO_2 , [HCO ₃ -] (art.)	Did not differ at any time
Mean arterial pressure	Did not differ at any time (t-test with Welch correction)
Habitus	pRBCT was better from t=8 to t=48
Appetite	pRBCT was better at t=24

Oxyglobin® has mostly equivalent efficacy to pRBCT in the management of severe anaemia induced by *B.canis rossi*, although certain parameters are superior during the middle treatment period for pRBCT. On the basis of this study, Oxyglobin® appears at least as effective as blood as a supportive therapy for this disease.

Ethics Committee Approval V001/04.

The haematological kinetics of canine babesiosis

<u>E Scheepers</u>¹, AL Leisewitz¹, MM Christopher², P Thompson¹ (elrien.scheepers@up.ac.za)

Departments of Companion Animal Clinical Studies and Production Animal Studies, Faculty of Veterinary Science,
University of Pretoria, Private Bag X04, Onderstepoort 0110, South Africa
Department of Pathology, Microbiology & Immunology, School of Veterinary Medicine,
University of California, Davis, USA

The course of the haemopoetic response during canine babesiosis caused by *Babesia canis rossi* has never been studied. This prospective, descriptive longitudinal study on clinical cases describes the haematological kinetics during the first six days following treatment of a natural babesiosis infection.

Ninety client-owned dogs diagnosed with *Babesia canis rossi* infection, based on examination of a Cam's Quick-Stain stained thin capillary blood smear and confirmed by PCR analysis, were included. At first consultation, 24 hours, three days and six days after first consultation, or until death, an EDTA sample was collected from the jugular or cephalic vein and submitted for a full blood count, using a CELL-DYN 3700 analyzer. Based on the treatment protocol, the dogs were divided into a blood transfusion (B) group, and a non blood transfusion (NB) group.

The anaemia remained normocytic normochromic throughout the study period, and the haematocrit was still below normal for both groups by Day 6. The reticulocyte counts were moderately increased with a peak value at Day 3 and a significant (P=0.0001) decrease by Day 6. Neutrophilia with a left shift occurred more commonly in the more anaemic dogs. A degenerative left shift neutrophilia (i.e. immature neutrophil count increased, with normal or low mature neutrophil count) occurred more commonly than a regenerative left shift neutrophilia in both groups. By Day 3, less than 50% of those dogs showing a neutrophilia with a left shift, showed a regenerative response. Both groups of dogs were severely thrombocytopenic on Day 0, with normal thrombocyte counts on Day 6. Multiple regression models showed that older dogs had lower lymphocyte counts throughout the study period and that more anaemic dogs had higher immature neutrophil counts on Days 0 and 1. For the NB group, the reticulocyte counts were significantly (P<0.05) lower throughout the study period, compared to findings by Reyers *et al* (unpublished data), who described an acute normovolaemic phlebotomy-induced canine model of anaemia. These authors found a rising reticulocyte count over the first week.

The lower reticulocyte counts in our study were unexpected, as a more pronounced reticulocyte response is expected in a haemolytic compared to a blood loss anaemia. The occurrence of mostly degenerative left shift neutrophilias was also unexpected, as a haemolytic anaemia normally stimulates a regenerative inflammatory response. The reason for the moderate erythrocytic response, the decrease in the erythrocytic response within the first week and the apparent inappropriate degenerative inflammatory leucocytic response in this parasite induced haemolytic anaemia, is still unclear and warrants further investigation.

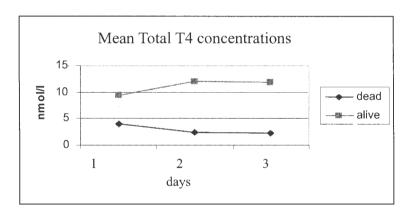
Low serum thyroxine concentrations correlate with mortality in canine critical illness

<u>JP Schoeman</u>¹, A Goddard¹, ME Herrtage² (johanp.schoeman@up.ac.za)

Department of Companion Animal Clinical Studies, Faculty of Veterinary Science,
University of Pretoria, Private Bag X04, Onderstepoort 0110, South Africa
Department of Clinical Veterinary Medicine, University of Cambridge, Cambridge, United Kingdom

Altered thyroid function in non-thyroidal illness is a well-recognised finding. However, its prevalence and prognostic value in canine critical illness is unknown. Human studies showed positive correlations between low thyrotropin (TSH) and low total thyroxine (TT4) concentrations and mortality in critical illness. Canine studies have shown more severe illness to be associated with lower TT4 concentrations.

A prospective study was undertaken to determine the serial daily serum TT4 concentrations of puppies admitted to a high care isolation ward with severe parvoviral diarrhoea and observed the associated mortality. One hundred and eight patients were studied. The diagnosis of parvoviral diarrhoea was confirmed by the detection of viral particles by faecal electron microscopy. Serum samples were taken prior to the initiation of treatment and daily thereafter until discharge or death. TT4 concentrations were determined by a commercial canine radioimmunoassay kit (Coat-a-count®, USA). Normal reference range for the laboratory has been established as 15 – 45 nmol/L.



Overall mortality was 22%. Mean serum TT4 concentration on the day of admission (day 1) was 8.2 nmol/L for the whole cohort. Mean day 1 TT4 concentration was significantly different between the groups, at 9.4 nmol/L in the dogs that survived and 4.1 nmol/L in the dogs that died (P<0.002). However, on day 2 the disparity between the mean TT4 concentrations was more marked, at 12.1 nmol/L in the dogs that survived and 2.5 nmol/L in the dogs that died (see table). Nineteen of the 24 dogs that died had serum TT4 concentrations of < 0.2 nmol/L on the day of death.

This study demonstrated the significant association between hypothyroxinaemia and mortality in a defined population of puppies with critical illness and reaffirmed the findings of significant perturbations in thyroid function in canine critical illness. Further studies are necessary to determine whether this functional hypothyroidism is primary or secondary in nature and whether it is accompanied by a concomitant decrease in free T4.

Epidemiological studies on gammaherpesviruses in goats and sheep causing malignant catarrhal fever

M Stokstad^{1,2}, AM Bosman², M van Vuuren² (maria.stokstad@veths.no)

Department of Production Animal Clinical Sciences, The Norwegian School of Veterinary Science,
Private Bag 8146 Dep, 0033 Oslo, Norway

Department of Veterinary Tropical Diseases, Faculty of Veterinary Science, University of Pretoria,
Private Bag X04, Onderstepoort 0110, South Africa

Malignant catarrhal fever (MCF) is a usually fatal gammaherpesviral disease of cattle, deer and other large ruminants. Several viruses are capable of inducing MCF, and these closely related viruses exist in nature as endemic subclinical infections in certain ruminants, to which the viruses are well adapted. The two most well known carriers of these viruses are wildebeest and sheep. In South Africa, wildebeest-associated disease is the most common, but sheep are also responsible for some cases of MCF. In a worldwide perspective, the ovine herpesvirus type 2 (OvHV-2) is believed to be responsible for most cases of MCF. Caprine herpesvirus 2 (CpHV-2) is another recently recognized gammaherpesvirus in domestic goats, and has been observed to cause clinical MCF in certain species of deer.

One of the key elements in understanding the risk factors for MCF is to know the epidemiology of the causative viruses in the carrier populations. The sheep and goats viruses have this far eluded isolation in conventional cell cultures, and only now, with the use of molecular methods, is it possible to carry out such investigations.

Specimens were collected from a Norwegian farm with both sheep and goats during 2005, and approximately 15 kids and lambs from birth and onwards were included. Their mothers were sampled twice. DNA was extracted from buffy coat samples, nasal swabs and conjunctival swabs. All DNA samples were examined for OvHV-2 and CpHV-2 by use of Polymerase Chain Reaction (PCR) techniques, and all serum samples for antibodies against gammaherpesviruses by use of an indirect fluorescent antibody test.

The results showed that most of the adult sheep were positive for OvHV-2 in all DNA samples at both occasions, and for antibodies in serum. Buffycoat collected from lambs at birth were negative, and they did not turn positive until 6-7 months of age, which corresponds with the time they seroconverted. Interestingly, the results from nasal swab/eye swabs did not correspond with this, as more than half of the lambs were positive for OvHV-2 in nasal swabs and/or eye swabs at birth. This indicates that in utero transmission is more common than previously described. In general, it was more common to find OvHV-2 in nasal and eye swabs than in blood. Only very few of the sheep samples were positive for CpHV-2. The results of the investigation for CpHV-2 in goats were quite similar to what was found for OvHv-2 in sheep. However, fewer kids were infected in utero, and the age they turned positive were a bit younger than in the lambs.

In general, there seemed to be little correspondence between the findings in the swabs and the buffycoat samples taken from the same animal at the same time, indicating that investigation of only blood samples may give a wrong conclusion. The results indicate that the epidemiology of these viruses in sheep and goat populations are more similar to the epidemiology of gammaherpesviruses in wildebeest than earlier suggested.

Molecular phylogenetic analysis and vector identification of a Hepatozoon organism infecting Nile crocodiles

DP Gomersall¹, NJ Smit¹, MC Oosthuizen², BL Penzhorn² (920203059@student.uj.ac.za)

Department of Zoology, Faculty of Natural Science, University of Johannesburg, P.O. Box 524, Auckland Park, 2006, South Africa

Historically the macroscopic simplicity of parasitic protozoan organisms has meant that the fine details of their taxonomy and phylogeny can be confusing. One such example is the genus *Hepatozoon*, which has experienced many taxonomic re-evaluations since its description, and in fact the true species-level taxonomy of many of the individuals of this genus is still unclear. Molecular biology and bioinformatic advances have made it easier to clarify the evolutionary relationship between organisms.

Haemogregarines matching the morphological and morphometric description of *Hepatozoon pettiti* were observed in blood smears taken from Nile crocodiles captured in the Okavango Delta. DNA was extracted from these stained smears, after which portions, and eventually, the full length of the 18s rRNA gene was amplified using multiple *Hepatozoon*-specific primers. Sequence analysis was performed using the Staden Package and results were aligned with published sequences of other representatives of *Hepatozoon*, using ClustalX. The phylogenetic position of *H. pettiti* was determined through the construction of trees using a combination of the neighbor-joining and bootstrap methods.

The resulting trees demonstrated that *H. pettiti* possesses the closest evolutionarily relatedness to *Hepatozoon* species infecting snakes, and then to other reptiles. The full length, 18S rRNA gene sequence from *H. pettiti* will be submitted to GenBank, to be available for use in future phylogenetic analysis. Results from the research into the life cycle of *H. pettiti* in the Okavango, showed that leeches infesting infected crocodiles contained the *H. pettiti* 18S rRNA target gene, thus demonstrating that leeches do acquire this blood protozoan when feeding on crocodiles and might thus be one of its vectors.

Future plans for this research include molecular analysis on *H. crocodinilorum* infecting American alligators in Florida, USA; and *H. caimaini* infecting black caimans in Brazil. The results of such a study, in combination with traditionally acquired data, would produce the most definitive taxonomic evaluation of crocodilian *Hepatozoon* species yet. Also, the sequencing of additional mitochondrial and genomic genes from both the parasites and their respective vertebrate hosts could be used as the basis of a model for evaluating host-parasite co-evolutionary relationships.

