UNIVERSITY OF PRETORIA

FACULTY OF VETERINARY SCIENCE

FACULTY DAY SEPTEMBER 6, 2007

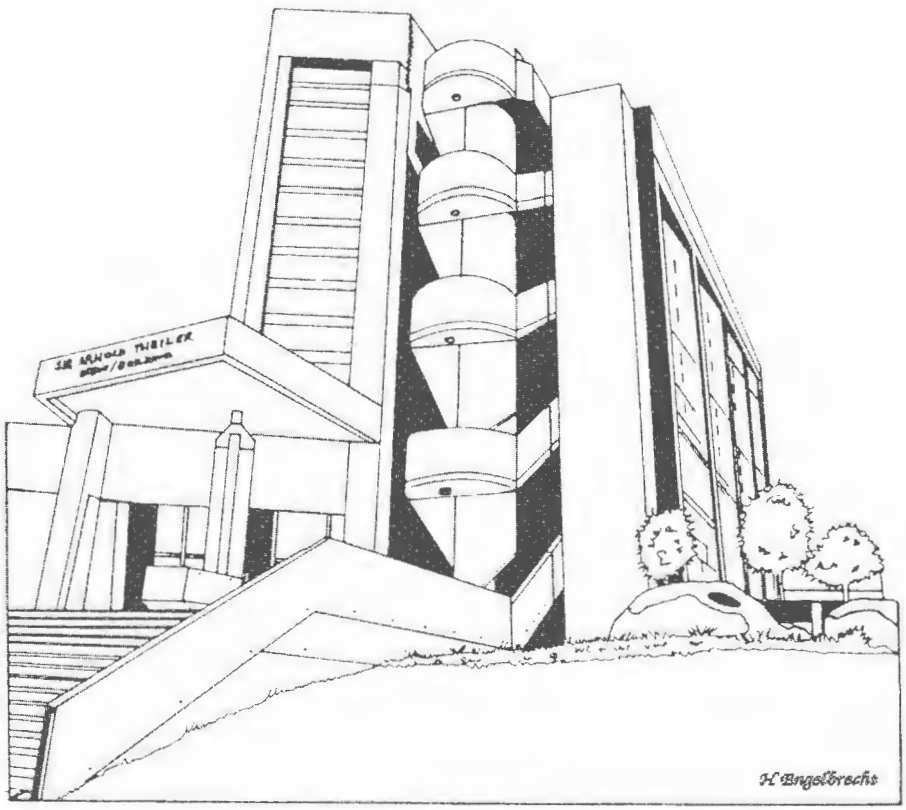
PROGRAMME AND SUMMARIES

FACULTY DAY

FACULTY OF VETERINARY SCIENCE

UNIVERSITY OF PRETORIA

6 SEPTEMBER 2007



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BRIEF HISTORY OF FACULTY DAY

Faculty Day of the amalgamated Faculty of Veterinary Science reflects a proud tradition, which had been nurtured by the original Faculties of Veterinary Science of both 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.

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MESSAGE FROM THE DEAN

Prof G E Swan

Faculty Day is a very important annual event during which we acknowledge the research contributions of staff and postgraduate students. Young researchers and postgraduate students are afforded the opportunity to present their research findings and to gain experience in the skill of scientific presentation. This year's contributions reflect a high standard, a continued increase in research output in the Faculty and an emphasis on more in-depth research incorporating the use of advanced technology. In 2006 the Faculty took some important decisions related to its research effort, among others, to make research a primary thrust, aiming to stimulate and focus research on unique problems, which will give us a leading edge. Another decision was to make postgraduate training one of the primary thrusts in the next four years.

Research at the Faculty has been growing steadily over the past year. Subsidy units which are earned from the Department of Education based on scientific outputs have increased by 38% compared to the previous year and especially gratifying is the fact that the subsidy per staff member reached 0,59, edging closer to the UP goal of 1,0. A noteworthy achievement is that all the Faculty's publications were published in the higher category ISI-accredited journals. This resulted in an increase of 51% in the allocation for research received from the University. Growth is also reflected in a steady increase in the number of NRF-rated staff members. Of critical importance is the increased number of postgraduate students which is reflected by a 30% increase in the number of postgraduate bursaries allocated this year. No less than 60% of these bursars are from previously disadvantaged groups, boding well for the future.

The UP'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. May these awards motivate you to further excel.

Through our continued eagerness and passion for research we will be able to become truly internationally competitive. Maybe we can take note of a quote by Celia Green stating that "the way to do research is to attack the facts at the point of greatest astonishment".

I believe that all participants and those who have come to support them will have a most satisfying and inspiring day. Appreciation also goes to the organizers of the day and our sponsors. Your contributions and commitment are instrumental in making this day possible. Welcome to Faculty Day 2007.

PROF GERRY SWAN
DEAN

Prof. dr. Albert WCA Cornelissen

Prof. dr. Albert WCA Cornelissen

Prof. dr. Albert W.C.A. Cornelissen (1951) is Dean of the Faculty of Veterinary Medicine, University of Utrecht. He graduated in biology from the University of Amsterdam in 1979 and received his PhD in parasitology in 1982 from the Faculty of Veterinary Medicine (Utrecht University). He subsequently worked as a researcher at the University of Edinburgh (1982 – 1983), The Netherlands Cancer Institute (1983 – 1986) and as group-leader at the Max – Planck-Institut für Biologie (1986 – 1991).

Since 1991 A.W.C.A. Cornelissen has been Professor of Parasitology at the University of Utrecht. He was appointed Chairman of the Department of Infectious Diseases and Immunology (1995 – 1999), Vice-Dean (1997 – 1999) and Dean (1999 – present) and also holds the chair of the Academic Biomedical Centre of Utrecht University (1999 – present).

He has published over 50 scientific articles and reviews in peer-reviewed journals, including the European Molecular Biology Organization (EMBO) Journal and Cell. At present, 24 PhD theses have been prepared under his guidance. He is editor of Research in Veterinary Sciences and has served on peer review panels of funding agencies, advisory boards, boards of non-governmental organizations, boards of professional societies and the National Research Council (NOW/Wotro).

FACULTY OF VETERINARY SCIENCE, UNIVERSITY OF PRETORIA

FACULTY DAY

THURSDAY 6TH SEPTEMBER 2007

PROGRAMME

07:45-08:00 **Registration and Coffee**
Master of Ceremonies: *Dr SL Fourie*

08:00-08:15 **Welcome and Opening Address**
Dean: Prof GE Swan

08:15-09:15 **RESEARCH PROGRAMME: ORAL PRESENTATIONS I**
SESSION CHAIRPERSON: ***Dr A Goddard***

The role of insulin in the blood glucose perturbations seen in canine babesiosis
P Rees, JP Schoeman

Evidence of a novel *Babesia* parasite in a domestic cat in South Africa
A-M Bosman, JCA Steyl, MC Oosthuizen, EH Venter, BL Penzhorn

Serial daily serum thyrotropin, thyroxine and free thyroxine in puppies with severe parvoviral diarrhea
JP Schoeman, ME Herrtage

Mandibular salivary gland sialoadenosis in dogs infected with *Spirocerca lupi*: A retrospective study
LL van der Merwe

Clinicopathological differences between dogs with benign and malignant spirocercosis-induced oesophageal nodules
E Dvir, RM Kirberger, V Mokarera, LL van der Merwe, SJ Clift

09:15-10:05 **Sir Arnold Theiler Memorial Lecture:**
"What makes an excellent Faculty of Veterinary Medicine?"
Prof AWCA Cornelissen

10:05-10:45 Awards Presentation: Lecturer of the Year; Nursing Lecturer of the Year; Researcher of the Year; Young Researcher of the Year; Prizes for academic achievements

10:45-11:30 **TEA** and Viewing of Posters, Commercial Exhibits and Photographic Exhibition

11:30-12:30 **RESEARCH PROGRAMME: ORAL PRESENTATIONS II**
SESSION CHAIRPERSON: ***Prof M van Vuuren***

The prevalence of blood parasites in South African felids
A-M Bosman, EH Venter, BL Penzhorn

Molecular characteristics of a *Theileria sp.* isolated from dogs in South Africa
PT Matjila, AL Leisewitz, MC Oosthuizen, F Jongejan, BL Penzhorn

Characterization of South African *Theileria equi* and *Babesia caballi* isolates based on 18S rRNA gene sequences
R Bhoora, E Zweygarth, AJ Guthrie, L Franssen, F Jongejan, MC Oosthuizen, BL Penzhorn, NE Collins

Molecular detection of *Ehrlichia ruminantium* variants which do not cause heartwater found in areas of southern Africa free of heartwater and *Amblyomma hebraeum* ticks
MTEP Allsopp, MF van Strijp, E Faber, AI Josemans, BA Allsopp

Extensive recombination occurs in the field between different genotypes of *Ehrlichia ruminantium*
MTEP Allsopp, BA Allsopp

12:30-13:00 **RESEARCH PROGRAMME: PRESENTATION OF POSTERS**
SESSION CHAIRPERSON: ***Prof C McCrindle***

P1. **Microscopic comparison of mammalian blood platelets**
L du Plessis

P2. **Serial daily blood lactate concentrations in puppies with parvoviral diarrhoea**
M van Schoor, JP Schoeman

P3. **Serial plasma glucose concentrations in canine bite wound cases**
CJ du Plessis, PN Thompson, JP Schoeman

P4. **Assessment of adrenal function in cheetah (*Acinonyx jubatus*)**
LS Köster, JP Schoeman, DGA Meltzer

P5. **Survey on filariosis of domestic cats in KwaZulu-Natal, South Africa**
EV Schwan, WB Hyman

P6. **Effect of altitude on venous proinflammatory cytokine production in Thoroughbred racehorses with exercise-induced pulmonary heamorrhage**
MN Saulez, J Godfroid, A Bosman, KW Hinchcliff, JL Stiltner, CC Breathnach, DW Horohov

P7. **New perspectives on the bacteriology and antimicrobial susceptibility of the bacteria of infected and non-infected dog bite wounds**
B Meyers, JP Schoeman, A Goddard, E Seakamela, JA Picard

P8. **Mapping of antigenic sites on a SAT2 foot-and-mouth disease virus using a chicken antibody phage display library**
PA Opperman, FF Maree, J Theron, W Vosloo

P9. **Genetic diversity of South African *Theileria parva* isolates**
KP Sibeko, N Collins, M Oosthuizen, D Geysen

P10. **Towards a recombinant vaccine for heartwater**
N Thema, A Pretorius, J Liebenberg, M van Kleef

P11. **Investigating the role of cellular receptors in foot-and-mouth disease virus adaptation to *in vitro* growth**
M Chitray, B Blignaut, FF Maree, W Vosloo

P12. **Determination of buffalo and giraffe heart weights**
S van Sittert, G Mitchell, JD Skinner, CH Moeller, OL van Schalkwyk

P13. **Correlation between tetracycline resistance in *Escherichia coli* isolated from impala (*Aepycerus melampus*) and *E. coli* isolated from their water source**
V Mariano, JA Picard, CME McCrindle, B Gummow, B Cenci-Goga

P14. **Ultrastructure of the interstitial vasculature of the ostrich testis**
MZJ Elias, JT Soley, TA Aire

P15. **Present trends in udder health and emerging mastitogenic pathogens in South African dairy herds**
I-M Petzer, J Karzis, JC Watermeyer, TJ van der Schans, R van Reenen, I de Goede

13:00-13:45 **Light LUNCH in Cafeteria**

13:45-15:00 **RESEARCH PROGRAMME: ORAL PRESENTATIONS III**
SESSION CHAIRPERSON: ***Prof C Botha***

Diclofenac: A molecular mechanism of toxicity
V Naidoo, GE Swan

Induction of *in vitro* angiogenesis in bovine endothelial cells and the effect of Paclitaxel on neovessel formation
P Mabeta, MS Pepper

Development of botanicals with insect repellent and toxicant properties for control of tick infestations
KG Mawela, D Luseba, JN Eloff, S Magano

Anthelmintic activity of the crude methanol extract of *Xylopia aethiopica* against *Nippostrongylus brasiliensis* in rats
MM Suleiman, M Mamman, YO Aliu, JO Ajanusi

Antidiarrhoeal activity of the methanol extract of *Annona senegalensis* Pers. (*Annonaceae*)
MM Suleiman, T Dzenda, CA Sani

15:00-16:00 **RESEARCH PROGRAMME: ORAL PRESENTATIONS IV**
SESSION CHAIRPERSON: ***Dr H Annandale***

An update on the 2007 Onderstepoort Feedlot Challenge
DE Holm

Effect of lumpy skin disease virus in bull semen on *in vitro* embryo production
PC Irons, I Luther, K Ebersohn, A Bosman, CH Annandale, E van Wilpe, B Colenbrander, EH Venter

Effect of the acidic buffer 2-[N-morpholino] ethanesulfonic acid (MES) on bull semen
I Luther, E Botha, D Gerber

The value of reproductive tract scoring as a predictor of fertility and production outcomes in beef heifers
DE Holm, PN Thompson, PC Irons

The isolation and characterization of a *Babesia bovis* stock for potential use in immunization
C Olds, A Latif, N Collins, E Zweygarth

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: *Dr S Clift*

Variable number of tandem repeat genotyping of *Mycobacterium bovis*: evaluation of the epidemiological relevance and comparison with IS*6110* RFLP and spoligotyping
C Allix, K Walravens, C Saegerman, J Godfroid, P Supply, M Fauville-Dufaux

A census survey of Western Cape ostrich farms to identify risk factors associated with seropositivity to H5 avian influenza virus
PN Thompson, M Sinclair, B Ganzevoort

The occurrence of antimicrobial drug resistance in enteric bacteria from exposed poultry abattoir workers and broilers fed antimicrobial growth promoters
JW Oguttu, CM Veary, JA Picard

Neurocysticercosis: A possible cause of epileptiform fits in people residing in villages served by the Bethanie clinic in the North West Province
SN Manoto, CM Veary

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 submitted work will be judged by experienced photographers.
   Organisors: Sr R van Reenen and Sr I de Goede

2. EXHIBITS BY SPONSORS

3. SCIENTIFIC POSTERS

What makes an excellent Faculty of Veterinary Medicine?