²Department of Veterinary Tropical Diseases, Faculty of Veterinary Science, University of Pretoria, Private Bag X04, Onderstepoort, 0110, South Africa

The incidence and economic significance of persistently infected feedlot cattle with bovine viral diarrhoea virus

<u>T Meiring</u>¹, L Prozesky², E de Preez³, SJ Clift² (thelma.meiring@up.ac.za)

¹Vetpath, P.O. Box 8464, Pretoria, 0001

Department of Paraclinical Sciences, Faculty of Veterinary Science, University of Pretoria,
Private Bag X04, Onderstepoort 0110, South Africa

³Vision Pharmaceuticals, P.O. Box 650391, Benmore, 2028

Bovine Viral Diarrhoea (BVD) is one of the most significant viral infections in both the beef and dairy industry today. The virus is associated with various clinical syndromes including a subclinical benign diarrhoea, peracute highly fatal diarrhoea, a haemorrhagic and thrombocytopaenic syndrome, reproductive failure including abortions and malformations and fatal mucosal disease in persistently infected (PI) animals.

Financial losses associated with PI animals are well known to feedlot owners in the USA and Europe, but nothing is known regarding the situation in South Africa. It was therefore decided to determine the incidence of PI animals submitted to a large feedlot in the Gauteng Province of South Africa as well as animals treated for respiratory infections using Immunoperoxidase (IMP) staining on ear notch samples.

Skin samples were fixed in formalin for two days to one month. Routine cutting and hydration of histological specimens were done. Sections were cut 3-4 microns thick and mounted on a pre-treated Superfrost Plus glass slide and dried overnight at 58 degrees Celsius. The ABC Vector Elite IMP staining was performed. After dewaxing and hydration antigen was unmasked according to proteolytic enzyme digestion (Pronase). One drop of rabbit serum with added 10 drops of PBS/BSA buffer was added to the sections for 20 minutes. Excess solution was shaken off slides and primary monoclonal BVD antibody is applied to the sections at a dilution of 1:1000 to stain for one and a half hours. The sections were then rinsed twice (once in distilled water and once in PBS/BSA buffer). Peroxidase Conjugated Avidin was then applied and incubated for 30 minutes. It was rinsed twice as before. Sections were placed in NovaRED substrate for approximately one minute. The sections were then counterstained with haematoxylin for 3-4 minutes, rinsed for 10 minutes, mounted and coverslips applied.

Immunoperoxidase staining of tissue is a quick, cost effective and reliable technique for the routine diagnosis of various diseases. It allows by means of a simple detection system the visualization of BVD antigen in the form of a coloured reagent product in various cell types i.e. smooth muscle cells, mononuclear cells (macrophages) and keratinocytes, using a light microscope.

Preliminary results on 1018 samples collected from weaned calves entering the feedlot revealed positive testing for BVD antigen in 39 cases (3, 8%) which is much higher than the international figure of between 1-2 %. It is also postulated that PI animals are predisposed to develop respiratory infections. This aspect is currently being investigated.

An outbreak of Dermatosparaxis in a commercial Drakensberger cattle herd in South Africa

<u>DE Holm</u>¹, E van Wilpe², C Harper³ (dietmar.holm@up.ac.za)

Department of Production Animal Studies,

Electron Microscopy Unit, Department of Anatomy and Physiology, and

Onderstepoort Veterinary Genetics Laboratory, Faculty of Veterinary Science, University of Pretoria,

Private Bag X04, Onderstepoort, 0110, South Africa

Dermatosparaxis, also known as cutaneous asthenia or Ehlers-Danlos syndrome, is a heritable collagen dysplasia causing hyperextensibility and fragility of the skin. This autosomal recessive defect has been reported in various species, but only in humans and sheep in South Africa. The genetic mutation was studied in Belgian Blue cattle, and described as a 3 bp change followed by a 17 bp deletion in the gene coding for the enzyme Procollagen 1 N-Protease (pNPI).

This outbreak in a commercial Drakensberger herd in the Northern Free State followed approximately one year after a new bull had been introduced in 2000. In July 2001, the bull started to develop severe skin lesions, which were unresponsive to treatment. Later that year the bull was culled. Some of his calves started to develop similar lesions from about 4 months of age; One of his sons, born in 2001, which was kept as a breeding bull, developed a slow healing lesion on the preputium after a fight with another bull. His offspring also developed skin lesions, but no mortalities occurred amongst the affected calves.

Two affected calves were referred to the Production Animal Clinic (OVAH) in October 2005. Detailed clinical examination revealed only skin abnormalities. During a farm visit, 18 cases with skin lesions were seen in the herd of 146 animals (12.3%). The lesions seen were either large skin lacerations with sharp skin edges that could be torn easily in acute cases, slow healing defects with contracted edges in sub acute cases or large, but thin scars in chronic cases. Lesions were mostly seen on the lateral extremities of the thorax and abdomen, and on bony protrusions elsewhere. Another abnormality was a thin, inelastic skin, that could easily be stretched, and that seemed to be poorly attached to the sub cutis. Animals with lesions were exclusively born during 2001, 2002, 2004 or 2005.

Transmission electron microscopy examination of skin biopsies revealed haphazard arrangement of collagen fibrils within collagen bundles with the fibrils not being as tightly packed as normal and some having a curved appearance. The fibrils showed size variation and slight irregular outlines were evident on cross-section, consistent with mild Dermatosparaxis.

DNA samples of some animals in the herd were analysed using fluorescently labelled primers to amplify the region of the pNPI gene that contained the mutation described in Belgian Blue cattle, but this mutation could not be demonstrated (homozygous or heterozygous) in any of them.

It is concluded from this case study that a milder, delayed form of Dermatosparaxis with a different gene mutation to that described in Belgian Blue cattle was found in Drakensberger cattle in South Africa, which possibly also explains the milder ultrastructural abnormalities. Further investigation needs to be undertaken to describe this mutation.

Active neosporosis with intestinal presence of suspected unsporulated oocysts in an 11-month-old Labrador Retriever bitch despite prolonged treatment on 3 different recommended antibacterials

JH Williams¹, E Van Dyk², M Böhm³, E Van Wilpe⁴, S Prinsloo⁵ (june.williams@up.ac.za)

Section of Pathology, Department of Paraclinical Sciences,

Department of Companion Animal Clinical Studies,

Electron Microscopy Unit, Department of Anatomy and Physiology, and
Department of Tropical Diseases, Faculty of Veterinary Science, University of Pretoria,

Private Bag X04, Onderstepoort 0110, South Africa

Domestic dogs are the natural definitive host of the apicomplexan protozoan parasite *Neospora caninum*. Oocysts have been found in the faeces of naturally-infected dogs and experimentally-infected coyotes (*Canis latrans*) but the enteric sexual stage of the parasite has not been seen. Other wild canids are also likely final hosts. Sources of infection in dogs include oral ingestion of oocysts from infected faeces, ingestion of tissue phases (bradyzoite-containing cysts or tachyzoites) of the parasite in meat or foetal tissues from infected intermediate or final hosts, and transplacental/vertical transmission.

A 7 week old pedigreed Labrador Retriever bitch bred in the Kloof district of Kwazulu Natal was brought to Pretoria for training as a gundog. Her growth, behaviour and training proceeded normally. At 6 months of age, she became reluctant to run out, sit, and jump over low obstacles, and then progressively ataxic, at times completely somersaulting and lying dazed. Radiography of hips, limbs and spine at 7 months of age was normal as was cervical myelogram; however, cerebro-spinal fluid tested strongly positive for *Neospora caninum* antibodies by immunofluorescent technique, whilst being negative for toxoplasma and distemper antibodies. Treatment consisted of a course of clindamycin 45mg/kg bid for 10 weeks. Two weeks after commencement, trimethoprim/ sulphamethoxyzole was added to the treatment (10 mg trimethoprim per kg bid) for a two week period. This was followed by trimethoprim only (same dose rate) until euthanasia. There were days when the bitch could sit and jump low obstacles, but the ataxia worsened during treatment. She became depressed and showed personality changes despite a continuing appetite. She was euthanased in a good body condition.

Full necropsy was macroscopically unremarkable. Histopathology, immuno-histochemistry and electron microscopy showed active tissue neosporosis. Unsporulated oocysts, which did not stain immunohistochemically for either *Neospora* or *Toxoplasma gondii*, and suspected to be those of *N.caninum* were found in the lumen of the ileum, caecum and colon. Multifocal viable cysts containing bradyzoites occurred in the cerebellar foliae, with fewer numbers sporadically in the cerebrum, lumbosacral spinal cord and a single lumbar spinal nerve root. There was multifocal chronic-active granulomatous non-suppurative myelitis and encephalitis. Free tachyzoites were visible in places when stained immunohistochemically. The tips of several cerebellar foliae showed marked tissue destruction with loss of architecture. Lymphoid hyperplasia was generalised as was the presence of eosinophils throughout most lymphoid tissues and the lamina propria of the gastro-intestinal tract.

It was clear that the treatments employed were not effective for the tissue phases of the organism. Analysis of faecal samples for confirmation of *N. caninum*, using the polymerase chain reaction (PCR) is currently being undertaken.

Exercise-induced pulmonary haemorrhage in South African Thoroughbred racehorses

MN Saulez¹, AJ Guthrie², KW Hinchcliff³, PS Morley⁴, D Macdonald⁵ (montague.saulez@up.ac.za)

Department of Companion Animal Clinical Studies, and

Equine Research Center, Faculty of Veterinary Science, University of Pretoria,

Private Bag X04, Onderstepoort 0110, South Africa

College of Veterinary Medicine, The Ohio State University, USA

College of Veterinary Medicine and Biomedical Sciences, Colorado State University, USA

The National Horse Racing Authority, South Africa

Impaired athletic performance and epistaxis are frequent complaints in racehorses with Exercise-Induced Pulmonary Haemorrhage (EIPH). Definitive diagnosis includes the presence of epistaxis, detection of blood by tracheobronchoscopy, and cytology of tracheal aspirates and bronchoalveolar lavage fluid. The precise cause of EIPH is uncertain, but rupture of pulmonary capillaries in the dorsocaudal lung lobes results in haemorrhage followed by pulmonary inflammation, fibrosis and angiogenesis. Although EIPH-related epistaxis occurs more often at sea level, the effect of altitude on the prevalence of EIPH as detected by tracheobronchoscopy is unknown. Furthermore, during maximal exercise at high altitude, the prevalence of EIPH may be exacerbated by hypoxemia. Our hypothesis was that racing at high altitude would increase the prevalence and severity of EIPH in Thoroughbred racehorses. We therefore investigated the prevalence and severity of EIPH in South Africa, where racing occurs at both sea level and at high altitude.

A cross-sectional study of pre-enrolled, unsedated Thoroughbred racehorses competing in flat races at high altitude (1,450m above sea level) and at sea level was performed. Tracheobronchoscopic examinations were performed within 2 hours after racing and recorded onto digital video disc and the presence and severity of EIPH was graded using a previously described system:

EIPH grade	Tracheobronchoscopic findings
0	No blood present in pharynx, larynx, trachea, or main stem bronchi
1	Presence of one or more flecks of blood or d" 2 short [<1/4 the length of the trachea] narrow [<10% of the tracheal surface area] streams of blood in the trachea or main stem bronchi
2	One long stream of blood [(> $\frac{1}{2}$ length of the trachea) or > 2 short streams occupying less than $\frac{1}{3}$ of the tracheal circumference
3	Multiple, distinct streams of blood covering more than $\frac{1}{3}$ of the tracheal circumference without blood pooling at the thoracic inlet
4	Coalescing streams of blood covering >90% of the tracheal surface with blood pooling at the thoracic inlet

Tracheobronchoscopic examinations were performed on 1,014 racehorses (mean \pm SD age 4.3 \pm 1.1 years {altitude} and 3.9 \pm 1 years {sea level}), competing at 5 race venues in 28 race meets over a race distance of 1,488 \pm 414 m (altitude) vs. 1,421 \pm 345 m (sea level). The severity of EIPH for 411 horses examined at high altitude and 603 horses examined at sea level was: grade 0 (49.6 vs. 41.5%), grade 1 (33.3 vs. 30.3%), grade 2 (9 vs. 13.1%), grade 3 (6.3 vs. 10.4%), grade 4 (1.7 vs. 4.6%) respectively. At sea level, EIPH was more prevalent (P = 0.002) with a greater proportion of racehorses having more severe EIPH (P<0.001). EIPH-related epistaxis was present in 6 of 35 (17.1%) horses with a grade 4 EIPH. Overall, racehorses examined at sea level were younger (P<0.001) and competed over shorter distances (P = 0.005).