Albert WCA Cornelissen *(a.w.c.a.cornelissen@vet.uu.nl)*

Faculty of Veterinary Medicine, Utrecht University, PO Box 80163, 3508 TD Utrecht

The environment of universities has changed dramatically. Universities participate in globalization. Therefore, universities have to respond rapidly to new challenges and new circumstances. Research universities are liable to lose their leading role unless they are able to form, or join, worldwide networks of researchers working at the frontiers of knowledge. Collaborations with other 'knowledge institutions' are essential. These collaborations do not only have to contribute to regional development, but also have to have positive effect on their research grant portfolio.

Globalization, the fast developments in information technology and the existence of large scientific databases, does not only make most of what universities do transparent, but is also used to judge their output on such as items as impact and quality. This is done on a large scale and is used to rank them. Students, Schools, Faculty management, University Boards and the responsible ministries, mostly the Ministry of Education, use this information. All our stakeholders use these data to determine their policies in education and research and distribution of budgets. Thus, universities have to act. The core of this message is that departments and faculty members must accept the need to change and reject the *status quo*. This brings me to the core of the lecture: What makes an excellent Faculty of Veterinary Medicine?

Schools have to have a proactive strategy and thus have to be aware of national and international developments and the relevant reports addressing veterinary medicine and more general reports on the status and strategy of universities. Today's rapid advancements in veterinary medicine, veterinary technology and medical information have spurred an increase in specialized services and practices. These developments have produced intense, continuous pressure on veterinarians to remain current with new trends. The future of the profession is diversification. The veterinary field will continue to diversify as new insights, technologies and treatments or preventive procedures are developed. Graduates are expected to accumulate more specific knowledge and to receive the appropriate training needed for the various sectors in the field. This has to have an impact on the development of veterinary curricula. This will be discussed on the basis of the present situation in European schools.

Research universities want to be internationally recognized for their teaching and research. This implies that they are not only evaluated at the level of the school and in national systems, but do fall under the rules of globalization. Their science has thus to be at the forefront of scientific developments. Here competition is harsh, but also challenging and – more importantly – a strong research position can be obtained. Reassessment of research programs should lead to a better strategy for acquiring external funding, boost the quality of research by enhanced chances for scientific innovation at the interface between disciplines and has to lead to an economic use of limited resources. This will enable schools to attract and retain high potential scientists and will also increase fund raising capacity. These managerial aspects of science will be discussed on the basis of the Utrecht experience.

Organization and management are also critical factors for success. Many European Veterinary Schools have here a traditional, mainly inward looking attitude. Schools have to change this position and open up, collaborate with other faculties, influence the policies of their university and talk intensively with the profession and partners with similar missions. Collaboration is more fruitful than competition and does strengthen an international position. It is not always easy, since it implies that they have to bridge the competencies of academic professionals. However, successes on the basis of collaboration are very convincing arguments. A progressive attitude brings much: it is easier to attract and keep top talent; productivity in research output and services increases; it can cut costs due to sharing of infrastructure and strengthen the grant position. Organization and management are also illustrated on the basis of recent developments in Utrecht.

The role of insulin in the blood glucose perturbations seen in canine babesiosis

P Rees, JP Schoeman (phil.rees@up.ac.za)

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

Hypoglycaemia has been identified as a life threatening metabolic complication in almost 20% of severely ill dogs suffering from babesiosis due to *Babesia rossi* infection, and has been associated with an increased risk of mortality.

Insulin is the primary hormone involved in glucose homeostasis, and lowers blood glucose concentration by facilitating intracellular movement of glucose. Hyperinsulinaemia as a result of inappropriate insulin secretion may precipitate hypoglycaemia, and has been suggested as a possible cause of hypoglycaemia in human and murine malaria. A similar phenomenon may exist in canine babesiosis. This prospective, cross-sectional, observational study, including 94 dogs with naturally occurring virulent babesiosis, sought to identify the presence of inappropriate insulin secretion in hypoglycaemic canine babesiosis.

Pre-treatment jugular blood samples were collected for simultaneous determination of plasma glucose and insulin concentrations. Plasma insulin concentration was determined using a commercially available radioimmunoassay kit previously validated for use in dogs. The reference range for blood glucose (BG) in dogs is 3.3-5.5 mmol/L. Animals were retrospectively divided into three groups: hypoglycaemic (BG< 3.3 mmol/L; n=16), normoglycaemic (BG 3.3-5.5 mmol/L; n=62), and hyperglycaemic (BG> 5.5 mmol/L; n=16). Data is expressed as median and interquartile range. The median insulin concentrations for the hypoglycaemic, normoglycaemic, and hyperglycaemic groups were 0.0 pmol/L (0.0-18.8 pmol/L), 2.2 pmol/L (0.0-29.53 pmol/L), and 21.7 pmol/L (0.0-45.74 pmol/L) respectively. Statistical analysis, using the non-parametric Kruskal Wallis one way analysis of variance on mean ranked insulin data, revealed no significant difference in insulin concentration between the three groups ($\text{Chi-square}_{k\text{-}w} = 2.418$, $p = 0.299$). Additionally, the median insulin concentration in the hypoglycaemic and normoglycaemic groups was below the detection limit of the assay (<11 pmol/L), suggesting that insulin secretion was appropriately low (i.e. undetectable) in these cases. Time since last meal (TLM) was available for 87 dogs. The median TLM was 24 hours, constituting a significant period of illness-induced starvation. Consequently, insulin secretion by pancreatic β-cells would be completely inhibited, thus accounting for the low insulin concentrations observed in this study.

We conclude that hyperinsulinaemia is an unlikely cause of hypoglycaemia in virulent canine babesiosis. Other causes of hypoglycaemia, such as increased glucose consumption, depletion of hepatic glycogen stores, and hepatic dysfunction with impaired gluconeogenesis, are likely to play more important roles in the pathophysiology of hypoglycaemia in canine babesiosis.

Evidence of a novel *Babesia* parasite in a domestic cat in South Africa

A-M Bosman[1], *JCA Steyl*[2], *MC Oosthuizen*[1], *EH Venter*[1], *BL Penzhorn*[1] (bossie.bosman@up.ac.za)

[1]Department of Veterinary Tropical Disease, and
[2]Section of Pathology, Department of Paraclinical Sciences, Faculty of Veterinary Science, University of Pretoria, Private Bag X04, Onderstepoort 0110, South Africa

Blood parasites of the genus *Babesia* occur in many different hosts of the family Felidae. The two most frequently reported species are *Babesia felis*, which causes clinical babesiosis in domestic cats, and *Babesia leo*, primarily reported from asymptomatic lions. Babesiosis, a tick-borne disease, is regarded as an important disease in domestic cats in certain parts of South Africa, particularly in the coastal areas of the Western Cape, Eastern Cape and KwaZulu-Natal Provinces. Infections found in inland areas are mostly in cats that had accompanied their owners on holiday to the coast, but an endemic focus occurs along the eastern escarpment at Kaapschehoop, Mpumalanga Province. The vector transmitting babesiosis in felids is unknown.

Identification of *B. felis* is done primarily on its morphology on blood smears and serology but nucleic-acid-based techniques, such as the reverse line blot (RLB) hybridization assay, prove to be more sensitive tools in characterising blood parasites. This technique has been successfully used for the detection and characterisation of *Theileria* and *Babesia* species in blood specimens from various mammal species as well as domestic and wild felids.

A 2-year-old male cat in Rustenburg, North West Province, died of suspected babesiosis. The carcase was sent to the faculty for postmortem examination. Specimens were taken for histopathology examination and for nucleic acid-based analysis. Blood smears showed a large *Babesia* parasite. DNA was extracted from frozen organs. PCR was performed using primers that amplified a variable region of the 18S rRNA of the parasite. The amplicons obtained were analysed with the reverse line blot (RLB) assay.

The samples tested positive for a *Babesia* parasite but did not react with any species-specific probe. The PCR products were sequenced. On phylogenetic analysis, these sequences did not group with *B. felis*, *B. leo* or other small blood parasites such as *B. microti*, but were in a group of their own. These results revealed that a further *Babesia* species may be present in domestic cats.

Serial daily serum thyrotropin, thyroxine and free thyroxine in puppies with severe parvoviral diarrhea

JP Schoeman[1], *ME Herrtage*[2] (johanp.schoeman@up.ac.za)

[1]Department of Companion Animal Clinical Studies, Faculty of Veterinary Science, University of Pretoria, Private Bag X04, Onderstepoort 0110, South Africa
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Thyrotropin (TSH), total thyroxine (TT4) and free thyroxine (fT4) have been shown to inversely correlate with mortality in human critical care. Previous veterinary studies have demonstrated that dogs with more severe illness had lower TT4 and fT4 concentrations and one previous study on dogs with parvoviral diarrhea have shown significant differences in TT4 concentrations between survivors and non-survivors. It is unknown whether these changes to the pituitary-thyroidal axis described in parvoviral diarrhoea is exerted at the level of the thyroid gland or whether it involves the pituitary gland and TSH production as well. The differences in fT4 and TSH concentrations between survivors and non-survivors of canine critical illness are unknown.

A prospective study was conducted on sixty-three puppies with parvoviral diarrhoea admitted to the high dependency isolation unit of the Onderstepoort Veterinary Academic Hospital. Diagnosis was confirmed by faecal electron microscopy. Basal hormone concentrations were measured upon admission (day 1) and every day until death or discharge and each dog was accorded a daily clinical score (CS). TT4 and fT4 concentrations were determined by commercial canine radioimmunoassay (RIA) kits (Coat-a-count®, USA), whilst TSH was performed with an immunoradiometric kit from the same manufacturer. Dogs were retrospectively assigned to two groups: survivors (n=50) and non-survivors (n=13). CS, TT4, fT4 and TSH 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. Significance was set at $p< 0.05$.

Significant differences between the two groups were demonstrated for CS, TT4 and fT4 from day 1 through to day 4. However, no significant differences in TSH concentrations were noted between the groups on any of the days. A significant positive correlation was detected between serum fT4 and TT4 concentrations on all 4 days (mean $r_s = 0.942$, $P<0.001$) as well as between CS and fT4 and TT4 on all 4 days (mean $r_s = 0.704$ and 0.685 respectively, $P<0.001$). Mortality was negatively associated with CS, TT4 and fT4 on all days, but not with TSH on any of the days.

This study demonstrated that fT4 by RIA was significantly different between survivors and non-survivors of canine parvoviral diarrhoea and that this hormone was affected to a similar degree than TT4 by critical illness. The lack of a sensitive canine TSH assay, especially its inability to accurately read concentrations of < 0.03 ng/ml, might offer an explanation for its inability to discriminate between the outcome groups. This study adds further prognostic indicators to the previously described serum cortisol and thyroxine concentrations in the canine parvoviral diarrhoea and canine *B. canis rossi* babesiosis models of critical illness.

Mandibular salivary gland sialoadenosis in dogs infected with *Spirocerca lupi*: A retrospective study

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Sialoadnenosis (sialadenitis, salivary gland necrosis, hypersialosis) is a secondary complication in dogs with oesophageal and gastric disease and also a primary condition in dogs diagnosed with limbic epilepsy[1,2]. The condition is managed with phenobarbitone. The objective of the study was to evaluate the incidence, presentation and therapeutic response of this syndrome in dogs with spirocercosis. Of 281 dogs diagnosed with spirocercosis based on thoracic radiographic and oesophageal endoscopic criteria between 2001 and 2005, 27 dogs were concurrently treated with phenobarbitone for hypersialosis. Phenobarbitone was used inappropriately in 3 of these cases which had neoplastically transformed nodules. Thus true sialoandenosis occurred in only 24 cases, an incidence of 8.5%. Only 19 cases had complete records for analysis.