Racing at high altitude does not appear to be associated with increased prevalence or severity of EIPH. We conclude that as yet unidentified factors contribute to this increased risk of EIPH at sea level, despite horses being younger and racing shorter distances.

Ultrasonographic determination of the relative kidney size in the dog

WM Wagner (wencke.wagner@up.ac.za)

Diagnostic Imaging Section, Department of Companion Animal Clinical Studies, Faculty of Veterinary Science,
University of Pretoria,
Private Bag X04, Onderstepoort 0110, South Africa.

Ultrasound imaging is usually one of the first studies performed to assess the kidneys because important anatomic information can be obtained. Despite this, up to date, there is no reliable method for determining normal kidney size by ultrasonographic measurements in the dog. Therefore, kidney size is judged primarily by subjective evaluation in dogs. Linear kidney measure-ments in the cat are more useful because there is less variation in body size. On radiographs, this dilemma has been avoided by giving quantitative measurements, correlating the kidney size to the animal's body size, hence using the animal itself as reference. This results in a reliable normal range of kidney size in the dog from 2.5 to 3.5 times the length of L2 measured on a ventrodorsal abdomi-nal radiograph. It was hypothesized that a normal ultrasonographic range of canine relative kidney size could be determined by measuring the kidney size times the length of a lumbar vertebral body.

Ten privately owned adult bitches presented as healthy animals to the Onderstepoort Veterinary Academic Hospital for spaying were used. Renal parameters (urea, creatinine, urine analysis), and clinical examination findings were within normal limits. Standard abdominal radiographs (ventrodorsal and right lateral views) were obtained and a standard abdominal ultrasonographic examination was performed using a multi-frequency phased array transducer (5-9 MHz) operated at 5 MHz to rule out any other ultrasonographic abnormal finding. Additionally, the resistive index of both kidneys, the length of the kidneys on a sagittal plane and measurements of each lumbar vertebra were recorded.

All ten animals were healthy. Kidney length measurements were consistent and the resistive indices were all within normal limits. Lumbar vertebrae could be easily visualized and were measured using two different landmarks. Identification of the last lumbar vertebra could be easily achieved, utilizing the lumbosacral junction as reference point. Visualization of the cranial lumbar vertebrae was more cumbersome, since "forward counting" was required for correct identification. This required continuous visualization of the entire lumbar spine, which could be hampered by gastrointestinal gas. Therefore, the caudal lumbar vertebrae were considered to be of better use (particularly L6) for the relative ultrasonographic measurement of the kidney in the canine patient. The left kidney to L6 ratio was 2.59 +/- 0.22 and the right kidney to L6 ratio was 2.72 +/- 0.27.

The results of the present study suggest that the lumbar vertebra can be used to give an ultrasonographic reference ratio for the relative kidney size in the dog similar to the one already existing for radiographic evaluation. Contrary to the radiological study, caudal lumbar vertebra proved to give more consistent and easier obtained results. Due to the small number of animals, this study must be considered as a preliminary study. Further studies on a larger number of healthy animals are needed to establish reference values. The results of this preliminary study are however promising. Additionally, it is envisaged to compare the reference values of healthy animals to those with acute and chronic renal disease. Further studies adapting other radiological relative canine measurements to ultrasound (relative small intestinal, large intestinal and gastric diameter) are planned by the author.

An analysis of clinicopathological data in the prediction of mortality in an equine neonatal intensive care unit (NICU)

MN Saulez¹, B Gummow², NM Slovis³, TD Byars⁴, M Frazer³, K MacGillivray³, FT Bain³ (montague.saulez@up.ac.za)

Department of Companion Animal Clinical Studies, and

Section of Epidemiology, Faculty of Veterinary Science, University of Pretoria,
Private Bag X04, Onderstepoort 0110, South Africa

Hagyard Equine Medical Institute, Lexington, KY, USA

Byars Equine Advisory, LLC, Georgetown, KY, USA

In order to establish whether clinicopathological data on venous blood collected on admission may predict mortality in critically ill equine neonantes, a retrospective, longitudinal, observational study was done on 62 equine neonates (survivors: n=41, non-survivors: n=21) admitted to a neonatal intensive care unit.

The following analyses were carried out on blood samples collected by venipuncture on admission of the foal: Lactate, WBC and platelet count, Ht, Na⁺, K⁺, Cl⁻, Ca²⁺, PO₄²⁻, PCO₂, BUN, creatinine, glucose, fibrinogen, total plasma protein, GGT, SDH, ALP, AST, LDH, CK, total bilirubin, ammonia, aniongap, albumin, A/G ratio and IgG. Descriptive statistics were performed on each variable and the median with 95% confidence interval (CI) and range reported. Median scores between the survivor and non-survivor groups were compared using the Wilcoxon Rank-Sum Test for difference in medians.

WBC count, PCO₂, anion-gap and ALP were found to differ significantly (P<0.05) between groups. WBC count, PCO₂ and ALP were higher and anion-gap lower in the survivor group. No significant difference could be found between median scores for the other parameters.

Logistic regression using (Hierarchical) Forward Switching was performed to access the correlation between the variables and mortality. Significant correlations (p<0.05) were found for an ion-gap (OR=0.56, 95% CI: 0.34 to 0.93) and K⁺ (OR = 0.18, 95% CI: 0.04 to 0.83); while less significant correlations (p<0.10) were seen with A/G ratio (OR=5.19, 95% CI: 0.92 to 29.40), CI (OR=0.74, 95% CI: 0.53 to 1.03) and lactate (OR=1.79, 95% CI: 0.99 to 3.23).

Receiver operator curves (ROCs) were calculated for those variables that had significant correlations for mortality. The area under the ROCs for lactate, K^+ and anion-gap corresponded to 0.61 (CI = 0.44 to 0.74), 0.62 (CI = 0.45 to 0.74) and 0.66 (CI = 0.5 to 0.78) respectively, indicating that none of the variables proved to be good predicators of mortality.

Although increased anion-gap, K⁺, Cl⁻, A/G ratio and lactate may correlate with mortality; overall, it is not possible to accurately predict mortality based on individual clinicopathological data collected on admission.

Urinary steroid analysis in the Nile crocodile (Crocodylus niloticus)

LC Bekker¹, JG Myburgh¹, JH Spies², CJ Botha¹, LJ Guillette³, GE Swan¹ (lizette.bekker@up.ac.za)

Department of Paraclinical Sciences, Faculty of Veterinary Science, University of Pretoria,
Private Bag X04, Onderstepoort 0110, South Africa
Department of Chemical Pathology, Faculty of Health Sciences, University of Pretoria,
P.O. Box 2034, Pretoria 0001, South Africa
Department of Zoology, University of Florida, PO Box 118525, Gainsville, FL 32611-8525,
United States of America

Excretion of steroid metabolites has been well studied in humans. We however have no information in this respect in the Nile crocodile.

Juvenile crocodiles, two years old, were used. Urine was collected by using a urine catheter. Eleven samples were pooled together when individual samples showed low sensitivity. For each urine sample, one octadecyl solid phase extraction (SPE) cartridge was primed with methanol, after which de-ionized water was aspirated. Samples were loaded onto designated cartridges, which were then washed with de-ionized water. The conjugated steroids were eluted into tubes with methanol. The eluates were dried under nitrogen gas, keeping the tubes in a heating block at 37 °C.

Enzymatic hydrolysis was achieved by adding 100 ml methanol, 5 ml of a sodium acetate/acetic acid buffer (0.2 M, pH 4.6) and 200 ml glusulase to each dried eluate, incubating overnight at 50 °C. SPE was repeated with the hydrolyzed mixtures, adding aminopropyl cartridges to facilitate sample cleanup. The free steroids were eluted with ethyl acetate into tubes, and the eluates dried under nitrogen gas at 37ÚC. Derivatization followed with addition of 100 μ l of a 10% *O*-methoxylhydroxylamine hydrochloride solution in pyridine to each dried residue, incubating the mixtures at 60 °C for 15 minutes. Addition of 100 μ l N-Trimethylsilylimidasole followed and additional incubation was allowed at 100 °C for two hours.

An HP 6890 gas chromatograph utilizing an HP 5973 mass selective detector (Agilent technologies, Palo Alto, CA, USA) was used for gas chromatographic—mass spectrometric analyses (GC-MS). Data collection and integration was achieved with HP Chem Station software. Helium, 5.5 N, was employed as carrier gas, with a flow rate of 1.2 milliliter per minute. The ion source of the mass spectrometer was set to an electron voltage of 70 eV.

The total ion chromatogram obtained more than 30 peaks of which no mass spectra were identical. Four of the electron impact mass:charge spectra obtained from the peaks on the total ion chromatogram showed similarities to endogenous human steroids (androsterone/etiocholanolone, pregnanediol, pregnanetriol and 16á-OH-DHEA). The other spectra are still unidentified. These results demonstrate that crocodilian urinary steroid metabolites are detectable when employing this sample preparation method and makes possible the establishment of a database of endogenous steroid metabolite concentration ranges.

Steroid profiling in humans can be indicative of numerous endocrine disfunctions, including reproductive and thyroid disorders, enzyme deficiencies/excesses, hypo-and hypercortisolism (including tumours of the adrenal), and starvation. Qualitative and quantitative investigation of steroids in crocodiles from clean and polluted areas, may serve as a diagnostic tool to investigate similar problems in crocodilians.

Nutritional management of small-scale dairy farming systems in Central North West Province

PJ Sebei¹, CME McCrindle¹, L Prozesky¹, P Manzana² (julius.sebei@up.ac.za)

Department of Paraclinical Sciences, Faculty of Veterinary Science, University of Pretoria,
Private Bag X04, Onderstepoort 0110, South Africa
Department of Agriculture, Conservation and Environment, North West Province, South Africa

Dairy farming presents a unique set of problems for farmers that have been allocated land and financing through land restitution policies because it involves three sectors: agriculture, veterinary services and human health. Very few emerging farmers have access to such a broad spectrum of knowledge. Key factors that threaten their survival are the high input costs of feed and veterinary assistance as well as the quality and safety of milk produced for an informal market. Research into small-scale dairy farming in Central North West Province between 2002 and 2006 indicated that there were serious deficits in the nutritional management of dairy cows by small-scale farmers. The main problem was a reactive rather than pro-active mindset: the farmers tended to wait until the milk production had dropped before supplementing the cows, rather than feeding towards the optimum possible production level. Rations were not correctly balanced and testing of feed by the ARC laboratory at Irene, revealed that feed ingredients were substandard.

Participatory rural appraisal, an economic opportunity survey and a diagnostic surveillance survey were done with small-scale farmers (n=15) in Central North West Province between 2002 and 2006. In terms of the project, a small-scale dairy farm is defined as one that produces less than 500 litres of milk per day, irrespective of the size of land, number of cows or number of people involved. From participatory consultations with small-scale dairy farmers, established commercial dairy farmers, state veterinary and agriculture staff, feed manufacturers and distributors and the commodity organization (MPO), three possible farming systems for dairy were designed and field tested. The three farming systems were, firstly, a fodder flow system based on farm-grown rations with minimal purchased inputs (Option A), secondly, a total mixed ration and zero grazing (Option B) and thirdly, a dual purpose system based on traditional management practices with minimal inputs, veld grazing and winter supplementation (Option C).