Breeds affected included Fox or Jack Russell Terriers (7/19) and Staffordshire Terriers (4/19), terrier breeds thus accounting for 11/19 cases. Clinical history was prolonged and included retching, coughing, hypersialosis, gulping and apparent choking which worsened with stress, excitement or external throat palpation. Signs resulted in dysphagia and a reluctance to eat with food aversion noted in two cases. All the dogs showed weight loss. Mandibular salivary gland size was recorded in 17 cases and they were enlarged and firm in 16 (94%). Costo-abdominal respiration was noted in 6/19 (32%) cases and included all the Staffordshire terriers. The vomitus was generally described as a clear viscous or frothy fluid or as saliva and did not contain food. The medians of all haematological parameters fell within the normal ranges. Of the 18 cases which had thoracic radiographs taken, a mass lesion was identified in 15/18 (83%) cases, aortic aneurysm in 4/18 (22%) and vertebral spondylitis in 1/18 (6%). In 17 cases which had oesophageal endoscopy an oesophageal mass was found in 15 cases, a gastric mass in one and no mass in one case. The endoscopically negative case had a mediastinal mass with spondylitis on radiographs. Salivary gland biopsies were not taken but salivary gland fine needle aspirates performed in 4 cases demonstrated hyperplasia without inflammation. Treatment in all patients included doramectin and phenobarbitone. Outcome was recorded in 14 dogs. Clinical signs decreased within 48 hours of initiating phenobarbitone treatment (2mg/kg/bid) in 11 cases, 3 cases took longer to respond, one of which was ultimately euthanased.

This study shows that sialoadenosis as a complication of spirocercosis is not infrequent and can be easily managed in most cases if accurately diagnosed. The dysphagia and vomition due sialoadenosis has very different characteristics compared to the regurgitation caused by an oesophageal mass. A distinct susceptibility of the terrier breeds is also apparent.

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Clinicopathological differences between dogs with benign and malignant spirocercosis-induced oesophageal nodules

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Spirocerca lupi is a nematode infecting the canine oesophagus, where it induces the formation of a nodule that can be transformed into a neoplasm. Benign nodules are characterized by degenerate neutrophils and variable numbers of eosinophils, macrophages and lymphocytes. In mature nodules fibroblast proliferation predominates. Tumours are characterized by neoplastic osteoblasts or fibroblasts.

The following retrospective study compared the clinical presentation, haematology, serum albumin and globulin and radiology of benign and malignant cases of spirocercosis. Categorical parameters were compared by Chi-square test and continuous parameters by T-test. $P<0.05$ was defined as significant. Malignant cases (n=32) were diagnosed histologically (n=28; 16 osteosarcoma, 9 fibrosarcoma, 3 undifferentiated) or by diagnosis of a typical caudal oesophageal mass with metastases and spondylitis on thoracic radiographs (n=4). Fifteen dogs had metastases. Benign cases (n=30) were defined based on histology of the whole mass (n=11) or endoscopic diagnosis and marked response to treatment (n=19).

Dogs with spirocercosis-induced sarcoma were significantly older (6.48 ±1.93 years) compared to benign cases (4.79±2.81). In the malignant cases there were significantly more spayed females (10/32) and fewer intact males (4/32) compared to 2/30 and 13/30, respectively, in the benign cases. Hypertrophic osteopathy was observed in 38% of the malignant cases and in none of the benign cases (p=0.0002). Common clinical signs included weight loss, regurgitation, anorexia, pyrexia (T ≥ 39.5°), respiratory complications and salivation and did not differ in prevalence between groups. Serum globulins were higher in the benign group (50.97±16.5g/l vs. 41.15±9.06) but albumin was not significantly different. On haematology, the malignant group had a lower haematocrit (0.34±0.08 vs. 0.41±0.07), higher white cell count (31.6±27.83 vs. 17.71±13.18 $x10^3/\mu l$), higher mature neutrophil count (26.06±26.08 vs. 12.23±9.96 $x10^3/\mu l$) and lower eosinophil count (0.5±0.5 vs. 0.86±0.81 $x10^3/\mu l$). There were no differences in the mean corpuscular volume and immature neutrophil count. On radiology, the mass length was not significantly different, but the height and the width of the malignant masses were significantly bigger (63.21±15.22 and 74.14±20.57 mm) compared to the benign group (44.75±22.91 and 47.75±25.21, respectively). Spondylitis was more prevalent in the malignant group (66% vs. 40%, borderline significance, $p = 0.06$). Examining secondary pulmonary changes revealed significantly higher prevalence of bronchial displacement in the malignant group (52% vs. 17%).

Hypertrophic osteopathy appeared to be a very specific but relatively rare (poor sensitivity) marker of malignancy. The rest of the clinicopathological parameters had a large range of overlap between the malignant and benign groups.

The prevalence of blood parasites in South African felids

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Suspected *Babesia* positive blood samples from domestic cats, cheetahs (*Acinonyx jubatus*), lions (*Panthera leo*), black-footed cats (*Felis nigripes*), servals (*Felis serval*), caracals (*Felis caracal*) and a leopard (*Panthera pardus*) were submitted to the Biotechnology Laboratory, Department of Veterinary Tropical Diseases, Faculty of Veterinary Science, University of Pretoria, by various collaborators. These blood samples were collected in EDTA, and stored at -20 °C.

DNA was extracted from whole blood using the commercially available QIAamp® DNA Mini Kit (Qiagen, Southern Cross Biotechnologies, South Africa), according to the manufacturer's instructions. The PCR was performed as described by Gubbels *et al.*[1] using primers that amplified a 460 to 520 bp fragment in the V4 variable region of the 18SSU rRNA of *Theileria* and *Babesia* species. The PCR amplicons were verified using agarose gel electrophoresis before they were analysed by RLB hybridization.

In total, 127 of the felid samples tested positive for a *Babesia* species. Results varied from 66 (52.0%) samples that tested positive only for a *Babesia* parasite, 34 (27.6%) positive only for *B. felis*, 21 (16.5%) positive only for *B. leo* and 6 (4.7%) had mixed infections of *B. felis* and *B. leo*. *Babesia felis* was detected in domestic cats, cheetahs, lions and a serval while *B. leo* was detected in lions, cheetahs, a domestic cat and a leopard. Mixed infections of *B. felis* and *B. leo* were found in lions and a domestic cat. *Babesia felis* and *B. leo* occurred in cheetahs, but not as mixed infections. A high number of cheetah samples hybridized only with the genus-specific probe for *Babesia*. The results showed that *B. felis* and *B. leo* occurred more frequently in the host from which they had initially been described, namely domestic cats and lions, respectively, but were also detected in other felid species. A number of samples reacted only with the *Babesia / Theileria* genus-specific probe. This is an indication that further *Babesia / Theileria* parasites are present, but no probes exist as yet to identify these to species level.

The numerous *Babesia* species signals found in cheetah samples in this study indicate that although these parasites are morphological similar to *B. felis* their sequences in the 18SrRNA gene, where the *B. felis* and *B. leo* probes were designed, are different. This also applies for the genus-specific results were found in lions, black-footed cats, servals and caracals. The *Babesia* species signal can be an indication of one or more different *Babesia* parasites in felids. These results can only be solved if more data are known and therefore all these samples will be further analysed using sequencing and phylogenetic analysis.

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Molecular characteristics of a *Theileria* sp. isolated from dogs in South Africa

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Canine babesiosis, a haemolytic disease, is the most frequently encountered tick-borne protozoal infection of dogs in South Africa. The parasites associated with canine babesiosis in South Africa are *Babesia rossi* and *Babesia vogeli*. *Babesia rossi*, which causes a severe disease that can be life threatening, is the most prevalent species isolated from dogs presented at the Onderstepoort Veterinary Academic Hospital (OVAH). Severity of infection caused by *B. vogeli* in South Africa has not being well documented, except that *B. vogeli* was detected from dogs that were presented at the Outpatients Clinic, OVAH, and diagnosed with clinical babesiosis. Until recently there had not being any reports on pathogenic *Theileria* sp. in dogs. *Theileria annae* was the first *Theileria* species to be associated with a haemolytic disease of dogs in Spain. Our report describes a *Theileria* sp. isolated from dogs originating from two localities in South Africa: Pietermaritzburg and Onderstepoort.

Blood samples from dogs (total: 192) were collected monthly in EDTA tubes over a six-month period from the Pietermaritzburg area. Two clinical samples were collected from dogs presented at outpatients of the OVAH during the months of early 2005. One more clinical sample was collected at the OVAH in January 2007. DNA was extracted from 200 µl of each blood sample using the QIAmp® blood and tissue extraction kit (Qiagen, Hilden, Germany), following the manufacturer's protocols. The polymerase chain reaction (PCR) was performed with primers RLB-F2 and RLB-R2 from the 18S rRNA gene spanning the V4 region (hypervariable – conserved?). The PCR-amplified products were tested with the Reverse Line Blot (RLB) assay and products that did not hybridize with any of the known probes but hybridized with the *Theileria* catchall probe were selected at random from the Pietermaritzburg group of samples and from the Onderstepoort clinical samples. These samples were sequenced. Sequence data for the full 18S rRNA was assembled using the GAP 4 of the Staden package. The sequences were analysed with sequences of related genera using Clustal X. Neighbor-joining and the maximum parsimony methods were used for the construction of phylogenetic trees.

Initial processing of blood samples from Pietermaritzburg using the RLB revealed that 67 blood samples were positive for a *Theileria* sp. by hybridizing with a *Theileria/Babesia* catchall probe as well as the *Theileria* catchall probe. Partial and full sequencing of samples revealed that the samples were similar to the previously described *Theileria* sp. characterized from a Sable antelope and provisionally named *Theileria* sp. (sable). These current findings indicate that the dog samples were infected with a similar *Theileria* sp. More samples and data need to be collected and analysed to understand the full clinical relevance of the *Theileria* sp. we will cautiously call "*Theileria* sp (dog)" in South Africa.

Characterization of South African *Theileria equi* and *Babesia caballi* isolates based on 18S rRNA gene sequences

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Equine piroplasmosis is a tick-borne protozoal disease of equids that is caused by two different blood parasites, *Theileria equi* and *Babesia caballi* Currently, the diagnostic tests that are prescribed by the OIE for equine piroplasmosis for the international trade of horses include the Indirect Fluorescence Antibody test (IFAT) and the competitive Enzyme-linked Immunosorbent Assay (cELISA). Both tests detect antibodies to the parasites and not the parasite itself and may therefore result in false positive results due to the detection of maternal antibodies in negative foals, or antibodies in animals in which the infection has cleared. This study was therefore undertaken to develop a real-time PCR assay for the detection of *Babesia caballi* and *Theileria equi*.

SimpleProbes based upon sequence differences in *B. caballi* and *T. equi* 18S rRNA genes were used in real-time PCR assays to detect *B. caballi* and *T. equi* infections in blood samples from two experimentally infected ponies. The real-time PCR assays were able to detect *T. equi* DNA from day 5, post infection (p.i.) and *B. caballi* DNA from day 8 p.i. The real-time PCR assays were further tested on samples that were positive in culture for either *B. caballi* or *T. equi*. However, the probes were not able to detect parasite target DNA in all of these samples. Parasite 18S rRNA genes were therefore amplified from these samples and sequence analysis revealed variation in the sequences, which explained the failure of the real-time PCR assay to detect all samples.

The sequence diversity of the 18S rRNA gene was assessed in a total of 477 samples collected from horses and zebras from different geographical locations around South Africa. Samples were screened using the reverse line blot hybridization assay, which detects parasitic infections on a species level and also aids in the identification of samples with novel genotypes. Thirty-six of the samples hybridized only to the *Theileria/Babesia*-genus specific probe and not to the *B. caballi* or *T. equi* species-specific probes. These samples were selected for further sequence analysis. The full-length 18S rRNA gene was amplified and sequenced.

Results indicated that extensive sequence variation occurs in the 18S gene of *T. equi* and *B. caballi* isolates in South Africa. It is also evident that it will not be possible to design a real-time PCR assay to detect all *T. equi* and *B. caballi* isolates based on the 18S rRNA gene, and that alternative candidate genes will need to be identified for that purpose.

Molecular detection of *Ehrlichia ruminantium* variants which do not cause heartwater found in areas of southern Africa free of heartwater and *Amblyomma hebraeum* ticks

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In 1994, Boergoats from three farms in the Groblershoop district of the northern Cape Province of South Africa, a region free of heartwater and of the only South African heartwater vector tick, *Amblyomma hebraeum*, were screened for heartwater using ELISA and IFA tests. Fifty two percent of the animals tested positive for heartwater by either or both of the tests. A PCR assay which could detect five different *E. ruminantium* 16S genotypes gave positive results for 54% of the animals, suggesting that apparently non-pathogenic *E. ruminantium* variants existed in this heartwater-free area. One farm had contributed 47 animals, of which one was seropositive by ELISA, 19 were IFA positive, and 43 (91%) were probe positive for one or more of the *E. ruminantium* 16S genotypes.