Of the 11 farmers given a choice, three selected option A, three selected Option B and five selected Option C. After 12 months, both of the farmers that had chosen option B were using Option C. The explanation given by the farmers was that the high input cost and risk associated with buying a total mixed ration was not sustainable. Economic evaluation of Option C showed that it was probably the most profitable option for small-scale dairy farmers. The chief constraints to the application of option C are that it requires more land/cow for grazing than the other two and that management of milk hygiene, cow fertility and milk production could be considerably improved.

It was concluded that further research into Option C is justified because it is a low-input system with low risk, yet there is a great deal of room for improvement in productivity, reproduction and milk hygiene. With optimisation of the system described in Option C, it is postulated that milking as few as 5 cows and selling their calves at weaning, could result in a living wage for a small-scale farmer.

Cellular tropism of Equine Encephalosis Virus

<u>AD Pardini</u>¹, SJ Clift², MME Smit², E van Wilpe³, L Prozesky², AJ Guthrie¹ (anne.pardini@up.ac.za)

¹Equine Research Centre, ²Pathology Section, Department of Paraclinical Sciences, and ³Electron Microscopy Unit, Department of Anatomy and Physiology, Faculty of Veterinary Sciences, University of Pretoria, Private Bag X04, Onderstepoort 0110, South Africa

Equine encephalosis virus (EEV) is an orbivirus of horses and other equidae in Southern Africa. EEV appears to cause mild or inapparent infection in the majority of exposed horses and the pathogenesis is poorly understood. The tissue and cellular tropism of the virus was hitherto unknown.

In this study, archival tissues from three experimentally infected ponies were stained for virus using immunohistochemistry and compared with tissues from two uninfected controls. Previously, virus had been isolated from the blood, spleen and liver of all three inoculated ponies. Archived tissues sampled for electron microscopy were also available for examination. A group-reactive polyclonal rabbit-anti-EEV antibody² directed against all 7 serotypes of EEV was used on over forty formalin-fixed tissues from each pony. The immunoperoxidase staining was applied using routine methods of the immunohistochemistry laboratory, Pathology section of the Department of Paraclinical Sciences. Nova Red stain was used as the substrate to give a pink-red colour signal discernible under the light microscope.

Positive staining was characterized by crisp granules tinged red-brown or pink-brown in the cytoplasm of infected cells. Positive staining was observed in intravascular monocytes in microvessels of liver, spleen and lung of all three ponies. A moderately high density of positive staining was seen in spleen, whereas only a few isolated positive cells were identified in liver and lung tissues. The pink-red colour is readily distinguished from endogenous pigments in cells. No viral particles could be demonstrated in the sections examined under the EM, however, one of the infected spleen sections showed a high proportion of apoptotic cells by comparison with controls.

The immunoperoxidase test is able to detect cells infected with EEV in tissues. It was established that EEV can infect monocytes but it is unclear whether the virus replicates efficiently in these cells. Infected monocytes seem to aggregate in the spleen and there is some suggestion that apoptosis is induced in this organ. The significance of apoptosis observed is not clear, but it may be a protective mechanism that halts replication rather than an indication of pathogenicity. The ability of the virus to infect cells other than leukocytes was not demonstrated.

References

- 1. Howell P G, Guthrie A J, Coetzer J A W 2004 Equine Encephalosis In: Infectious diseases of livestock in southern Africa. 2nd Ed: Coetzer, JAW & Tustin, RC. Oxford University Press, Cape Town. pp. 1247-1251.
- 2. Crafford J E, Guthrie A J, Van Vuuren M, Mertens P P C, Burroughs J N, Howell P G, Hamblin C 2003 A group-specific, indirect sandwich ELISA for the detection of equine encephalosis virus antigen. *Journal of Virological Methods* 112:129-135.

Habitat preferences and suppression of the tsetse flies, Glossina austeni and G. brevipalpis, in South Africa

JR Esterhuizen^{1,2}, K Kappmeier Green^{1,2}, E Nevill¹, P Van den Bossche^{2,3} (helsgate@iafrica.com)

Agricultural Research Council-Onderstepoort Veterinary Institute, Onderstepoort 0110, South Africa

Department of Veterinary Tropical Diseases, Faculty of Veterinary Science, Private Bag X04,

University of Pretoria, Onderstepoort 0110, South Africa

Institute of Tropical Medicine, Veterinary Department, Antwerpen, Belgium

In South Africa, livestock trypanosomiasis occurs in Zululand, KwaZulu-Natal Province and affects approximately 16000km². There are two tsetse species present, namely *Glossina austeni* and *G. brevipalpis*. To assist with the management and control of the vectors and the disease, the tsetse flies' seasonal distribution was studied in Zululand.

The H-type tsetse trap, designed specifically for the KZN conditions, was used for tsetse fly collections. The H-trap is a rectangular shaped 'tent', made from blue cloth, with two horizontal collection cones made from white netting. This trap differs from tsetse traps used in other African countries, in being more horizontally oriented towards the collection of the low flying, forest inhabiting *G. austeni* and *G. brevipalpis*. Thirty six H-traps were deployed in an 11km longitudinal transect through three habitat types (open grassland, exotic plantations and indigenous forest) on the Ndlozi peninsula in Lake St Lucia, where large populations of both tsetse species are present.

Results indicated a difference in habitat preference between the two species. The apparent abundance of *G. brevipalpis* was highest in indigenous forest and open grassland but was significantly lower in exotic plantations. *Glossina austeni*, on the other hand, seemed to move very little between habitats as the majority was captured in indigenous forest.

The implications are that for future tsetse control operations, all habitats (including less wooded areas) should be treated to control *G. brevipalpis*, while for *G. austeni* special attention will have to be paid to the forested areas.

After the habitat preference study, a tsetse population reduction trial using odour-baited (synthetic oxodours) and insecticide (0.8% deltamethrin) treated targets, was initiated. H-traps were used to monitor tsetse populations before, during and after the reduction trial. Three treatment regimes were followed. In the first year (2001-2002), targets were deployed at 4 per km², in the second year (2002-2003), 8 per km², and finally in 2003-2004, 12 per km².

About 14 months after initial target deployment, the density of *G. austeni* in the treatment area was reduced by 99% and maintained at this level for 30 months. The control of *G. brevipalpis* was less successful with a reduction in catches of between 60-89%, only apparent for short periods. Removal of the targets resulted in a fast reinvasion and recovery of the *G. brevipalpis* population. In contrast, *G. austeni* catches in the previously treated area remained low for more than 14 months after target removal.

Findings indicate the difficulty in reducing populations of a mobile fly such as *G. brevipalpis*, which moves between habitats and disperse over longer distances. This would require area-wide treatment of all habitats for effective population reduction. Area wide control would simultaneously effect the control of *G. austeni* in the wooded and forested areas.

Genetic diversity of South African Theileria parva isolates

KP Sibeko¹, N Collins¹, M Oosthuizen¹, D Geysen² (s24469582@vetstud.up.ac.za)

¹Faculty of Veterinary Science, Department of Veterinary Tropical Diseases, University of Pretoria,
Private Bag X04, Onderstepoort 0110, South Africa

²Department of Animal Health, Institute of Tropical Medicine, Antwerp B-2000, Belgium

The polymorphic immunodominant molecule (PIM), p67, ribosomal RNA internal transcribed spacers (ITS), small subunit rRNA (SSUrRNA) and large subunit rRNA (LSUrRNA) genes have been analysed in search of discriminatory sequence differences between buffalo- and bovine-associated *T. parva* isolates. In this study, restriction fragment length polymorphism (RFLP) and sequence analysis of the variable regions of the parasite antigen genes p67, p104 and PIM were used to differentiate *T. parva* isolates.

Cattle and buffalo blood samples from different areas in South Africa were investigated. A total of 109 samples were characterized, including 105 field samples from cattle and buffalo and four experimentally infected bovines. Primers for amplification of the variable regions of the antigenic genes p67, p104 and PIM were designed, and the polymerase chain reaction (PCR) was used to amplify these regions. The sizes of the p67 amplicons were determined by agarose gel electrophoresis. Restriction enzymes *BcI*I and *Alu*I were used to digest p104 and PIM amplicons respectively and RFLP profiles were analysed by polyacrylamide gel electrophoresis. Amplicons from p67 and PIM genes from 12 selected isolates were purified, cloned into the PGEMT- Easy plasmid vector and sequenced.

The presence or the absence of the 130 bp insert in the p67 gene has been used as a marker to distinguish between cattle- and buffalo-associated *T. parva* isolates in East Africa. However, both large and small p67 PCR products were obtained from many of the buffalo-associated *T. parva* isolates in this study, indicating that this criterion cannot be used to distinguish between buffalo- and cattle-derived isolates in South Africa. p67 gene sequences could be used to "track" profiles of *T. parva* parasites in transmission experiments. Ticks were fed on infected buffalo or cattle and the parasite was transmitted to susceptible cattle. The p67 sequences obtained from parasites extracted from these animals remained identical to those of the parasite in the original animal.

The p104 profiles for the majority of the isolates from buffalo were typical of the buffalo-associated *T. parva* allele as previously found in other southern African isolates. However, the Welgevonden isolate had a new profile that has never been observed before. PIM profiles from some isolates were homogenous and resembled the Muguga isolate, a cattle-derived isolate from Kenya. Most of the buffalo-derived isolates had highly polymorphic PIM profiles, except for those from the Hluhluwe buffalo which were homogenous. PIM gene sequences from clones which appeared to share the same PIM profile were sometimes very different, implying that the PIM profile may not necessarily be an indication of the number of strains, or the heterogeneity, within an isolate.

It is evident that p67, p104 and PIM PCR-RFLP profiles cannot be used to distinguish between cattleand buffalo-associated isolates in South Africa. However, these profiles may assist in tracking *T. parva* infections in South Africa.

Sero-prevalence of toxoplasmosis in sheep in South Africa

<u>N Abu Samra</u>¹, CME McCrindle¹, BL Penzhorn, B Cenci-Goga³ (nada.nada@gmx.de)

¹Section of Veterinary Public Health, Department of Paraclinical Sciences, and ²Department of Veterinary Tropical Diseases, Faculty of Veterinary Science, University of Pretoria, Private Bag X04, Onderstepoort 0110, South Africa ³Department of Public Health, Faculty of Veterinary Science, University of Perugia, Italy

In 1978 the overall sero-prevalence of toxoplasmosis in human patients in South Africa was found to be 20%. Toxoplasmosis is a zoonotic disease with severe manifestations in HIV positive human patients. In South Africa, toxoplasmosis in these patients is known to be a cause of sometimes fatal complications, such as encephalomyelitis and ocular lesions. According to the literature, mutton infected with the cysts of *Toxoplasma gondii* is an important route of transmission to humans who ingest under-cooked meat, or eat with unwashed hands after working with meat. There is no data on the sero-prevalence in sheep in South Africa, although this is available for most other countries, including Zimbabwe.

The aim of this study was to estimate the sero-prevalence of *T.gondii* in sheep. Three-stage cluster sampling was done where five different provinces randomly chosen from all the provinces in South Africa were the primary units; Gauteng, KwaZulu-Natal, Free State, Eastern Cape and Western Cape. Two sheep abattoirs and one rural location per province, selected randomly from a list supplied by the provincial Departments of Agriculture, were the secondary units. A total of 600 serum samples from these sheep were tested for IgG using an Immuno Flourescence Agglutination test (Diagnostic & Technical Services CC, Randburg, South Africa).

The sero-prevalence per province was found to be: Gauteng 6%, Eastern Cape 7.75%, Western Cape 6.0%, KwaZulu-Natal 6.3% and Free State 2.7%, with an overall prevalence of 5.6%. From the results it appears that toxoplasmosis in sheep has a lower sero-prevalence in South Africa than in other countries. Zimbabwe has an average sero-prevalence in sheep of 67.9%.