To further characterise the organism(s) we returned to the same area in 2002 and 2003 and collected 51 blood samples and 212 ticks from cattle and sheep on two farms. Blood stabilates were prepared *in situ* and were frozen immediately in liquid nitrogen for attempted infection studies. The ticks were maintained alive for between three weeks and three months to digest their blood meals. Individual ticks were washed in ethanol and air dried before DNA extraction. We also examined 113 ticks from zebra, horses, eland and scrub hares collected in during the early 1980s from heart-water-free areas of Namibia and the Eastern Cape, which had been alcohol-preserved before blood meal digestion. The objective was to determine whether the "positive heartwater" results were new or long-standing phenomena. DNA samples were subjected to a PCR for amplification of the *E. ruminantium*-specific pCS20 region and amplicons were slot blotted and probed. Samples giving strong pCS20 signals were selected for pCS20 and 16S V1 loop sequencing. Blood stabilate from one pCS20 positive sheep was injected i.v into a pCS20-negative sheep to check for pathogenicity. The temperature of the animal was monitored daily and blood was collected for culture.

Of the 2002-2003 samples 29% of the animals and 10.4% of the ticks were pCS20 positive, and 74% of the preserved ticks were pCS20 positive. Four tick species were found during all the surveys: *Rhipicephalus evertsi evertsi*, *R. evertsi mimeticus*, *Hyalomma truncatum* and *H. marginatum rufipes*. Probing results for *E. ruminantium* 16S genes indicated that all the animals appeared to be carrying more than one genotype, whereas the ticks rarely carried more than one. pCS20 and 16S sequences identical to those of known *E. ruminantium* strains were identified. The clean sheep injected with blood stabilate had a mild febrile reaction seven days post inoculation but showed no signs of distress and the level of infection was not sufficient for the establishment of cultures.

A variety of non-pathogenic *E. ruminantium* variants apparently exist in some areas free of heartwater and *A. hebraeum* ticks. The high prevalence of *E. ruminantium* in ticks which had not digested their blood meal suggests that animals other than ruminants may be the natural reservoirs for these variants, while ticks which had digested their blood meal apparently harbour the organisms for at least three months. The variants are of long-standing origin and their existence accounts for some of the serological anomalies experienced in the area. It is possible that the vectors of these organisms may be *Rhipicephalus evertsi* spp.

Extensive recombination occurs in the field between different genotypes of *Ehrlichia ruminantium*

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A wide variety of genotypes of *Ehrlichia ruminantium* varying from virulent to avirulent occur in the field. Immunological cross-protection between them varies widely from total to minimal. Because future vaccines will need to incorporate components from different genotypes information on the extent of genetic variation in the field for the characterization of present and future isolates of *E. ruminantium* is important. As outer membrane protein *map*1 gene was found to be highly variable and showed no correlation with virulence, immunogenicity or geographical origin we examined core function genes and targeted eight of these from 12 different cultured stocks originally isolated in different areas of Africa and the Caribbean.

Genomic DNA was prepared from *E. ruminantium* culture supernatants and the following genes were amplified by PCR with suitable primers: 16S rRNA, *glt*A, *gro*EL, *fts*Z, *sod*B, *nuo*B, *rnc* and *cta*G. Amplicons were sequenced, the sequences were aligned using CLUSTALW, and maximum likelihood phylogenetic analysis was performed using PHYML. A concatenated alignment of the eight genes was also constructed and analysed separately. In all cases the corresponding *E. canis* ortholog sequences were included in the alignments to root the trees.

Only 11 stocks yielded sequences for all eight genes, one stock failed to give an amplicon for *glt*A. There were fewer individual sequences for each gene than there were stocks, suggesting that inter-genome recombination has occurred between different stocks. Of the southern and eastern African stocks, all except Kümm1 showed evidence of recombination between themselves, but none with the western African stocks. The South African Kümm1 stock is anomalous as it shares no orthologs with any other southern or eastern African stock, but shows recombination with all the western African stocks. Gardel, isolated in the Caribbean was unable to recombine with African genotypes. Recombination may occur during the period when the organisms are extracellular within the tick, immediately after feeding and before intracellular infection is established.

Although varied, the topologies of the phylogenetic trees inferred from individual gene sequences consistently showed each tree to exhibit two major clades, one consisting of stocks of southern and eastern African origin (the S&E clade), the other consisting of west African stocks plus the southern African Kümm1 stock (the W clade). In the concatenated tree of 8 sequences from 11 stocks the two major clades were also clearly separated, and Gardel fell into the S&E clade, but deeply branching and with low bootstrap support. The prominent two clade pattern suggests that there may be some restriction to gene flow between the corresponding geographical areas. Highest population densities of the most important African tick vector, *A. variegatum*, are indeed on the eastern and western sides of the African continent. A region of lower tick population density separates these two areas and this could be the reason for the restricted gene flow.

The genetic variability of the stocks in southern and eastern Africa is much greater than that among the western African isolates, which suggests that the organism originated in the southern or eastern region of the continent. The common ancestor of the western African stocks possibly became established in the region after passing the central African genetic bottleneck. The genotypically western stock, Kümm1 appears to have been recently introduced into the region. The unique Gardel stock belongs to neither the S&E clade nor the W clade, and may be descended from an ancestral stock from west central Africa. The existence of a large reservoir of shared genetic diversity among *E. ruminantium* stocks has important implications for vaccine development.

Diclofenac: A molecular mechanism of toxicity

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Diclofenac, a commonly used veterinary drug on the Asian Subcontinent has caused a massive decline in the vulture populations in the region. Although the use of diclofenac was recently banned, following conclusive proof supporting the safety of meloxicam, the potential toxicity of other veterinary non-steroidal anti-inflammatory drugs (NSAIDs) needs to be determined. In the first step in creating an *in vitro* model, the mechanism of toxicity of diclofenac had to be determined. Three hypothesis were proposed and tested: (a) vasoconstriction of the renal portal veins with subsequent necrosis; (b) increased production of reaction oxygen species (ROS) with subsequent activation of the mitochondrial apoptotic cycle; and (c) a decrease in the secretion of uric acid by renal tubular epithelial (RTE) cells. To test these hypotheses ex-*vivo* tissue models were established using tissue from either the domestic chicken (*Gallus gallus*)(a validated model) or vulture (*Gyps africanus*).

Using chicken isolated cranial renal portal veins, equilibrated in organ baths with a physiological salt solution, we were able to demonstrate that both diclofenac and meloxicam were physiological antagonists of noradrenalin within the renal portal vein. This effect was irreversible despite numerous washings of the organ baths. The results support medical literature, it which it has been demonstrated that the NSAIDs are vasodilatory in certain renal vascular beds due to the inhibition of PGf_2 alpha production i.e. inhibition of the noradrenaline-prostaglandin coupled constriction mechanism.

Using isolated chicken and vulture RTE cells, both diclofenac (all doses) and meloxicam (high doses) were directly toxic to the cells following 48hrs of incubation. This toxicity was associated with an increased production of ROS at 12 hours, which was temporarily ameliorated by pre-incubating cultures with uric acid, a known anti-oxidant. Although the exposure period simulated the pharmacokinetics of diclofenac in the vulture, it failed to consider the mean residence time of 2 h in the chicken. More importantly meloxicam, which is safe in both species, is also characterised by short half-lives. When cultures were incubated with either drug for only two hours, meloxicam was shown to be non-toxic while diclofenac retained its toxicity. In both cases no increase in ROS production was evident, which suggests that toxicity is not completely related to ROS.

Diclofenac and meloxicam was shown to influence the excretion of uric acid by interfering with p-amino-hippuric acid channels in both vulture and chicken RTE cells grown on permeable anipore membranes to simulate the renal tubule,. The effect of diclofenac was irreversible as uric acid excretion continued to be inhibited following the removal of the drug. More importantly diclofenac induced net re-absorption of uric acid without decreasing apical concentrations i.e. diclofenac depleted intracellular uric acid concentrations.

We therefore conclude that the toxicity of NSAIDs in avian species results from the depletion of uric acid, an important intracellular anti-oxidant, from within the RTE cells. The greater susceptibility of the vulture appears to arise from constant exposure to diclofenac which increases ROS production from 12 hours post exposure.

Induction of *in vitro* angiogenesis in bovine endothelial cells and the effect of Paclitaxel on neovessel formation

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Angiogenesis, the process by which blood vessels are formed, plays a critical role during embryological development and is required for the maintenance of functional and structural integrity of tissues during postnatal life. Angiogenesis is also a dominant feature in various pathological conditions including arthritis, and has been cited as a central mechanism underlying tumour growth and metastasis. The establishment of models to study the mechanism of angiogenesis may lead to better understanding of this process and to the development of novel therapies to target diseases characterized by excessive angiogenesis. The purpose of this study was to induce angiogenesis *in vitro* using bovine microvascular endothelial cells as a model, and to determine the effects of Paclitaxel (an antineoplastic microtubule-disrupting drug) on this model.

Bovine microvascular endothelial (BME) cells were grown in alpha-modified minimum essential medium (α-MEM) supplemented with 15% heat-inactivated donor calf serum and 1% penicillin-streptomycin at 37 ^{0}C in a humidified atmosphere containing 5% CO_2. Cells were then harvested and grown on collagen gels. Cells either received no treatment (control), or were treated with 10 ng/ml basic fibroblast growth factor (bFGF), or a combination of bFGF (10 ng/ml) and Paclitaxel (0.01– 0.3 μM) for 6 days. At termination, cultures (n = 6) were fixed with 2.5% glutaraldehyde and photographed. *In vitro* angiogenesis was quantitated by determining the total additive length of all cell cords that had penetrated the underlying collagen gel.

Untreated BME cells formed a monolayer on the surface of the collagen gel. In cultures treated with bFGF, BME cells infiltrated the collagen matrix, and organized into networks of capillary-like tubular structures within the matrix. No capillary-like tube formation was observed in cultures treated with bFGF and Paclitaxel. These drug-treated cells formed a monolayer and appeared similar in morphology to control cells. Quantitative analysis showed that Paclitaxel inhibited BME cell invasion in a dose-dependent manner, with significant inhibition observed at doses of 0.01 to 0.3 μM.

Basic fibroblast growth factor induced BME cells to infiltrate the underlying collagen matrix, where they formed an extensive network of capillary-like tubular structures, a phenomenon that closely mimics the angiogenic process occurring *in vivo*. Paclitaxel inhibited growth factor-induced in vitro angiogenesis. These observations were confirmed by quantitative analysis. This assay of *in vitro* angiogenesis may be useful in the study of cellular and molecular pathways involved in angiogenesis, and in screening for potential antiangiogenic agents.

Development of botanicals with insect repellent and toxicant properties for control of tick infestations

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Aloe marlothii, Aloe ferox, Clerodendrum glabrum, Jatropha curcas, Ricinus communis and *Strychnos madagascariensis* which are used by farmers for insect control were selected and investigated for tick repellent and toxicant properties. The extracts were evaluated against the livestock tick, *Rhipicephalus appendiculatus*.

Different concentrations of the plant extracts were prepared using organic solvents ranging from polar to non-polar (methanol, acetone and dichloromethane). Infusions (paraffin-water-soap) and aqueous decoctions were also prepared.

Preliminary results of the tick – climbing repellency bioassay illustrated that 30 % (w/v) of dried crude acetone extracts of *A. ferox, A. marlothii* and *C. glabrum*; DCM extracts of *R. communis* and *A. marlothii* and MeOH extract of *J. curcas* repelled the ticks. The aqueous extracts were did not repel them. At lower concentration of 10%, only acetone extracts of *C. glabrum* were effective. Acetone itself had no climbing repellency effect.

Three types of contact bioassays were conducted for toxicant activity i.e. Type A (dipping), Type B (topical) and Type C (dry extract application). Organic extracts were not effective in the toxicity assay. Aqueous infusions of *A. ferox* and *S. madagascariensis* had a strong topical toxicity effect at 35.5 % w/v whilst infusions of *A. ferox* and *J. curcas* showed strong dipping toxicity effect. Both *A. ferox* and *S. madagascariensis* were still effective at a concentration of 30% in topical and dipping bioassays.

Based on the results, organic extracts were good repellents and aqueous extracts were good toxicants. Acetone was a good extractant leading to lower yields but higher percentage repellency. Polar aqueous extracts are powerful extractants giving high yields but poor percentage repellency. It appears that volatile compounds present in organic extracts are repellents and non-volatiles compounds present in aqueous extracts are toxins.

The results confirm the ethnoveterinary use of aqueous extracts to protect animals against ticks. It is not sure if extracts at the effective levels would be tolerated well by the hosts or if application of aqueous extracts would work under natural conditions. Efforts are in place to isolate biologically active compounds for structure elucidation.

Anthelmintic activity of the crude methanol extract of *Xylopia aethiopica* against *Nippostrongylus brasiliensis* in rats

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Parasitic helminths affect animals and man, causing considerable hardship and stunted growth. The prevalence of helminth diseases in sub-Saharan Africa is very high, especially during the wet season when infection is as high as 100% in cattle. Such high infection rates prevent optimum productivity. The major control strategy adopted against helminth parasites is the use of anthelmintics. However, the high cost of modern anthelmintics and development of resistance has limited the effective control of these parasites. A practical solution to this is to develop effective drugs from reasonably less expensive and available raw materials. This can rationally be approached through the study of indigenous traditional plant remedies. *Xylopia aethiopica* is a plant which is used as a traditional vermifuge for roundworms in Nigeria. This study was undertaken to test the anthelmintic activity of the crude methanol extract of the seeds of *X. aethiopica* against experimental *Nippostrongylus brasiliensis* infection in rats.

Safe doses of methanol seed extract of *X. aethiopica* as determined by acute toxicity testing was screened for anthelmintic activity in worm-free rats experimentally infected with a rat-adapted nematode (*N. brasiliensis*) using worm count method. Anthelmintic efficacy was calculated as follows: (C-T)/C x 100 where C and T represent the worm burdens of the untreated and treated groups of rats, respectively. The Student t-test was used to determine the significance of the comparison.

The extract exhibited a significant and consistently high anthelmintic effect. The anthelmintic effect produced by *X. aethiopica* was dose-dependent. At doses of 0.8 g/kg, 1.0 g/kg, 1.2 g/kg, 1.4 g/kg, 1.7 g/kg and 2.0 g/kg the extract was 21%, 47%, 51%, 50%, 63% and 76% effective, respectively, when compared to untreated control rats. At doses higher than 2.0 g/kg, the extract caused signs of toxicity in rats. Therefore, a dose of 2 g/kg was considered to be the maximum tolerated dose in this study.

The study clearly indicated anthelmintic activity of the crude methanol extract of *X. aethiopica* against *N. brasiliensis* in rats. There is also the possibility that the extract might be efficacious against other species of helminths, as it is known that *N. brasiliensis* is more resistant to anthelmintics than are most other helminth parasites of rats.

Further studies to isolate and reveal the active compound(s) contained in the crude extract of *X. aethiopica* and to establish the mechanism(s) of action are our focus of further studies. Moreover, there is a need to conduct detailed toxicological studies of the extract in both laboratory and target animal species to justify clinical investigation in target species.

Antidiarrhoeal activity of the methanol extract of *Annona senegalensis* Pers. (*Annonaceae*)

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Diarrhoea has been recognized as one of the most important health problems in developing countries and remains the number one killer disease among children aged 1-5 years, and worldwide the disease accounts for 4-5 million deaths among humans annually. Treatment of diarrhoea is generally non-specific and is usually aimed at reducing the discomfort and inconvenience of frequent bowel movements. The World Health Organisation (WHO) has included a programme for the control of diarrhoea, which involves the use of traditional herbal medicine. *Annona senegalensis* Pers. (Annonaceae) popularly called "*gwandar daaji*" in the Hausa language in Nigeria is found widely distributed in central and west Africa. The plant possesses several medicinal uses which includes traditional treatment of diarrhoea. In the study described herein, the antidiarrhoeal effect of methanol extract of the stem bark of *A. senegalensis*, using both *in vivo* and *in vitro* experimental models was evaluated. In addition, an effort was made to assess the safety of the plant extract *in vivo*.

Different doses of methanol extract of fresh stem bark of *A. senegalensis* were screened for acute toxicity in mice. The spasmolytic effects of varying concentrations of the extract were evaluated *in vitro* using isolated rabbit ileum; these effects were evaluated on both spontaneous contractions of the ileum and that induced by acetylcholine and histamine. In addition the *in vivo* antidiarrhoeal effect of different doses of the extract was tested using gastrointestinal transit time measurement in charcoal fed mice. For the in vivo test, one-way analysis of variance (ANOVA) was used to analyse differences in means using 5 % as a criterion for significance.

The extract at doses of ≤ 5 g/kg did not cause any apparent toxic signs. It also attenuated spontaneous contractions of the rabbit ileum and that induced by acetylcholine and histamine in a concentration-dependent fashion. The extract at the dose of 10 mg/kg significantly ($p<0.05$) reduced the transit time of the charcoal meal when compared with untreated control mice. However, no significant difference was observed with higher doses.

Substances that are not toxic at doses ≤ 5000 mg/kg are considered relatively safe. The plant extract was, therefore, considered to be non toxic at doses used. The antidiarrhoeal effect of *A. senegalensis* at a dose of 10 mg/kg was found to be comparable to loperamide (a standard antidiarrhoeal agent). Surprisingly, the extract when administered at higher doses (>100 mg/kg) caused increased peristalsis in charcoal fed animals. Similar mechanism(s) of action of the extract with that of morphine was suggested as morphine is know to cause antidiarrhoeal effect in a similar fashion. It is likely that the extract was acting as an antagonist of either neurotransmitter to block their effect by preventing the release of Ca^{2+} from the cisternae and hence its entry into the cell to activate smooth muscle contraction as suggested from the *in vitro* study.

The study clearly demonstrated that the methanol extract possesses an antidiarrhoeal effect. Further studies are required to completely understand the mechanisms of the extract antidiarrhoeal action, and to isolate the active component(s) in the crude extract.

An update on the 2007 Onderstepoort Feedlot Challenge

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In the past there was no need to promote rural veterinary practice as a career, as this was one of only a few options. Today's veterinary students are from various cultural backgrounds, with various different interests, the only common link being a passion for animals. Recently concerns have been raised about the survival of the rural veterinarian, and there is now a need to actively recruit and motivate students and young veterinarians to follow a career in rural practice.

A questionnaire was compiled for the BVSc II class in January 2007, to probe their backgrounds and career prospects. Thirty-seven male and 60 female students participated in the survey. More than half of the respondents indicated that they would prefer to start their careers in mixed animal practice, and male students were more likely to go into mixed practice. There was poor correlation between farming background and career option, and almost 50% of respondents indicated that exposure to new (previously unknown) fields has changed their career prospects. A few respondents indicated that they were likely to start their careers in state veterinary service, the police or the SANDF due to bursaries that they hold.

Possible strategies to promote a production animal based career include selecting more students from farming backgrounds, providing financial support for students in the form of loans and bursaries, promoting quality of life and job satisfaction of rural practitioners and stimulating the interest of students in production animal studies through leadership and mentorship.

To achieve the latter, the Department of Production Animal Studies has re-introduced the Onderstepoort Feedlot Challenge in the Bovine Health and Production (BHP500) curriculum this year, which will run from 25 May to 5 October 2007. Some of the aims of the feedlot challenge are:

1. To improve the morale of veterinary students through rewarding teamwork.
2. To develop general skills such as leadership, communication, responsibility and business- and management skills of the students.
3. To stimulate the interest of students in production animal practice by using a practical project that is competition based.
4. To expose students to farming, country life and the role that they can play in the community.

Five groups of students will compete in this year's challenge, by feeding 4 calves per group. Each group has one or two coordinators, and all responsibilities including selecting and purchasing the animals, preconditioning and processing, ration formulation and mixing, and feeding as well as marketing were given to different individuals within a group. Groups are to be evaluated on various aspects which help to decide the winning group. Students will be individually evaluated within a group. The reaction to this way of teaching has been mostly positive, and the enthusiasm amongst students to learn and gain experience in this field is noticeable.

It is the responsibility of the educator to stimulate the interest of our students in various aspects of veterinary science, in particular in rural veterinary practice and in food production, to ensure a balance in veterinary science in the years ahead.

Effect of lumpy skin disease virus in bull semen on *in vitro* embryo production

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This study investigated the risks associated with the use of semen infected with lumpy skin disease virus (LSDV) in *in vitro* fertilisation (IVF).

Six straws of frozen-thawed semen from uninfected bulls were spiked with LSDV and two straws from experimentally infected bulls shedding LSDV in their semen were subjected to swim-up and then tested for virus by polymerase chain reaction (PCR) and virus isolation. The samples from infected bulls were negative, but the spiked samples all tested positive by PCR but not virus isolation after swim-up. Virions could be demonstrated by electron microscopy, of which 9% were attached to sperm cells.

Semen was spiked with virulent LSDV and used for the fertilisation of oocytes from abattoir ovaries. Control and treated groups consisted of 497 and 488 oocytes respectively.

Results of PCR-testing for LSDV of media and embryos in batches of 100 cumulus-oocyte complexes fertilised with uninfected semen (control) and those fertilised using semen spiked with LSDV (treated) are as follows:

Lumpy skin disease virus was detected by PCR at all steps of the embryo production system in the treated group, and in three control batches of 100 presumptive zygotes each at day two and five following fertilisation, but not day seven or ten. Virus was isolated from two batches of day ten embryos.

Of the 187 control and 798 treated oocytes, 100 (53.5%) and 415 (52.0%) underwent cleavage ($p = 0.75$). Of the 497 control and 488 treated presumptive zygotes, 146 (29.4%) and 173 (35.5%) developed to embryos by day 7 respectively ($p<0.05$). Of 146 and 173 embryos in the control and treated groups, 26 (17.8%) and 12 (6.9%) had hatched by day ten ($p<0.01$).

Lumpy skin disease virus adheres to and enters the sperm cell. If oocytes are fertilised using infected semen, viable virus does persist and infected embryos are produced, but viral titers are low implying a low risk of infection of susceptible embryo recipients. Embryo development is negatively affected by the presence of virus once hatched from the zona pellucida.

Effect of the acidic buffer 2-[N-morpholino] ethanesulfonic acid (MES) on bull semen

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The aim of this research project was to test the effect of exposing fresh bull semen to the organic acidic buffer MES (2-[N-morpholino] ethanesulfonic acid) on its motility, acrosomal- and plasma membrane integrity before and after cryopreservation. It has been shown previously that denuded IVP (*in vitro* produced) embryos can be rendered free from foot and mouth disease virus (FMDV) by exposure to MES at a pH of 5.5 for a minimum of 30 seconds and a maximum of 1 minute.[1]

The effects of MES on fresh bovine semen to be used for *in vitro* fertilization, artificial insemination or cryopreservation are unknown.

Thirty ejaculates were collected and half of each was assigned to a control (Co) or to a treatment group (Rx) respectively; evaluations were performed after MES treatment. Aliquots from Co and Rx were frozen (Cryo), and evaluated post-thaw for motility, acrosomal- and plasma membrane integrity. In the treatment group a fresh semen aliquot was added to MES buffered saline (pH 5). After 60 seconds 20ml of PBS were added to restore the pH (pH 7). Acrosomal- and plasma membrane integrity were evaluated by mean of Eosin-Nigrosin (E/N) and FITC-PNA stains. There was no difference in the progressive motility of fresh (Fr) semen from Co (70.74 ±1.78) & Rx (65.54 ±1.78) groups ($p>0.05$). Post thaw the motility of Cryo-Co (37 ±2.63) & Cryo-Rx (22.5 ±2.63) differed ($p<0.05$). Evaluation of Live/Dead spermatozoa on eosin-nigrosin (E/N) smears differed between Fr-Co (157.42 ±4.42) & Fr-Rx (125.91 ±4.42) and between Cryo-Co (120.36 ±4.96) & Cryo-Rx (101.45 ±4.96) groups ($p<0.05$). FITC-PNA smears evaluated for acrosomal integrity showed no difference between any of the groups or within any of these groups ($p>0.05$). However E/N smears did show a difference in the percentage of smooth and ruffled acrosomes in both Fr-Co (155 ±3.11) & Fr-Rx (128.36 ±3.11) and Cryo-Co (110.12 ±4.67) & Cryo-Rx (94.96 ±4.67) groups ($p < 0.05$).

It is concluded that semen can survive this treatment although it has a detrimental effect. The main damage of the treatment occurred on the sperm membranes; therefore the effect was worse in frozen thawed semen, where the membranes were exposed to the additional stress of freezing and thawing, than in fresh semen. The described method can possibly be refined, e.g. shorter exposure time or a different pH, to reduce the detrimental effect it has on sperm membranes. It is likely that semen treated with MES can be used for IVF or AI, but this needs to be confirmed with additional trials.

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The value of reproductive tract scoring as a predictor of fertility and production outcomes in beef heifers

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In this study 272 beef heifers (Bovelder breed) were studied from just prior to their first breeding season (median age 431 days), through their second breeding season and until just after they had weaned their first calves in March 2005.

Reproductive tract scoring (RTS) by rectal palpation was performed one day before the onset of the first breeding season. Scores were allocated using the 5-point scoring system described by Anderson et al.[1] that uses a combination of diameter and tone of the uterine horns, and size and structures of the ovaries. The effect of RTS on several reproduction and production outcomes was tested, and the results are summarised in the table below (The Chi-square test, ANOVA and Kruskal-Wallis one-way ANOVA were used to compare proportions, means and medians respectively).

		Days to first AI	Pregnancy rate to AI	Days to calving	Weaning mass (calf)	Pregnancy rate to subsequent AI
RTS	**n**	**median**	**%**	**median**	**mean (kg)**	**%**
1	16	6^{ab}	31^{a}	53.5^{ab}	194^{ab}	63^{ab}
2	70	11^{b}	40^{a}	52^{a}	186^{b}	61^{b}
3	81	8^{ab}	53^{a}	28^{bc}	213^{ac}	72^{ac}
4	74	8^{a}	70^{b}	15^{c}	207^{ac}	85^{ac}
5	30	6^{a}	80^{b}	18^{c}	213^{ac}	90^{ac}

^{abc} Superscripts that differ: $P < 0.05$
n Numbers at onset of trial

The association of RTS with the outcomes was compared to the associations of other input variables such as mass, age, body condition score (BCS) and Kleiber ratio (growth rate/mass$^{0.75}$) using multiple or univariable linear or logistic regression.