There is an 80% sero-prevalence in sheep in France and 20-30% sera prevalence in different states in the USA. There was no significant difference between the levels in rural and commercial sheep at the 95% confidence level in South Africa, although it appeared superficially to be more prevalent in commercial sheep.

It can be concluded that the lower sero-prevalence of toxoplasmosis in sheep in South Africa, as compared with international levels, was probably due to more extensive methods of sheep farming and the relatively low rainfall in southern Africa.

Software-based decision-support for sustainable management of haemonchosis in small ruminants

DP Revnecke, JA van Wyk (dean.reynecke@up.ac.za)

Department of Veterinary Tropical Diseases, Faculty of Veterinary Science, University of Pretoria,
Private Bag X04, Onderstepoort 0110, South Africa

Resistance to anthelmintics in gastro-intestinal (GIT) parasites of small ruminants, specifically *Haemonchus contortus*, is increasingly becoming a major constraint to production in sheep flocks on both commercial and developing farms. Targeted selective treatment, a new paradigm in resistance management, has gained wide acceptance as a non-invasive technique to retard the frequency of transmission of resistant alleles in GIT parasites. This research was initiated to evaluate the feasibility of using software-based simulation techniques to provide higher resolution in the anthelmintic treatment decision-making process. A Monte-Carlo simulation model to assess, and thus predict, intensity of worm infection, has been developed in the present project.

A systems-based risk analysis was applied to data available from individual farms, including class of animal, FAMACHA (FC) score, body mass, rainfall, and anthelmintic treatment. The analysis was undertaken using @Risk version 4.5 software (Palisade Corporation), based upon the probabilistic risk assessment method as described by the OIE (2005). Variables in the model were entered as statistical distributions according to the distribution functions generated by @Risk. All simulations were undertaken using Monte-Carlo sampling, and each simulation consisted of 10000 iterations of the model. A previously published deterministic dose-response model was parameterised with field observation data¹, and stochasticity was introduced into the model for simulation of the probability of clinical disease at the time of the sample.

The model indicated that the more body weight is correlated with the model output, the lower the risk of disease; that the more skewed the model output, the higher the risk of disease, and that a high number of FC classes does not necessarily indicate higher risk than a lower number of FC classes in a sample. The model proved to be sensitive to blanket drenching events, as a lower intensity of infection was predicted immediately after blanket drenching. Further findings were that the 95% confidence limit of the simulated worm burden was highly correlated with rainfall values processed into a Shannon diversity index, which allowed the spread and evenness of rainfall among rainfall events to be accounted for. It is proposed that these findings will allow producers to make empirically supported decisions to optimise selective anthelmintic drenching in affected flocks.

References

- 1. Roberts JL, Swan RA 1982 Quantitative studies of ovine haemonchosis.
- 2. Relationship between total worm counts of *Haemonchus contortus*, haemoglobin values and bodyweight. *Veterinary Parasitology* 9:201-209.

A pilot study to compare the bioavailability of a long-acting and conventional flunixin meglumine injectable formulation in cattle

RJ Peter¹, V Naidoo², L Bekker², GE Swan², CJ Botha² (rose@nexcorp.co.za)

Argos Veterinary Science, P O Box 1726, Mt. Edgecombe 4300, South Africa

Section of Pharmacology, Department of Paraclinical Sciences, Faculty of Veterinary Science,
University of Pretoria, Private Bag X04, Onderstepoort 0110, South Africa

Flunixin ([2(2'-methyl-3'-trifluoromethylamino) nicotinic acid]) is a commonly used non-steroidal anti-inflammatory drug (NSAID) in animals and functions by reversibly inhibiting the cyclo-oxygenase pathway of eicosanoid formation. Flunixin also has inhibitory effects on serum thromboxane (TBX_2) and exudate PGE_2 concentrations and β -glucuronidase (β -glu) activity. NSAIDs such as Flunixin meglumine are used to treat a number of musculoskeletal conditions and inflammatory disorders in cattle. In addition flunixin is also known to attenuate the endotoxin-induced, eicosanoid-mediated changes of endoxaemia. With a combination of both potent visceral analgesic and anti-endotoxic activity, flunixin has become a preferential aid on the management of equine intestinal ischaemia.

The pharmacokinetics of flunixin meglumine has been extensively described in adult cattle: The drug is characterised by a small volume of distribution (0.16 to 0.55 ℓ /kg), a relatively short elimination half-life (1.6 to 6 h). Flunixin has an age specific profile and is characterised by an extensive volume of distribution (2.11 \pm 0.37 ℓ /kg) and elimination half-life (6.87 \pm 0.49 h) in calves. The limited half-life of the drug tends to make treatment more difficult as more frequent treatments may be needed to maintain suitable control of inflammation. Bomac Laboratories Ltd., New Zealand believed they had created a sustained release formulation that could prolong the mean residence time of flunixin to a few days. The obvious benefits of this formulation would have been a longer inter-administration period and less animal handling.

This new injectable formulation was evaluated in single dose, three group parallel study using nine adult Brahman Steers. Using the conventional formulation as a comparison the plasma pharmacokinetic (Cmax, AUC_{last} , Tmax, AUC_{inf} , MRT and $T\frac{1}{2}B$) and plasma pharmacodynamics (the extent and duration of serum TBX_2 inhibition) was evaluated for both formulations i.e. the PK/PD relationship was used to determine the total period of the formulation's inhibitory activity.

No differences were evident for the pharmacokinetic parameters of the long-acting and standard flunixin formulations (at both tested doses). The long acting formulation failed to achieve a depot effect (flip flop pharmacokinetics) and dumped the entire concentration in the flunixin into the central compartment within 30 minutes of administration. Mean residence times were variable within and between the three groups and appeared to be as a result of higher exposure in the long-acting group rather than a depot effect.

Based on the pharmacodynamic analysis TBX₂ concentrations returned to normal within 48 hours for the standard formulation regardless of the dose administered. One animal in the long acting group showed prolonged inhibition (72 hours) which was not considered significant. Although a trend was observed it was unlikely that the new formulation would produce a prolonged effect in treated animals.

Protocol number V022/04

Diclofenac: A proposed mechanism of toxicity

<u>V Naidoo</u>¹, GE Swan² (vinny.naidoo@up.ac.za)

¹Department of Paraclinical Sciences and ²Office of the Dean, Faculty of Veterinary Sciences, University of Pretoria, Private Bag X04, Onderstepoort 0110, South Africa

Three species of vultures endemic to South Asia are on the brink of extinction in both India and Pakistan. Although the devastation was first noticed in the early 1990s, it was not until 2004 that Oaks *et al.* linked the vulture death to the veterinary use of diclofenac. Remarkably this exposure occurred via drug residues in their food at a minor exposure level of 0.8 mg/kg. To help ensure the species survival, an extensive study was undertaken to find a suitable drug to replace diclofenac. This study concluded that meloxicam was extremely safe in vultures as both a pure drug in formulation and as residues. With diclofenac being so dangerous and meloxican appearing so safe one question remained: Why is diclofenac toxic to vultures?

To answer this question, a study was undertaken in chickens on the assumption that toxicity was avian specific and not necessarily raptor specific. In an initial model validation study five roosters, in a controlled study, were exposed to four different doses of diclofenac by oral gavage. None of the treated birds died following treatment. The bird treated at 2.5 mg/kg did, however, show clinical signs of moderate depression. On post-mortem this bird showed signs of mild visceral gout and nephrosis. From this study it was evident that chickens were less susceptible to toxicity, possibly as a result of lower bioavailability.

To rule out bioavailability as a variable a second study using the intramuscular route was undertaken at five doses (0.6, 1.25, 2.5, 5 and 10 mg/kg) in pullets in groups of six. No mortalities were seen in the lowest group, while two birds died in each of the three middle doses and three birds at the maximum exposure. All the birds that died showed signs of severe depression that persisted till death. From the probit analysis the intramuscular LD₅₀ was determined to be 9.3 mg/kg. From the specific clinical pathology no differences were seen in serum Ca²⁺ and Na⁺ levels. As expected uric acid levels was increased at 24 hours and remained high until death with a total exposure of 230mmol/l/hr for the period. For the 10mg/kg group blood gas analysis at 24h and at death indicated a normal pH of 7.4 initially which dropped terminally to 6.4. The terminal drop in pH corresponded to a massive hyperkalaemia. Post mortem signs were as described for the vultures and characterised by severe visceral gout and bilateral nephrosis. On re-evaluation of stored vulture samples, from a previous diclofenac study, the terminal spike in blood potassium and an overall uric acid exposure of 200mmol/l/h was present.

From the similarities in clinical, clinical pathological and post mortem signs the following hypothesis is put forward. The severe depression in the birds is most likely as a result of the acidosis, which is related to the massive increase in the plasma uric acid levels. Although acidosis is present and severe; death is probably as a result of the hyperkalaemia, which is a result of metabolic compensation for acidosis. The spike in uric acid although still unexplained may result from diclofenac's known ability to inhibit the organic anionic transporter (URAT1) in the proximal convoluted tubules. Although this pump is involved in the re-absorption of uric acid in people, its function in rodents is purely excretory. Inhibition of the pump as an excretor will result in the accumulation of uric acid. It is therefore proposed that diclofenac is toxic in vultures as a result of its ability to inhibit a uric acid excretory URAT1 pump in the proximal convoluted tubule.

Comparison of morphine and carprofen administered alone or in combination for post-operative analgesia in dogs undergoing ovariohysterectomy

<u>TB Dzikiti</u>¹, KE Joubert^{1,2}, LJ Venter¹, LN Dzikiti³ (brighton.dzikiti@up.ac.za)

Department of Companion Animal Clinical Studies, Faculty of Veterinary Science, University of Pretoria,
Private Bag X04, Onderstepoort 0110, South Africa
Fourways Veterinary Hospital, P.O. Box 68190, Bryanston 2021, South Africa
Computational Biology Unit, Department of Biochemistry, Faculty of Natural and Agricultural Sciences,
Lynnwood Road, Hillcrest, Pretoria 0002, South Africa

This study compared the analgesic efficacy of two common analgesics – the pure agonist opioid morphine and the cyclo-oxygenase type-2-selective carprofen.

Forty-five dogs undergoing ovariohysterectomy were randomly assigned to three groups: The morphine group received morphine 0.4 mg/kg subcutaneously pre-operatively and 0.2 mg/kg subcutaneously every 4-6 hours post-operatively. The carprofen group received once-off carprofen 4 mg/kg subcutaneously pre-operatively and 0.1ml saline subcutaneously every 4-6 hours post-operatively. The morphcarp group received similar dosages of both morphine and carprofen. After premedication with acepromazine 0.01 mg/kg subcutaneously, anaesthesia was induced with thiopentone 5-10 mg/kg intravenously or propofol 4-6 mg/kg intravenously and maintained with halothane. A blindfolded anaesthesiologist assessed pain in the dogs over a 24-hour period using a pain-scale modified from the University of Melbourne pain-scale using the Glasgow pain-tool. Pain was assessed just before sedation (baseline), just before induction, and at 1, 2, 4, 6 and 20 hours postoperatively. Dogs in extreme discomfort at any assessment point (pain-score above 10 out of the possible maximum of 20) were excluded and treated with morphine 0.4mg/kg subcutaneously. Respiratory rate, pulse rate and body temperature were taken together with pain-scores. Median pain-scores were analysed (P<0.05) using the Kruskal-Wallis rank-sum test and when necessary the Pair-wise Wilcoxon rank-sum test with a Bonferroni adjustment for multiple testing was used.