RTS was associated with pregnancy outcome to the 50 day AI season ($P < 0.01$), days to calving ($P < 0.01$), calf weaning mass ($P < 0.01$) and pregnancy rate to the subsequent breeding season ($P < 0.01$). These associations were mostly independent of associations with mass, age and BCS before the onset of the first breeding season. RTS was a better predictor of fertility than was Kleiber ratio, and similar in its prediction of calf weaning mass.

It was concluded from this study that RTS is a unique predictor of heifer fertility, compares well with (but is independent of) other traits used as a predictor of production outcomes and is likely to be a good predictor of production in the cow.

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The isolation and characterization of a *Babesia bovis* stock for potential use in immunization

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Cattle farming forms an important part of South Africa's agricultural industry and is at risk of incurring severe losses due to babesiosis caused by either *Babesia bovis* or *B. bigemina*. Current vaccination against babesiosis makes use of a live blood vaccine, an approach that has a number of limitations. Consequently research is focused on developing a safer more reliable recombinant vaccine. To be able to identify candidate genes for use in vaccines, the microaerophilous stationary phase (MASP) culture technique is used. This technique allows for the *in vitro* cultivation of *B. bovis* parasites for prolonged periods of time facilitating the isolation of secreted protein antigens with potential protective properties.

This study focused on farms in the Swartberg region of KwaZulu-Natal, where, despite vaccinations, outbreaks of babesiosis still occur. It was important to determine whether a field isolate was responsible for these outbreaks or if it was a case of vaccine failure. A polymerase chain reaction (PCR) test based on two single copy variable *B. bovis* genes (Bv80 and BvVA1 genes) was used to discriminate between *B. bovis* vaccine or field isolates using the size differences between amplified PCR products. Bv80 PCR products were sequenced to determine the relationship between size difference and sequence variation. In addition to this, the 18S rRNA V4 hypervariable region was sequenced for each strain to ascertain if changes in the Bv80 sequences were reflected in changes in the 18S rRNA gene sequences.

The Bv80 variable region profiles created for a number of South African *B. bovis* strains indicated that the vaccine strain was not responsible for the outbreaks being experienced in the region. This result was confirmed by the 18S rRNA gene analysis, alignments of the V4 hypervariable region showed a high degree of sequence homology with variations indicating strain differences.

The use of these field strains to create a laboratory adapted *B. bovis* strain and thus to develop a MASP technique suited to South African strains is a high priority. The adaptation of the isolated Swartberg field strain as well as other field isolates could provide protective antigens required for the development of a recombinant *B. bovis* vaccine. *In vitro* cultivation of the vaccine and field strains according to published methods yielded limited success. The use of bovine serum supplemented medium did not support *B. bovis* growth at all whilst equine serum supplemented medium supported growth for a limited period of time only. Preliminary results using ALBUMAX® supplemented medium have shown promising results thus far. This adaptation of the Swartberg strain is the first step in the isolation of potentially protective antigens to be used for the development of a recombinant vaccine against bovine babesiosis.

Variable number of tandem repeat genotyping of *Mycobacterium bovis*: evaluation of the epidemiological relevance and comparison with IS*6110* RFLP and spoligotyping

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Transmission routes of the pathogen *Mycobacterium bovis* remain unclear for many animal and human disease cases. A major limitation is the lack of sufficiently informative or epidemiologically well evaluated molecular methods for typing like IS*6110* RFLP and spoligotyping.

Here, we report an evaluation of a MIRU-VNTR high-throughput method based on 29 MIRU-VNTR loci to genotype 127 *M. bovis* isolates from cattle of 77 different Belgian farms, representative from a nation-wide collection obtained from 1995 to 2003.

MIRU-VNTR stability was demonstrated by analyzing a series of 72 isolates in total, obtained from different animals from a same farm or from different farms with an identified epidemiological link. The genotyping results and the genotypic diversity (h) were compared with those obtained by IS*6110* RFLP and spoligotyping. Among 68 isolates with no known epidemiological link, MIRU-VNTR typing discriminated better than RFLP and spoligotyping, taken individually (32 vs 16 and 17 genotypes; h=0.91 vs 0.73 and 0.85, respectively) or in combination (32 vs 28 genotypes; h=0.91 vs h=0.92). The maximal resolution was already achieved with a subset of 9 loci.

The observed congruence of the genetic relationships between IS*6110*-RFLP, spoligotyping and MIRU-VNTR markers is consistent with a clonal population structure of *M. bovis*. These results support MIRU-VNTR typing as a convenient and discriminatory technique to analyse the population structure of *M. bovis* in much greater details, and to address some still unsolved issues in the epidemiology of the pathogen.

A census survey of Western Cape ostrich farms to identify risk factors associated with seropositivity to H5 avian influenza virus

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Avian influenza (AI) is a growing public health concern. Highly pathogenic AI (HPAI) has only been associated with H5 and H7 AI virus subtypes and these are the only subtypes notifiable to the World Organisation for Animal Health (OIE). After the July 2004 isolation of H5N2 HPAI virus from ostriches (*Struthio camelus*) in the Eastern Cape Province, a national serological survey was conducted until May 2005. It was found that 16.3% of ostrich farms in the Western Cape Province of South Africa were seropositive to H5 AI virus, resulting in a year-long ban on exports.

In order to identify risk factors associated with farm-level seropositivity, a census survey was performed by means of a questionnaire. The study population consisted of all export registered ostrich farms in the Western Cape Province that were still registered at the end of 2005 (435 farms, of which 367 were available for questioning). For each farm, information was collected on the ostrich population, movements of birds, management practices, and frequency of contact between ostriches and various wild bird species. Logistic regression models were developed for the whole province and also for the two largest ostrich farming regions, "Klein Karoo" and "Southern Cape", in order to identify statistically significant risk factors.

Of the 367 farms surveyed, 82 (22.3%) had been classified as seropositive during the 2005 survey. Seroprevalence differed between areas, being highest in the Klein Karoo (31.6%). In all three models, increased risk of farm-level seropositivity was associated with increasing numbers of ostriches (excluding chicks) on the farm. Increased risk of seropositivity was associated with reduced frequency of cleaning of feed troughs (<1x/week vs. >1x/week), both overall (odds ratio (*OR*) = 4.49, $P = 0.007$) and in the Southern Cape (*OR* = 53.6, $P = 0.005$), and with failure to clean and disinfect transport vehicles, both overall (*OR* = 2.28, $P = 0.03$) and in the Klein Karoo (*OR* = 2.62, $P = 0.04$). Increased risk of seropositivity was also associated with increasing frequency of contact of ostriches with certain wild bird species: overall with white storks (*Ciconia ciconia*), in the Southern Cape with gulls (*Larus* spp.), and in the Klein Karoo with Egyptian geese (*Alopochen aegyptiaca*).

Previous studies have suggested that outbreaks of AI in ostriches may have originated in wild waterfowl, via faecal contamination of feed or water. The results of this study are consistent with this theory, but also suggest that mechanical transmission may occur via contaminated transport vehicles. Proper management of feeding (and possibly also watering) troughs in order to reduce faecal contamination by wild birds, as well as biosecurity measures to reduce mechanical transmission between premises, may reduce the risk of seropositivity.

The occurrence of antimicrobial drug resistance in enteric bacteria from exposed poultry abattoir workers and broilers fed antimicrobial growth promoters

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The usage of antimicrobials in food animals, such as chickens, increases the prevalence of antimicrobial drug resistance among enteric bacteria of these animals and exposed humans. In this study antimicrobial drug resistance was investigated to selected enteric bacteria from broilers raised on feed supplemented with antimicrobial performance enhancers (AMPE), and the people who carry out evisceration, washing and packing of intestines in a high throughput poultry abattoir in Gauteng, South Africa.

Six farms were chosen and from each of these farms, 100 broilers were randomly selected five minutes after slaughter and their caecae collected aseptically. The contents were then selectively cultured for *Escherichia coli*, *Enterococcus faecium*, *E. faecalis*, and vancomycin-resistant enterococci (VRE). The minimum inhibitory concentration (MIC) micro broth dilution test as prescribed by the Clinical and Laboratory Standards Institute, USA was used to determine the susceptibility of the isolates to the following antimicrobials: vancomycin, virginiamycin, doxycycline, trimethoprim, sulphamethoxazole, ampicillin, bacitracin, enrofloxacin, erythromycin, fosfomycin, ceftriaxone and nalidixic acid. The same was done on faeces of 27 abattoir workers exposed to potentially resistant microorganisms from broilers and 28 persons used as controls, who had not been equally exposed to potentially resistant microorganisms from broilers. Both of the human populations had not been treated with antimicrobials three months prior to sampling. Statistical analysis was done by the Fishers exact test.

No salmonellae were cultured. A total of 168 *E. coli*, 20 *E. faecalis* and 96 *E. faecium* from the broiler caecae and 54 (28 and 26) *E. coli*, 25 (21 and 3) *E. faecalis* and 12 (2 and 10) *E. faecium* from humans were subjected to antimicrobial susceptibility testing. The figures in brackets represent isolates from the abattoir workers and human controls respectively. The majority of *E. coli* isolates from broilers had MIC values that were considered to be resistant to most of the antimicrobials tested. Low resistance was observed among broiler enterococci isolates to vancomycin, virginiamycin, trimethoprim and ampicillin. Although abattoir workers had higher median MICs to a number of antimicrobials than the control persons, a significant difference was only observed for the following antimicrobials: for *E. faecalis*: enrofloxacin ($p = 0.019$), for *E. faecium*: trimethoprim ($p = 0.01$) and enrofloxacin ($p=0.029$), while in the case of *E. coli*: trimethoprim ($p = 0.012$) and ampicillin ($p = 0.036$). The study confirms that people working with animals fed AMPE such abattoir workers tend to carry a higher level of resistance. It could not, however, be positively concluded, that abattoir workers were at a higher risk of acquiring resistance than persons not associated with the poultry industry.

Neurocysticercosis: A possible cause of epileptiform fits in people residing in villages served by the Bethanie clinic in the North West Province

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A study to detect human taeniasis and cysticercosis was conducted in four village communities served by the Bethanie clinic in the North West Province. It was selected because of reports of people being diagnosed with epileptiform episodes. The total population of the four villages is estimated at 13 947 and many house holders rear pigs in small numbers for both meat and an immediate income.

The primary aims of the work were to conduct a survey of all small scale pig producers and a census of rural village consumers in the study area – both by means of structured questionnaires. In the former, to review pig husbandry practices, slaughter and marketing of pigs and in the latter, to provide information on pork consumption, sanitation as well as people's knowledge of *T. solium*. From the questionnaires the total number of patients with recorded seizures in the study area, within the selected time frame were determined. Stool samples from consenting participants were screened for *T. solium*. A descriptive analysis of retrospective data was conducted to determine the proportional morbidity of neurocysticercosis from the medical records of patients diagnosed with seizures in an attempt to establish possible sources of infection and routes of transmission.

Secondary objectives were to determine more accurately the total pig population in the study area and to determine the prevalence of cysticercosis in pigs through inspection of those slaughtered at an approved abattoir – surprisingly all found to be negative.

The questionnaires revealed a poor understanding of the disease, poor sanitation and hygiene, poor methods of pig husbandry and poor meat inspection and control in rural smallholder communities. There was no significant statistical difference in the proportion of households reporting evidence of epilepsy, between those who owned pigs and those that did not. The incidence of high risk behaviour is common, and there is a strong evidence of a tendency towards an association between epilepsy, consumption habits and various epidemiological factors which were considered as possible risk factors. The fact that no taenia proglottids were found in the faecal samples collected is elaborated on. It is considered unlikely, but possible that the consumer/farmer information days played a significant role in the outcome of this study.

Microscopic comparison of mammalian blood platelets

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Blood platelets are the main mediators of haemostasis. Human blood platelets have been well characterised in contrast to those of other mammalian species, especially wildlife. The aim of this study was to provide comparative morphological information on blood platelets in selected mammalian species and man.

Blood samples were taken from healthy humans and animals (impala, cheetah, black rhinoceros and elephant) in accordance with accepted ethical procedures. Smears for light microscopy were made at the time of blood collection while samples for scanning and transmission electron microscopy (SEM and TEM) were processed within an hour after collection using standard methods.

Light microscopy showed the platelets of the various species to be pleomorphic with granules scattered throughout the cytoplasm. Occasionally pseudopodia were noted. Platelets from different species varied in size with those of impala the smallest and the cheetah's the largest. With SEM, human and cheetah platelets appeared similar as bilaterally flattened discs, but the cheetah platelets were bigger in design. Those of the impala were small with biconvex sides. The black rhinoceros sample displayed two types: small oval discs and a few large putative proplatelets. Elephant platelets displayed no distinctive shape and had numerous pseudopodia. TEM showed the platelets as discoid-shaped cells containing granules and various organelles throughout the cytoplasm. Alpha-granules were present in all species but differences in size, shape, number and electron density were observed. Dense bodies were rare in human platelets. Microtubules were present in all except the elephant platelets. The surface connecting canalicular system (SCCS) appeared well-developed in humans and cheetahs, poorly developed in the black rhinoceros and absent in both impala and elephant platelets. Glycogen granules were present in all species studied.

The blood platelets of the different species appeared similar when viewed by light microscopy and SEM. However, TEM revealed differences in the type and structure of the characteristic platelet cytoplasmic organelles (alpha-granules, microtubules, SCCS) which suggests that the different species use diverse mechanisms to fulfill their basic functions in haemostasis.

Serial daily blood lactate concentrations in puppies with parvoviral diarrhoea

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Elevated blood lactate concentrations have been shown to predict mortality in many human (septic- and circulatory shock) and canine conditions (gastric dilatation/volvulus and canine babesiosis caused by *B.canis rossi*). However, the initial concentrations and serial changes in blood lactate concentrations in puppies with parvoviral diarrhoea and their association with mortality have not been studied.

A prospective study was undertaken to determine the serial daily blood lactate concentrations of puppies admitted to the high care isolation ward at the Onderstepoort Veterinary Academic Hospital (OVAH) with severe parvoviral diarrhoea and observed the associated mortality. Sixty-three client-owned puppies were studied. The diagnosis of parvoviral diarrhoea was confirmed by the detection of viral particles by faecal electron microscopy. Blood samples were taken prior to treatment and daily thereafter until discharge or death. Blood lactate concentrations were determined by using the Accutrendâ analyser. Normal canine reference range was taken as 0.5 – 2.5 mmol/L. Lactate concentrations were compared between survivors (n=50) and non-survivors (n=13) using the independent samples T-test for parametric data. Laboratory data is presented as mean ± standard deviation. Significance was set at $p < 0.05$.

Overall mortality was 21% (13/63). Median age and bodyweight was 4 months and 5 kg respectively. Median duration of illness prior to admission was 3 days. Mean blood lactate concentration on the day of admission (day 1) was 3.1 mmol/L (± 1.55) for the whole cohort. Blood lactate concentrations decreased markedly to mean concentrations of 1.1 mmol/L (± 0.16) by day 7 in all dogs and especially in survivors. Mean day 1 blood lactate concentration was not significantly different at 3.94 mmol/L (± 2.32) in the non-survivors versus 2.87 mmol/L (± 1.2) in the survivors ($p = 0.135$). Mean lactate concentrations were consistently higher in non-survivors, but this difference failed to achieve significance between the two groups on day 2 or 3 ($p = 0.225$ and $p = 0.460$ respectively), even though the paired samples T-test showed that day 1 and day 2 lactate concentrations in the non-survivors did not decrease significantly over time ($p = 0.269$).

Blood lactate concentrations were generally lower in pups with parvo viral diarrhoea compared to other canine critical illnesses. Lactate concentrations showed a definite trend to be higher in non-survivors than in survivors of severe parvoviral illness. However, these concentrations failed to remain as significantly elevated beyond day 2 and up to death in non-survivors, as was the case in the *B. canis rossi* model of canine babesiosis. Further studies in a larger group of pups are necessary to prove or refute the association between blood lactate concentrations and mortality in parvoviral diarrhoea, as the relatively small sample size in this study may have led to a type II error (i.e. failure to reject the null hypothesis of no difference).

Serial plasma glucose concentrations in canine bite wound cases

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Dogs with severe dog bite wounds are generally admitted in a state of shock, with high sympathetic tone and resultant high glucose counter-regulatory hormone (adrenaline, glucagon, cortisol and growth hormone) concentrations. Hypermetabolism follows the ebb phase after injury and results in hyperlactataemia and hyperglycaemia due to glucose intolerance and insulin resistance. Conversely, in severe sepsis, hypoglycaemia can occur due to decreased glucose production, gram-negative bacterial endotoxin or increased utilization.

We hypothesized that a high incidence of hypoglycaemia would be found in severe dog bite wound patients. This study aimed to describe the serial changes in plasma glucose concentrations and document the incidence of hypo-, normo- and hyperglycaemia over a 72 hr period since trauma, in canine bite wound cases admitted to the Onderstepoort Veterinary Academic Hospital.

Twenty dogs with dog bite wounds, whose clinical condition warranted intravenous fluid therapy and admission to the Intensive Care Unit, were recruited. Relevant historical, signalment and clinical data was recorded. Blood was taken at admission for haematology and plasma glucose concentrations. Glucose was repeated in 8 hr increments and haematology in 24 hour increments since time bitten. Blood was collected into EDTA for haematology and evacuated Sodium Fluoride Oxalate tubes for plasma glucose determination. Analysis of glucose was performed in a single batch using the hexokinase method. The primary investigator was blinded to the plasma glucose concentrations for the duration of the study. No glucose containing fluids were administered to any of the dogs and alpha-agonist drugs such as medetomidine (Domitor®, Novartis) that could potentially have affected plasma glucose concentrations were avoided. The median glucose for each category of a variable was compared using Kruskal-Wallis one-way ANOVA on ranks. Significance was set at $p < 0.05$.

At admission, 5% of the dogs (1/20) were hypoglycaemic, 40% (8/20) normoglycaemic and 55% (11/20) hyperglycaemic. No other dog showed hypoglycaemia (<3.3 mmol/l). The median glucose concentrations were in the hyperglycaemic range (5.5 mmol/l – 6.3 mmol/l), at 8/10 collection points, excluding the 56 hr and 64 hr collection points, which showed normoglycaemia (3.3 - 5.5 mmol/l). Puppies and thin dogs had significantly higher median plasma glucose concentrations than adult and fat dogs at 0 and 16 hours, respectively ($P < 0.05$ for both). Fifteen dogs survived the 72 hr study period. Three out of 4 dogs admitted as collapsed, died, whereas all dogs admitted as either alert or depressed, survived ($P = 0.004$).

The low incidence of hypoglycaemia was surprising. The high incidence of mild hyperglycaemia is consistent with the findings in human critical care and constitutes the first evidence of serial hyperglycaemia in critically ill canine bite wound cases. The high death rate in the collapsed group and the higher plasma glucose concentrations found in puppies and thin dogs warrant investigation with a larger group of animals.

Assessment of adrenal function in cheetah (*Acinonyx jubatus*)

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It has been proposed that stress could play a major role in the development of helicobacteriosis-associated gastritis in captive cheetah. Captive cheetahs have statistically significantly higher baseline faecal corticoid concentrations and show adrenal cortical hypertrophy as compared to free-ranging cheetahs. Faecal steroid concentrations are influenced by intestinal transit time and time until faeces is collected prior to processing. Thus a more direct method of adrenocortical assessment, such as an ACTH stimulation test, would be advantageous.

The aims of this study were to determine: 1) serum cortisol concentrations in a group of clinically normal captive cheetah, 2) whether a standard dose of 500 ug of ACTH produces a consistent stimulation of the adrenal gland, and 3) the optimum time interval between the collection of the baseline and the peak post-stimulation blood sample.

A formulation of synthetic ACTH available in South Africa was tested, with a dosing protocol used previously in domestic cats. The serum cortisol response was assessed in eight captive cheetahs, of varying ages, after the intravenous administration of 500 µg (11.1 to 33.3 µg/kg) of tetracosactide (Synacthen Depot®) while maintained under general anaesthesia using Tiletamine/Zolazapam (Zoletil®) at 5.5 mg/kg intramuscularly (ACTH group). Additionally, eight control cheetahs were subjected to an intravenous saline placebo treatment in order to establish baseline cortisol concentrations at similar times of anaesthesia (control group). Data distribution was non-parametric. Within-group data was analysed by the Friedman's test for related samples and between-group time points were compared using the Mann-Whitney U test. Values are expressed as median and interquartile range.

Median basal cortisol for the whole group was 120 nmol/l (78-173) and did not differ between groups ($p = 0.248$). There was no significant difference in the median cortisol concentration between any of the timepoints in the control group ($p = 0.238$). There was, however, a significant overall difference between the median cortisol concentrations of the time points following ACTH administration in the ACTH group ($P < 0.01$). The peak median cortisol, which occurred at 180 minutes, was 769 nmol/l (532 – 927) and this peak did not differ significantly from the median cortisol concentrations at time points 120 and 150 minutes after ACTH stimulation ($p = 0.867$).

Although there appeared to be inter-cheetah variation in the timing of the peak cortisol response, this study found that cheetah should be sampled somewhere between 120 and 180 minutes after intravenous ACTH administration to obtain a serum cortisol peak that should be in the region of 700 nmol/l, which is considerably higher than in the domestic cat.

Survey on filariosis of domestic cats in KwaZulu-Natal, South Africa

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The objective of the survey was to determine the occurrence and prevalence of filarial helminths in domestic cats in the coastal areas of KwaZulu-Natal. Filarial helminth species reported from domestic cats in Africa are *Dirofilaria repens* and *Brugia patei*. Of these only *D. repens* is currently known to be endemic in South Africa where infection is commonly diagnosed in dogs in the coastal areas of KwaZulu-Natal. Originally regarded as apathogenic, *Dirofilaria repens* has been recognized as a possible cause of pruritic dermatitis in cats and dogs and has also been incriminated in causing immunologic haemolytic anaemia in dogs. Humans can act as accidental hosts with over 400 cases reported worldwide.

Blood samples from 81 cats brought to private practices in selected towns and cities in the coastal areas of KwaZulu-Natal, namely Mtubatuba (8), Empangeni (2), Durban (64), Scottburgh (3) and Port Shepstone (4), were drawn into evacuated EDTA tubes and initially analysed by membrane filtration for the presence of microfilariae. Species identification was based on the somatic distri-bution of acid phosphatase activity in histochemically stained microfilariae concentrates.

Of the 81 blood samples examined for microfilariae, nine samples (11.1%) were positive on membrane filtration: Mtubatuba (2), Durban (5), Scottburgh (1), Port Shepstone (1). The pattern of the acid phosphatase staining activity revealed *D. repens* as the only species involved.

This is the first attempt to determine the prevalence and aetiology of filariosis in cats in South Africa.

Effect of altitude on venous proinflammatory cytokine production in Thoroughbred racehorses with exercise-induced pulmonary heamorrhage

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Horses performing strenuous exercise frequently develop exercise-induced pulmonary haemorrhage (EIPH). Intrapulmonary blood accumulation causes small airway inflammation and may impair gaseous exchange. Bronchoconstriction may develop secondary to the bronchiolitis leading to increased pulmonary resistance and exacerbation of EIPH. We have recently demonstrated that racehorses have increased prevalence and severity of EIPH at sea level compared to high altitude. Intra-pulmonary pro-inflammatory cytokines may modulate pulmonary inflammation and enter the venous bloodstream. Our aim was to identify venous inflammation by measuring cytokine mRNA expression in racehorses with EIPH in South Africa, a racing jurisdiction that does not permit race day administration of furosemide, and in which horses race at both sea level and at high altitude.

A prospective, cross-sectional study of pre-enrolled Thoroughbred racehorses competing in flat races at high altitude (1,450 m above sea level) and at sea level was performed between 1 August and 19 November 2005. After tracheobronchoscopy was performed within 120 minutes post-race, the presence and severity of EIPH was graded (0 to 4)[1] and venous blood collected from 10 horses in each grade classification at both altitude and sea level. Blood collection was repeated 72 h later.

Following RNA isolation and cDNA synthesis by reverse transcriptase, real-time PCR was used to detect equine cytokine-specific mRNA for interleukin (IL)-1, 6 & 10, interferon (INF)-γ, and tumor necrosis factor (TNF)-α. Refer to the primers and or methods used by publications. Data was normalized to the RQ of the grade 0 EIPH samples. Data was analyzed using Spearman's test, ANOVA and Holm-Sidak t-test for multiple comparisons. Significance was set at $p < .05$.

Compared to racing at altitude, horses racing at sea level had increased proinflammatory cytokine expression ($p < 0.001$) especially horses with a grade 3 vs. 2 and 0 EIPH ($p < 0.05$). Racehorses at sea level with a grade 4 vs. 0, 1 and 2 EIPH expressed increased IL-6 ($p < 0.05$) while greater IL-10 expression was present in horses with grade 3 vs. 0 and 2 EIPH ($p < 0.05$). Overall, there was greater expression of IL-6 ($p = 0.046$) and TNF-α ($p = 0.005$) at sea level which increased with increasing severity of EIPH.

Racing at sea level seems to be associated with increased inflammatory cytokine mRNA production in venous blood as compared with high altitude.

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New perspectives on the bacteriology and antimicrobial susceptibility of the bacteria of infected and non-infected dog bite wounds

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In spite of dog bite wounds being a common reason for dogs requiring veterinary care, there is surprisingly little data on the bacteriology of bite wounds. Thus, a prospective study was performed on dogs with various grades of bite wounds presenting at the Onderstepoort Veterinary Academic Hospital, University of Pretoria, and a nearby animal shelter.

Fifty dogs with bite wounds inflicted within the previous 72 hours were selected. This represented 104 wounds. Wounds were clinically graded according to severity and evaluated cytologically. Wounds were classified as infected or non-infected and swabs were collected from all wounds for bacterial culture. Infection was diagnosed if 2 of the following 3 criteria were met: macroscopic purulence, phagocytosed bacteria present or if the wounded dog had pyrexia. Non-infected wounds were either sterile (established by culture) or contaminated (culture positive but bacteria not phagocytosed on cytology). Swabs from all wounds were cultured aerobically and anaerobically and all aerobic cultures were evaluated for antimicrobial susceptibility using the Kirby Bauer disk diffusion test.