There were no differences in pain-scores between groups, and thus analgesia offered by the three analgesia protocols, but there were differences within groups across different assessment points. Baseline total pain-scores were lower than scores at all postoperative time points within all three groups, while 1hour postoperative total pain-scores were higher than pain-scores at 20 hours postoperatively within the Carprofen Group and the MorphCarp Group. Both morphine and carprofen provided good analgesia without obvious adverse physiological effects. No dog had to be excluded from the study for extreme discomfort.

We conclude that at dosages used, carprofen administered alone offers analgesia equal to that of morphine and that the two drugs combined do not result in better analgesia than either drug on its own in dogs undergoing ovariohysterectomy.

Protocol number 36/5/598

Isolation of antioxidant compounds from Combretum apiculatum

DT Kgatle, MA Aderogba, JN Eloff (s25466519@tuks.co.za)

Phytomedicine Programme, Department of Paraclinical Sciences, University of Pretoria, Faculty of Veterinary Science, University of Pretoria, Private Bag X04, Onderstepoort 0110, South Africa

The presence of oxidants such as reactive oxygen species (ROS) in tissues plays a crucial role in many illnesses of humans and animals. The use of *Combretum* species in many cultures in folk medicine for the treatment of several inflammatory conditions (abdominal pains, headache and toothache) and their established antimicrobial activities prompted us to investigate the antioxidant potential of this plant genus.

Bioactivity-directed fractionation was carried out on the leaf extracts of *Combretum apiculatum*. In this process, column chromatography using eluent solvents of differing polarities was used to fractionate the plant extract. Qualitative antioxidant potential was evaluated using a 1, 1-diphenyl-2-picrylhydrazyl (DPPH) assay. Following each column chromatography step, fractions were combined according to similar chemical compositions viewed on thin layer chromatography (TLC). TLC plates were developed and sprayed with 0.2% DPPH in methanol to detect antioxidant activity. DPPH is a free radical substance with a deep purple colour, and the presence of antioxidant compounds is noted by a decolourising effect when the free radical is scavenged, and the colour changes from purple to pale yellow.

The process of bioassay-guided fractionation led to the isolation of four antioxidant compounds from the ethyl acetate soluble fraction of *C. apiculatum* leaves. The structures of the isolated compounds were determined on the basis of spectral studies (¹H-NMR and ¹³C-NMR) and identified as: 2', 4'-dihydroxy-6'-methoxy chalcone (cardamomin), 5, 7-dihydroxyphenyl flavanone (pinocembrin), quercetrin, and kaempferol. When TLC separations of the isolated compounds were sprayed with DPPH solution, all the compounds instantly bleached the purple background colour of the DPPH free radical, thus showing free radical scavenging (antioxidant) potential.

The presence of these antioxidant compounds in *C. apiculatum* could provide rationale for the ethnomedicinal use of this plant for the treatment of inflammatory and other conditions in traditional medicine. The quantification of the activity of these antioxidant compounds in relation to well-known free-radical scavenging compounds such as vitamin C is planned. Other biological activities of the isolated compounds will also be investigated.

Erythrophleum sawdust: toxic bedding for pigs

LJ McGaw¹, JN Eloff¹, TW Naudé², J Segalés³ (lyndy.mcgaw@up.ac.za)

Phytomedicine Programme and ²Section Pharmacology and Toxicology, Department of Paraclinical Sciences, Faculty of Veterinary Science, University of Pretoria, Private Bag X04, Onderstepoort 0110, South Africa Centre de Recerca en Sanitat Animal (CReSA), Departament de Sanitat I d'Anatomia Animals, Facultat de Veterinària, Universitat Autònoma de Barcelona, 08193 Bellaterra, Barcelona, Spain

The genus *Erythrophleum* is endemic in tropical Africa and is widely used as a poison or an ordeal preparation for people suspected of committing serious crimes or witchcraft. A variety of alkaloids isolated from *Erythrophleum* species are known to have toxic effects, and some possess cardioactive properties. Wood from *Erythrophleum* species is popular in Europe for, among other uses, timber flooring, stairs and wooden bridges. In Spain it is known as "elondo" wood and may originate from one of several *Erythrophleum* species. Following reports of intoxication and death of pigs from a Spanish farm after sawdust from "elondo" was used as bedding for the animals, samples were analysed for the presence of potentially toxic alkaloids.

Alkaloids were extracted from sawdust samples obtained from the wood mill as well as from bedding material from the affected farm. Extracts were prepared from *Erythrophleum lasianthum* bark and leaves collected from Tembe Nature Reserve as a comparison. The extracts were evaluated for the presence of alkaloids by noting the formation of precipitates in alkaloid-containing samples with phytochemical reagents (Mayer's and Dragendorff's reagents). Thin layer chromatography (TLC) analysis and application of Dragendorff's reagent was employed to confirm the presence of alkaloids. The extracts were tested for cytotoxicity to Vero monkey kidney cells in a cell-line bioassay.

Each of the alkaloid detection procedures used verified the presence of alkaloids in the extracts prepared from the mill and the farm sawdust. TLC revealed that compounds producing a positive reaction for alkaloids were present at the same R_f values for the mill and farm samples as for the *E. lasianthum* extracts. The alkaloid extracts from the mill sawdust and the bedding material showed highly cytotoxic effects, with LC₅₀ values of 3.5 and 10.3 µg/ml respectively, supporting evidence of toxicity.

It was concluded that *Erythrophleum* alkaloids were present in the bedding samples responsible for the toxic reactions in the pigs. It is therefore not recommended for farmers to use as bedding for their animals sawdust originating from sawmills processing wood from trees known or suspected to contain toxic constituents.

Activity of invasive weed plants against plant pathogenic fungi

MM Meela, LK Mdee, P Masoko, JN Eloff (s26180783@tuks.co.za)

Phytomedicine Programme, Department of Paraclinical Sciences, Faculty of Veterinary Science, University of Pretoria, Private Bag X04, Onderstepoort 0110, South Africa

Plant pathogens are a major threat to food security worldwide, as at least 10% of global food production is lost to plant diseases. One major cause of plant disease is pathogenic fungi. The most important method of protecting plants against fungal attack is the use of fungicides, but the development of resistance towards synthetic fungicides is of great concern. Moreover, the health risks associated with the use of chemical fungicides increase the need to search for safe, efficacious and environmentally friendly fungicides. Plants produce antifungal agents by secondary metabolism to protect themselves from fungal attack, and therefore many plant species possess substantial antifungal activity. The use of plant extracts could enable the development of inexpensive and environmentally acceptable fungicides based on locally available natural products. This study was undertaken to select one or more plants based on antifungal activity for the development of plant-derived fungicides, with the focus on based on readily available, unwanted plant material in the form of invasive weed species.

Acetone leaf extracts from a selection of invasive plants were screened for antifungal activity against five plant pathogenic fungi, namely Fusarium oxysporium, Rhizoctonia solani, Penicillium expansum, Aspergillus niger and Aspergillus parasiticus, using bioautography and serial microplate dilution methods. For bioautography, thin layer chromatography (TLC) plates were developed under saturated conditions with eluent systems of varying polarities, sprayed with fungi, incubated overnight and sprayed with tetrazolium violet as a fungal growth indicator. The serial microplate dilution assay, again with tetrazolium violet indicator, was used to determine the minimum inhibitory concentration (MIC) values of the plant leaf extracts.

Some 40 clear zones on the bioautograms indicated growth inhibition by compounds present in several plants. The MIC results indicated promising antifungal activity of *Passiflora subpeltata* extract (average MIC = 0.08 mg/ml), as well as for *Chromoleana odorata* and *Passiflora suberosa* extracts, both with average MIC values of 0.10 mg/ml.

Bioautography results were not very good as compared to the MIC results, this is possibly due to the evaporation of volatile active compounds during removal of mobile solvents or disruption of synergism between active constituents during separation by TLC.

It appears that some of the invasive weed species have promising antifungal activity. The plant extracts with low average MIC values indicating good activity are the focus of further studies.

The immunoperoxidase staining technique as a diagnostic tool

SJ Clift, MS Smit, RM Phaswane (sarah.clift@up.ac.za)

Section of Pathology, Department of Paraclinical Sciences, Faculty of Veterinary Science, University of Pretoria,
Private Bag X04, Onderstepoort 0110, South Africa

Immunoperoxidase (IMP) staining of tissues is a quick and reliable technique for the routine diagnosis of infectious diseases and neoplasms, as well as for research. The purpose of IMP staining is to allow, by means of derived antibodies and a simple detection system, the visualization of a specific antigen in the form of an insoluble coloured reagent product in cells or tissues, using a light microscope. It is cost-effective and can be performed on formalin-fixed, paraffin-embedded tissues, cell cultures, snap frozen tissue and rapidly air dried smears or imprints of tumours. The sought-after antigen can be identified within target cells and, in tissue sections, often in association with a histological lesion, a crucial step in the establishment of disease causation. IMP can be performed on paraffin tissue blocks that have been stored for decades, facilitating retrospective as well as pathogenetic studies.

For certain diseases and cell markers IMP is quite simple to optimize and validate. IMP works extremely well, for example, in the diagnosis of rabies and canine lymphoma immunophenotype. In the former, IMP stains rabies antigen unmistakably (compared to H&E- and Acid Fast Methylene Blue staining), and can do so even in autolysed formalin-fixed tissues, making it a robust technique for rabies diagnosis in Africa. The use of IMP to differentiate between B and T cell lymphomas in dogs has been extremely useful since the immunophenotype has been significantly associated with response to chemotherapy as well as remission and survival times.

However, despite the success stories, it is important to note that some tests are difficult or even impossible to optimize. For example, the lumpy skin disease (LSD) IMP gave a large number of false positive results. In this case the test was remedied by removing contaminants from the primary polyclonal anti-LSD antiserum. The IMP test for IgG in the skin of suspected autoimmune skin disease patients is very unreliable and we no longer advise its use since the problem of false positive staining could not be adequately remedied. If used wisely, the immunoperoxidase staining technique is an extremely useful additional test for the diagnosis of disease by veterinary pathologists.

Prognostication in canine parvoviral diarrhoea using basal serum cortisol concentrations

<u>JP Schoeman</u>¹, LJ Venter¹, A Goddard¹, AL Leisewitz¹, ME Herrtage² (johanp.schoeman@up.ac.za)

Department of Companion Animal Clinical Studies, Faculty of Veterinary Science, University of Pretoria,
Private Bag X04, Onderstepoort 0110, South Africa
Department of Clinical Veterinary Medicine, University of Cambridge, Cambridge, United Kingdom

In human critical care, patients with the highest cortisol concentrations generally have the highest mortality rates, with resultant prognostic classifications based on basal cortisol and/or delta cortisol concentrations. In contrast, comparative data on canine critical illness does not exist. Parvoviral diarrhoea is a severe infectious disease inducing a sepsis-like state, predominantly in paediatric canine patients. The objective of this study was to evaluate the prognostic value of basal serum cortisol concentrations in canine parvoviral diarrhoea.

A prospective, *in vivo* study was conducted on clinical cases of parvoviral diarrhoea meeting the criteria for admission to a high care ward in a veterinary academic hospital. Forty-five patients were enrolled and had their basal serum cortisol concentrations measured at admission and daily until death or discharge. Mortality was assessed as a function of basal cortisol concentrations obtained at day 2 in the whole cohort. The results are indicated in the table below. Specificity and sensitivity data were obtained using empirical receiver operator curves (ROC).

Serum cortisol	Dead	Survived	Total
<224 nmol/L	2	34	36
>224 nmol/L	6	0	6
	8	34	42

Day 2 cortisol concentrations of >224 nmol/L had a specificity of 100%, a sensitivity of 75% and a 1.00 positive predictive value for mortality in paediatric canine patients with parvoviral diarrhoea.

Basal cortisol concentrations in canine patients are considerably lower than in human patients, reflecting the lower reference range (10–160 nmol/L) of this species. The results are in accordance with human critical care data, allowing for prognostication based on basal cortisol concentrations. This data contributes to the affirmation of the dog as a model for conducting studies in the human critical care field. Our data suggest that basal cortisol concentrations have a good prognostic value and could be helpful in identifying patients with parvoviral diarrhoea at high risk of death. Further studies will have to be conducted to determine whether the same is true for the adrenocorticotrophic hormone (ACTH) stimulation test, as is the case in humans.

Morphologic and molecular characterization of coccidia from the African buffalo (*Syncerus caffer*)

DH Lorom, MC Oosthuizen, BL Penzhorn (davelorom@gmail.com)

Department of Veterinary Tropical Diseases, Faculty of Veterinary Science, University of Pretoria,
Private Bag X04, Onderstepoort 0110, South Africa

Coccidia are extremely common parasites, having been found to infect every vertebrate ever intensively scrutinized. The vast majority of coccidia that infect the mucosa of the gastro-intestinal tract belong to two families, Eimeriadae and Cryptosporidiidae. Oocysts from both families have previously been reported in African buffalo but identification at the species level is lacking. Furthermore, species identification in buffalo has become increasingly important due to recent morbidity and mortality in boma-housed animals.

Faecal samples were collected from buffalo housed in bomas at five locations in South Africa. These samples were either taken directly from the rectum of the animal or pooled from animal defaecations in the bomas. Following collection, all faecal samples were stored in containers with $K_2Cr_2O_7$. An aliquot of each sample was placed in a Petri dish and oocyst sporulation was allowed to occur. The oocysts were separated from the faecal/dichromate mixture by flotation in saturated NaCl solution. Digital photomicrographs of oocysts were taken under 1000X magnification, measured and identified morphologically. A second aliquot was washed repeatedly with distilled H_2O and the oocysts purified for DNA extraction. PCR was then used to amplify the 18S rRNA gene. The PCR product was cloned, purified and subjected to gene sequence analysis. Data was analyzed using the Staden package and aligned with related genera and species using Clustal. The obtained consensus sequence was then matched to the corresponding photomicrograph.

Microscopic examination revealed clinically ill animals were infected with up to four species of coccidia. Identified species include members from families Eimeriadae and Cryptosporidiidae. Three oocyst forms resembled published descriptions from cattle, including *Eimeria bovis, Eimeria bukidnonensis*, and *Eimeria subsherica*. An as yet un-described *Eimeria* oocyst was also found. Sequence analysis of cloned genes confirmed the presence of multiple *Eimeria* genotypes. This sequence data when aligned with partial 18s rRNA sequences published in Genbank confirmed the morphologic identification of the three described and one un-described *Eimeria* species.

The use of gene sequence analysis has been proven a very effective approach to parasite identification, particularly when parasites are in very low numbers or morphologically indistinguishable. Further gene sequence analysis will greatly contribute to the clarification of the taxonomic position of *Eimeria* and related genera infecting the African buffalo and other wildlife.

Cardiovascular effects of lumbar epidural anaesthesia in isoflurane-anaesthetised pigs during surgical devascularisation of the liver

GF Stegmann (frik.stegmann@up.ac.za)

Department of Companion Animal Clinical Studies, Faculty of Veterinary Science, University of Pretoria, Private Bag X04, Onderstepoort 0110, South Africa

The combined use of epidural and general anaesthesia is a controversial issue as adverse effects such as hypotension¹ or advantages such as improved cardiovascular stability² were reported. This investigation is part of an ongoing study in the development of a bioartificial liver support system. Abdominal surgery involving ligating hepatic blood vessels may potentially result in intraoperative cardiovascular instability associated with tachycardia or hypotension. The purpose of this investigation was to examine the effects of an epidural ropivacaine on heart rate (HR) and mean arterial blood pressure (MAP).

Fourteen 4-month-old, female Landrace pigs with mean body weight of 27.6 kg were used in the investigation. The pigs were scheduled for surgical devascularisation of the liver. During surgery anaesthesia was maintained with either isoflurane (A-group, n=7) or a combination of isoflurane and epidural anaesthesia (AE-group, n=7) on a circle rebreathing anaesthetic circuit with CO₂ ab-sorption. Anaesthesia was induced by IM injection of a midazolam (Dormicum, Roche, 0.3 mg/ kg body weight) and ketamine (Anaket, Centaur, 10 mg/kg) combination. After the placement of an IV catheter in the ear vein, propofol (Diprivan, Astra Zenica, 3 mg/kg) was administered IV to effect for tracheal intubation. For the A-group the vaporizer was set at 2.5% and for the AE-group at 1.5%. Ventilation was controlled with the end-tidal CO₂ concentration maintained at 40 mmHg. Monitoring was performed with a multiparameter patient monitor (TL-101T, Nihon Kohden Medical Systems). Arterial blood pressure was measured with an electronic transducer connected to a catheter placed in the femoral artery. End-tidal CO₂ partial pressure was measured with an in-line sensor placed between the tracheal tube and anaesthetic machine. Epidural anaesthesia was obtained with ropivacaine (Naropin, Astra Zenica) at 0.3 ml/kg. Blood volume was maintained with the IV administration of a balanced electrolyte solution at an infusion rate of 10 ml/kg/min. Permission for the study was obtained from the Animal Care and Use Committee of the University of Pretoria (Protocol no. H1-04).

The mean (\pm SD) for HR and MAP for the A-group was 96 (\pm 21.7) beats/min. and 72.9 (\pm 13.4) mmHg and for the AE-group 77(\pm 16.2) and 90.8 (\pm 12.5) respectively. The differences between the treatment groups were statistically significant for HR (p = 0.048) and MAP (p = 0.006).

The combined administration of an epidural anaesthetic (ropivacaine) and general anaesthesia (isoflurane) resulted in a statistically significant lower HR and statistically significant higher MAP when compared to general anaesthesia only during abdominal surgery in pigs. Epidural anaesthesia allowed the use of lower inspired concentrations of isoflurane and resulted in improved blood pressure during general anaesthesia.

References

- 1. Borghi B, Casati A, Iuorio S, Celleno D, Michael M, Serafini P, Pusceddu A, Fanelli G 2002 Frequency of hypotension and bradycardia during general anesthesia, epidural anesthesia, or integrated epidural-general anesthesia for total hip replacement. *Journal of Clinical Anesthesia* 14: 102-106.
- 2. Veering B T, Cousins M J 2000 Cardiovascular and pulmonary effects of epidural anaesthesia. *Anaesthesia and Intensive Care* 28: 620-635.

Canine bladder trigone diverticulum

WM Wagner¹, B Meyers² (wencke.wagner@up.ac.za)

Diagnostic Imaging Section, and Surgery Section, Faculty of Veterinary Science, University of Pretoria, Private Bag X04, Onderstepoort 0110, South Africa

Diverticula of the bladder are pouch-like inversions or invaginations of the bladder wall, arising either as congenital defects or acquired lesions. Congenital and acquired bladder diverticula are uncommon abnormalities in the small animal patient. Three types of bladder diverticula exist in the canine and feline patient: 1) traumatic bladder diverticula, 2) vesicourachal diverticula, mainly in the feline patient, and 3) trigone bladder diverticula. Bladder diverticula in the dog and cat have been well described as a result of urachal abnormalities. These however occur at the apex of the bladder. Both previously described canine bladder trigone diverticula were assumed to be of congenital origin, and occurred in young intact male German Shepherd dogs. Both were clinically silent, and only presented because of secondary complications. In humans, bladder diverticula are located in the apex and trigonal area and are most common in adult men (acquired), less common in boys (congenital) and rare in females.

A 5-month-old intact male Rottweiler was presented to the Onderstepoort Veterinary Academic Hospital for intermittent urinary incontinence, which was particularly noticeable after lying down. There were no obvious abnormalities apparent on clinical and neurological examination. Urine collection via cystocentesis showed no abnormalities. A full hematology and biochemical profile showed a mild mature neutropaenia and mild lymphocytosis, which was thought to be non-significant.

A trigone bladder diverticulum could be demonstrated by contrast radiography, ultrasonography and computed tomography, and emphasized the importance of positional diagnostic imaging.

Since there was no history of a traumatic insult, or outflow obstruction and considering the young age of the animal, a congenital origin was assumed. Contrary to the numerous reports on urachal diverticula in the veterinary literature, there have only been two other canine bladder trigone diverticula described. These were considered to be of congenital origin by the authors. Both canine patients were also young (9 and 14 months old) and intact males. However, they were both German Shepherd dogs, with one having multiple diverticula. Results of excretory urography may be entirely normal without revealing the presence of a diverticulum because the latter may not fill with contrast medium or the urine residual within the diverticulum may dilute the contrast material sufficiently to obscure its presence. In this case, the bladder diverticulum could be nicely visualized, because prior insertion of a negative contrast medium into the bladder enabled visualization of the bi-compartmental appearance ("hunchback double bladder"). Taking the young age of the patient into consideration and since there was no history of traumatic insult and outflow obstruction, a congenital diverticulum was assumed. Unfortunately no histopathology was obtained. Even though there is a similarity to the human literature, some differences exist to the reported canine bladder trigone diverticula, which are extremely rare. This case also emphasizes the importance of positional diagnostic imaging procedures and is the first report describing CT findings in a canine bladder trigone diverticulum.

Distribution and structural features of Pacinian (Herbst) corpuscles in the non-glandular region of the palate of the ostrich (*Struthio camelus*)

<u>C Tivane</u>¹, JT Soley², HB Groenewald² (john.soley@up.ac.za)

Department of Pre-clinics, Veterinary Faculty, Eduardo Mondlane University, Maputo, Mozambique Department of Anatomy and Physiology, Faculty of Veterinary Science, University of Pretoria, Private Bag X04, Onderstepoort 0110, South Africa

Pacinian corpuscles are large ovoid structures found in the dermis and hypodermis and which function as "deep pressure receptors for mechanical and vibratory pressure". These structures have been demonstrated in the oral cavity of birds where they have been referred to as Herbst corpuscles, modified Herbst corpuscles or Vater-Pacini end-organs. The presence of these structures, however, has not been reported in the ostrich.

The heads of five 12-14 month old ostriches were obtained from a local ostrich abattoir. The heads were fixed in 10% buffered formalin, rinsed in running tap water and incised at one commisure of the mouth to expose the oro-pharynx. Sections of the median palatine ridge (a prominent longi-tudinal mucosal fold that extends rostrally from the choanae to the tip of the beak) and adjacent mucosa were excised and prepared for light microscopy using standard techniques.

The non-glandular mucosa of the palate consisted of a keratinised stratified squamous epithelium supported by a thick layer of irregular dense fibrous connective tissue (lamina propria). Beneath the lamina propria was a submucosa of loose connective tissue containing numerous large blood vessels and nerves. Variably-sized Pacinian corpuscles exhibiting round, ovoid or elongated profiles were randomly distributed throughout the mucosa and were located in the deeper region of the lamina propria, adjacent to the submucosa. However, these corpuscles were observed to be concentrated in the median palatine ridge, forming a U-shaped collection of between 10 to 15 large corpuscles (100 – 250 µm in diameter) around the submucosal core of the ridge.

Individual corpuscles displayed morphological features typical of Pacinian corpuscles. The neural component (nerve terminal) of the corpuscle was centrally situated and surrounded by a series of closely apposed lamellae forming a distinct zone, the inner core. This zone was also characterised by the presence of a number of Schwann cell nuclei. Surrounding the inner core was a series of loosely arranged, concentric lamellae separated by obvious spaces. This region (the outer core) formed the bulk of the capsule surrounding the neuronal component and displayed relatively few nuclei. The entire corpuscle was closely invested by a thin, fibrous connective tissue layer displaying numerous fibroblast nuclei. Closely associated with the wall of the corpuscle were numerous variably-sized myelinated nerves.

This study revealed that the Pacinian corpuscles observed in the non-glandular reion of the ostrich palate are structurally similar to those described in other birds and in mammals. However, the concentration of large numbers of these bodies in the median palatine ridge appears to be a unique feature, particularly when compared to the lateral location of Herbst corpuscles reported beneath the hard palate of the domestic fowl.

The arterial microvasculature of the distal *ductus deferens,* receptaculum ductus deferentis and phallus of the ostrich as revealed by India ink injection

MZJ Elias, TA Aire, JT Soley (john.soley@up.ac.za)

Department of Anatomy and Physiology, Faculty of Veterinary Science, University of Pretoria, Private Bag X04, Onderstepoort 0110, South Africa

Although the basic pattern of arterial supply and venous drainage of the male reproductive tract, and the micro-vasculature of the testis, epididymis and proximal ductus deferens of the ostrich has been established, the arterial microvasculature of the distal ductus deferens, receptaculum ductus deferens and phallus of the ostrich is unknown. This paper reports on the arterial micro-vasculature of these structures in the ostrich, using India ink injection.

The arterial system of the male reproductive tract was injected with India ink via the aorta in five ostrich torsos obtained from an abattoir. Small tissue blocks were trimmed from the distal ductus deferens, receptaculum ductus deferens and phallus (root, elastic vascular body and phallic sulcus) and then conventionally processed for light microscopy.

The distal ductus deferens consistently demonstrated subepithelial arterioles (1.5-5.5 μ m in diameter) located just beneath the epithelium. The peritubular connective tissue exhibited 2-3 rows of variably sized and sparsely distributed arteries (5-20 μ m in diameter). Larger peripheral arteries were situated beneath the serosa. The receptaculum ductus deferens, revealed a similar distribution of vessels to that observed in the distal ductus deferens. The root of the phallus was surrounded ventro-laterally by a sponge-like structure con-sisting of numerous lymph spaces traversed by connective tissue septa or struts. The struts were richly supplied with relatively large vessels. The elastic vascular body, which is located ventral to the body of the phallus, exhibited a few arteries (10-25 μ m in diameter) that were sparsely distributed in the elastic connective tissue. The walls of the phallic sulcus were composed of erectile tissue covered by a stratified squamous epithelium. Several smaller vessels (3-9 μ m in diameter) lay beneath the epithelium whereas the stroma displayed larger, randomly distributed vessels (15-250 μ m in diameter).

The distal ductus deferens of the ostrich reveals relatively fewer peritubular and peripheral arteries when compared to the turkey and rooster. The particular distribution of arteries in the spongy tissue around the base of the phallus, the presence of very few arteries in the vascular elastic body, as well as the rich arterial supply to the erectile tissue in the phallic sulcus, are noteworthy features not previously reported in birds.

Morphological and immunohistochemical characterization of the testicular capsule and peritubular tissue of ratite birds

PC Ozegbe, TA Aire, M-C Madekurozwa, JT Soley (madex@op.up.ac.za)

Department of Anatomy and Physiology, Faculty of Veterinary Science, University of Pretoria, Private Bag X04, Onderstepoort 0110, South Africa

The testis of most vertebrates is enclosed in a capsule while the peritubular tissue surrounds the seminiferous tubules. The testicular capsule and peritubular tissue of mammals, responsible for contractile, paracrine and transport functions, have received considerable attention recently but very little is known about these important tissues in birds. This study therefore characterizes the testicular capsule and peritubular tissue immunohistochemically and ultrastructurally in two ratite species, emu (*Dromaius novaehollandiae*) and ostrich (*Struthio camelus*).

Abattoir-obtained testicular tissue, of adult, sexually active, male ostriches (n = 5) and emus (n = 5) was immersion-fixed in either 4% glutaraldehyde in Millonig's phosphate buffer or 10% buffered formalin. The tissue blocks were subsequently processed, using conventional standard procedures for light and electron microscopy. Immunostaining (using a LSAB-plus kit; Dakocytomation, Denmark) against smooth muscle actin, cytokeratin, desmin and vimentin (at dilutions of 1:50, 1:100, 1:300 and 1:100, respectively) was performed.

Smooth muscle actin and desmin were co-expressed in the testicular capsule of the emu and the ostrich. Vimentin immunoreactivity was also identified in the testicular capsule and peritubular tissue of the emu, but not in the ostrich. However, the peritubular tissue of both birds was immunonegative for actin. The outer zone of the tunica albuginea of the testicular capsule displayed stronger immunostaining for actin and desmin than the inner zone in the ostrich. This zonal arrangement was reversed in the emu. Cytokeratin, however, was absent in both birds. The testicular capsule and peritubular tissue of both birds were similar in composition structurally, except for the dispersed melanocytes observed in the emu. The tunica serosa of the testicular capsule comprised squamous cells which displayed highly elongated and heterochromatic nuclei. The bulky tunica albuginea consisted of an outer zone of circularly-oriented smooth muscle cells that arborized and enclosed bundles of collagen, and an inner zone of mainly smooth muscle cells. The ostrich showed a much greater concentration of collagen bundles in this tunic than the emu. The peritubular boundary tissue in both birds comprised several layers of cells, unlike in the rat. The myoid cells of the peritubular tissue were similar to the smooth muscle cells of the testicular capsule, structurally.

The testicular capsule and the peritubular tissue of ratites contain cellular components that have typical contractile features. Contractility, with its associated sperm-transporting capability, therefore, appears to be one of the main functions of the testicular capsule and the peritubular tissue of the emu and ostrich.

The effect of rumen lesions caused by subclinical acidosis on growth in feedlot calves

P Thompson¹, A Hentzen², <u>W Schultheiss</u>³ (peter.thompson@up.ac.za)

Department of Production Animal Studies, Faculty of Veterinary Science, University of Pretoria,
Private Bag X04, Onderstepoort 0110, South Africa
Supreme Livestock Services, Heidelberg, South Africa
Schering-Plough Corp., Isando, South Africa

Subclinical (and sometimes clinical) rumen acidosis occurs as calves are introduced onto a high energy ration in the feedlot, often resulting in rumenitis, and the resultant lesions are commonly seen at slaughter. Lesions are expected to cause pain and discomfort resulting in reduced feed intake. However, there are no reports on their possible effect on growth. The aim of this study was to determine whether any of the rumen lesions seen at slaughter in South African feedlot calves were associated with a reduction in average daily gain (ADG).

The rumens of 1,284 calves from four South African feedlots were examined at slaughter and lesions were described and graded according to extent and severity. Lung scoring was also done for bronchopneumonia and pleuritis. The effect of each rumen lesion type on ADG over the entire feedlot period was estimated using multiple linear regression. Associations between the presence of rumen lesions and lung lesions were also assessed.

One or more lesions of the rumen mucosa were seen in 89.0% of all rumens, occurring mainly in the ventral sac. The adjusted effects of each lesion were as follows:

Lesion present	Prevalence	Effecton ADG	<i>P</i> -value
Papillar atrophy	71.5%	_	0.8
Diffuse mucosal lesions	66.6%	$-46 \mathrm{g}$	0.1
Stars	58.2%	-64 g	< 0.001
Perforation	0.8%	-35 g	0.1
Mucormycosis	5.4%	_	0.7

The prevalence of stars varied between feedlots, from 48.2% to 71.5%. However, their negative effect on ADG was remarkably consistent. It was not influenced by the number of stars present or their size. The effect of diffuse lesions varied between feedlots, but neither the size nor the chronicity of the lesions were associated with ADG. There was insufficient evidence for an association between the presence of rumen and lung lesions.

Lesions that were associated with more severe episodes of rumen acidosis consistently had a negative effect on growth rate over the entire feeding period. This is likely due to pain, resulting in reduced feed intake. Another way in which mucosal lesions may affect growth is via a reduction in the surface area for volatile fatty acid absorption. However, there was little evidence that the extent of diffuse rumen lesions affected ADG.

The results of this preliminary work will help in the development of a simple system for scoring rumen lesions at the abattoir. This will assist feedlot managers in adjusting their ration formulation and bunk management practices in order to achieve optimum rumen health and efficiency of gain.

The public health implications of farming cattle in areas with high background concentrations of vanadium

B Gummow¹, CJ Botha¹, JPTM Noordhuizen², JAP Heesterbeek² (bgummow@up.ac.za)

Department of Production Animal Studies, Faculty of Veterinary Science, University of Pretoria,
Private Bag X04, Onderstepoort 0110, South Africa
Faculty of Veterinary Medicine, University of Utrecht, Yalelaan 7, 3584 CL Utrecht, Netherlands

Vanadium (V) is a widely distributed transition metal used by industry primarily in the manufacturing of steel. 83% of industrial vanadium currently comes from mining vanadiferous magnetite in South Africa, China and Russia. The remaining 17% is recovered from the oil industry. Many fossil fuels contain vanadium – particularly coal (at concentrations of between 19-126 ppm in ash) and crude oil (at concentrations of between 3-257 ppm). The natural prevalence of vanadium exceeds that of such well-known metals as copper and lead and equals that of zinc and tin. So, the potential for it to enter the food chain is real.

Forty-two adult Brahman-cross cattle farmed extensively in two groups, immediately adjacent to and 2 km from a vanadium processing plant respectively, were slaughtered over a five year period at a nearby abattoir. Cattle were exposed to vanadium at close to no-adverse-effect levels. The dose of vanadium cattle were taking in prior to slaughter was calculated for each animal from environmental and physiological data using a stochastic risk assessment model. The median exposure doses in the month prior to slaughter ranged from 0.55 mg vanadium/kg bwt/d to 2.73 mg vanadium/kg bwt/d. A range of tissues was taken from the cattle at slaughter for vanadium determination and tissue levels of vanadium in muscle, liver and kidney are reported. The concentrations of vanadium in the milk of cattle from the same farm sampled over five years are also reported.

Concentrations were further modelled using a lognormal distribution function to look at possible extreme values that are likely to occur. The concentrations of vanadium in commonly consumed tissues ranged from <0.05 to 11.51 mg/kg (wet-mass basis). The median concentration of vanadium in milk was 0.23 mg vanadium/kg. People drinking milk were at highest risk. The potential oral daily intake of vanadium for people consuming these foodstuffs was modelled using a stochastic model. The model predicted that there is less than a 5% chance that the potential daily intake of vanadium from milk will be >0.44 $\mu g/kg/d$ for adults.

Based on this upper limit it was concluded from current knowledge of toxicity in humans that the tissue and milk residues from cattle should pose no health risk to the consumer.

Faculty of Veterinary Science University of Pretoria Faculty Day 2006 : Committees

Programme Convenor : Prof J T Soley

Scientific Review Committee: Prof R A Meintjes (Convenor)

Prof E H Venter Dr J E Crafford

Dr L van der Merwe

Judges (Scientific Papers) : Prof J O Nöthling

Dr D W Verwoerd Prof C M Veary Dr A Goddard

Judges (Scientific Posters) : Prof L Prozesky (Convenor)

Dr E-M Mostert Dr J H Williams Dr N E Collins

Master of Ceremonies : Prof N M Duncan

Audio-Visual : Mr J A Meiring

Exhibitors : Prof K Pettey

Tea/Coffee : Faculty Secretaries

Registration : Ms U du Plessis

Ms S M Botes

Campus Management : Dr P E A van Dam

Photographic Exhibition : Dr E van Dyk

Marketing : Ms L Prinsloo