The victims were predominately male, uncastrated, small breeds. Of the 104 wounds, 21 were judged to be infected and 83 non-infected. Seventeen (16%) of all wounds that were sterile were also classified as non-infected. This is statistically significant ($P = 0.02$). Of the 84% that were culture positive, 16% grew aerobes, 1% anaerobes and 67% a mixture of aerobes and anaerobes. A total of 211 isolates were cultured representing a mean of 2.1 isolates per wound. The aerobes cultured belonged to *Pasteurella* (22%), *Streptococcus* (20%) and *Staphylococcus* (17%) species, respectively. Within these groups, *Pasteurella multocida* (65%) and *Staphylococcus intermedius* (70%) were predominant. *Pasteurella canis* and pyogenic streptococci were common in infected wounds, whereas *Bacillus* spp., *Actinomyces* spp. and the oral streptococci were generally found in contaminated wounds. Three anaerobic genera were cultured, namely, *Prevotella*, *Clostridium* and *Peptostreptococcus*, and were usually associated with wounds with dead space. This was also the first documented case of *Capnocytophaga canimorsus* in an infected dog bite wound.

Clinical examination and cytological assessment were capable of establishing whether antibiotics were required or not. Although no single antibiotic was considered to be effective against all the bacteria, *in vitro*, potentiated sulphonamides, ampicillin and amoxycillin plus clavulanic acid gave the best results.

Mapping of antigenic sites on a SAT2 foot-and-mouth disease virus using a chicken antibody phage display library

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Of the seven distinct foot-and-mouth disease virus (FMDV) serotypes that exist, the South African Territories (SAT)-types, occurring in sub-Saharan Africa, show the greatest genetic and antigenic variation. The variation is geographically linked giving rise to topotypes within serotypes. Vaccines prepared from one topotype may provide only very specific protection within the same topotype and not across topotypes. Efficient FMD control, using vaccination, relies on discerning if the immune response elicited by a vaccine strain will protect against viruses circulating in the field. Current laboratory tests used to determine the protective nature of a vaccine strain are not only time-consuming but also hampered by the lack of knowledge concerning the neutralising antigenic determinates of the SAT viruses.

In this project the antigenic sites on the capsid of a SAT2 FMDV vaccine strain were determined by selecting antibody single chain variable fragments (scFv) showing affinity to the virion. Those scFvs that recognise neutralising antigenic sites will be utilised in an ELISA to rapidly determine whether the vaccine strain will protect and neutralise incoming field isolates.

The SAT2 vaccine strain, ZIM/7/83, was used for the selection of scFv binders from the naïve Nkuku chicken phage display library in five selection steps. Enrichment was confirmed via polyclonal and monoclonal phage ELISAs using populations of phages produced at each selection. Characterisation of individual phage clones from selections four and five revealed 40-61% of the bacterial clones tested secreted fusion phages, while 26-41% of the clones secreted soluble scFvs that specifically recognised the ZIM/7/83 antigen. Of the 85 clones selected and sequenced, three unique full-length clones were obtained, designated Binders 1, 2 and 3.

Studies were performed to determine the stability of the three binders both in small and large scale expression and differences were observed between the respective binders. The specificity, as well as the cross reactivity of Binders 1 and 2 to the other FMDV serotypes was tested using an ELISA. Both reacted only to the antigen and its parental virus with no reactivity to viruses from SAT-1 and SAT-3. Investigations are currently underway to determine if Binders 1 and 2 are recognising neutralising epitopes.

Genetic diversity of South African *Theileria parva* isolates

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Corridor disease is the most important form of cattle theileriosis in South Africa today after the eradication of East Coast fever (ECF) in the early 1950s. However, there is a concern that *Theileria parva* parasites that could cause ECF may still exist in the African buffalo reservoir host in South Africa. The presence or the absence of a 130 bp insert in the p67 gene is thought to distinguish between cattle- and buffalo-associated *T. parva* isolates in East Africa. The aim of this study was therefore to determine the p67 gene diversity of South African *T. parva* isolates in African buffalo and cattle.

Cattle and buffalo blood samples were collected from different areas in South Africa. Primers were designed for PCR amplification of the variable regions of the p67 gene. The sizes of the p67 amplicons were determined by agarose gel electrophoresis. Selected amplicons from different isolates were purified, cloned into a plasmid vector and sequenced.

In this study, up to four PCR products (ranging in size between 850 and 1200 bp) were obtained from isolates investigated. Four p67 profiles were identified including a "single", "double", "three" and "four" band profile. Sequence analysis revealed p67 gene sequences with a 130 bp insert and sequences without the insert, similar to the buffalo- and cattle-associated sequences, respectively, as already reported from East Africa. In addition, two novel p67 sequence variants were identified. Phylogenetic analysis of p67 gene sequences revealed three major groups and sequence variations within the insert were noticed between the three major groups.

Both large and small p67 PCR products were obtained from many of the buffalo-associated *T. parva* isolates in this study. Sequence analysis revealed that the smaller products lacked the 130 bp insert. The p67 sequence data from these isolates confirm the results previously obtained from two isolates from buffalo from the Kruger National Park which had p67 genes which lacked the insert. This indicates that the presence or the absence of the insert cannot be used to differentiate between cattle- and buffalo-associated *T. parva* isolates in South Africa, since many of the isolates that lacked the insert in p67 caused classical Corridor disease in cattle. However, it will be important to further examine the relevance of the various p67 genotypes in the epidemiology of theileriosis in South Africa.

Although the p67 gene profiles appear to be more complex than previously thought and probably cannot be used to distinguish between cattle- and buffalo-associated isolates in South Africa, these profiles may assist in tracking *T. parva* infections in transmission experiments.

Towards a recombinant vaccine for heartwater

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Ehrlichia ruminantium, the causative agent of heartwater, is an obligate intracellular pathogen and, as such, cell mediated immunity plays a key role in the control of bacterial replication and subsequent protection against the disease. The cellular responses induced in animals after vacci-nation can be measured by the secretion of cytokines such as interferon gamma (IFN-γ) and interleukin-4 (IL-4), which are strong indicators of Th1 (T helper 1 cells) and Th2 responses respectively. Of particular relevance to this disease, IFN-γ has been shown to be an inhibitor of *E. ruminantium* growth on endothelial cells *in vitro.* Thus, identification of antigens that preferentially activate T cells to proliferate and secrete IFN-γ need to be evaluated as vaccine candidates.

We have identified a cocktail of four open reading frames (administered as a DNA vaccine in sheep) that induce 100% protection and production of the cytokine IFN-γ, after needle challenge but only 20% protection after tick challenge in the field. Because only limited protection was obtained during this field vaccine trial our research is focused to improve this efficacy. Since secreted pro-teins are also reported to be major targets in the specific immune response we hypothesise that they may be potential heartwater vaccine candidates.

With the aid of bioinformatics tools five ORFs were selected encoding secreted *E. ruminantium* proteins from the Welgevonden stock genome sequence. The corresponding recombinant proteins were expressed in a bacterial expression system and assayed to determine whether they induce recall cellular immune responses *in vitro.* The peripheral blood mononuclear cells used in the assays were obtained from a naïve and heartwater immune sheep.

Only four recombinant proteins could be expressed and significant proliferative responses ($p \leq 0.01$) were evident for 3/4 recombinant proteins. IFN-γ production was determined using an ELISPOT assay and 3/4 recombinant proteins also induced IFN-γ production. Each recombinant protein had its own optimum concentration for inducing immune responses and the responses differed between animals. Thus these three proteins that induce proliferation and IFN-γ production may be important in protection against heartwater and will be tested in future vaccine studies.

Investigating the role of cellular receptors in foot-and-mouth disease virus adaptation to *in vitro* growth

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Foot and mouth disease (FMD) is a highly infectious disease of livestock affecting all cloven-hoofed species. The causative agent, FMD virus (FMDV), is a positive sense RNA virus from the family *Picornaviridae*, genus *Aphthovirus*. Previous investigations into the molecular epidemiology of FMDV in sub-Saharan Africa have focused mainly on the SAT types. To provide some insight in the genome variation of other serotypes prevalent in Africa, FMDV A and O type viruses isolated from bovine from different countries and regions in Africa were selected for genetic characterization of the capsid-coding region (P1). The receptors that are expressed on vaccine production cell lines i.e. BHK21 and IBRS2 cells play an important role during the selection and adaptation of FMDV as vaccine strains. This selection is hampered due to insufficient knowledge regarding these receptors and the amino acid motifs on the virus capsid that bind to them. This led to investigations into the level of mRNA expression coding for the known FMDV receptors (αVβ1, αVβ3, αVβ6, αVβ8 and HSPG) on cells used locally.

Nine FMDV-O and eight type A viruses from four different topotypes respectively where selected. Following RNA extraction, RT-PCR was performed for all samples where the entire P1 region was amplified. Sequencing was performed via a genome walking approach and complete contigs were aligned using Sequencher™ 4.7 followed by phylogenetic analysis. To determine the mRNA expression levels for the FMDV receptors, PCR primers were designed which amplified the β1, β3, β6, β8 and HSPG gene regions in BHK21 and IBRS2 cells using an RT-PCR approach. The mRNA expression levels were determined with increasing cell passage levels for both cell lines. The intensity of the PCR product on the agarose gel was calculated using GelTrak virtual densitometer while normalizing results using the b-actin as a house-keeping gene. In addition, the susceptibility of both cell lines for FMDV infection at early and late passage levels was investigated using plaque titration assays.

Phylogenetic analysis of the complete P1 regions indicated similar clusters as was previously found with partial VP1 sequence data. Following alignment of the newly generated P1 sequences with published data, we identified hypervariable regions in the outer capsid proteins corresponding to or in proximity to previously identified immuno-dominant sites on FMDV A and O types. The hydrophilicity and deduced surface exposure were considered for each residue. The information gained from the characterization of the P1 sequences added to our knowledge of the genetic variation within different capsid-coding regions as well as the amino acid sequences in the areas implicated in virus entry. There were differences in the expression levels for the different receptors at increaseing passage levels. The susceptibility of BHK21 and IBRS2 cells for FMDV infection revealed variable results. It was found that these results could not be related to the receptor mRNA expres-sion levels.

Determination of buffalo and giraffe heart weights

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Giraffe brains require a blood perfusion pressure of 100 mmHg (as in all mammals). However, their heads are >2 m above the level of the heart. To overcome hydrostatic pressure generated by the column of blood between the heart and the head, their hearts generate an average blood pressure of *ca* 200 mmHg. As mean arterial pressure (MAP) = cardiac output (CO) x peripheral resistance (PR), this high pressure may be derived either from a high PR or from a high CO.

Giraffe and buffalo were compared and certain correlations made in respect of heart mass (Mh), body mass (Mb) and neck length (Nl) in an attempt to investigate the origin of the high MAP in the giraffe. If neck length is the origin of high blood pressure, heart mass (Mh) should correlate better with neck length (NL) than body mass (Mb). If high blood pressure results from PR then Mh should correlate better with Mb. Thus in giraffe Mh should correlate with NL but in buffalo it should correlate best with Mb.

Table 1: Comparison of Mb and Mh

Species	Mb (kg)	Mh (kg)	Predicted Mh (Kg)	Mh: Mb (%)
Giraffe (N=9)	914.9 ± 330.6	5.0 ± 1.7	4.6±1.6	0.55±0.05
Buffalo (N=32)	468.1 ± 145.8	2.0±0.6	2.4±0.7	0.44±0.04

The Mh:Mb ratio is significantly higher in giraffe than in buffalo – giraffe hearts therefore form a significantly larger proportion of body mass than they do in buffalo. For this sample size giraffe hearts cannot be shown to be significantly larger than the allometric prediction. However for the larger buffalo sample size, buffalo hearts are significantly smaller than predicted values.

Table 2: Values for CV variables predicted from allometric equations assuming that MAP is 100 mmHg

Species	CO (l.min^{-1})	HR (min^{-1})	SV (ml)	TPR mmHg.l^{-1}.min^{-1}
Giraffe	46.4 ± 14.0	44.9 ± 5.0	1070 ± 410	~ 2
Buffalo	27.0 ± 7.1	52.9 ± 5.7	530 ± 180	~ 4

Predicted cardiac output (CO) and stroke volume (SV) in buffalo are significantly smaller than they are in giraffe, while the predicted HR of buffalo is significantly higher than it is in giraffe but CO's in both species are what can be predicted from allometric analyses. Thus CO is not a contributor to high blood pressure in giraffe. PR(MAP/CO) of giraffe is lower than it is in buffalo. (assuming MAP is 100 mmHg). For a MAP of 200 mmHg giraffe TPR would be ~ 4 mmHg.$l$min^{-1}, (same as in buffalo). It is probable that the TPR in giraffe is the origin of the high MAP.

In both buffalo and giraffe changes in Mh correlate significantly with changes in Mb (product-moment correlation). Mh is also related to neck length, but the correlation is higher in giraffe than in buffalo. Thus while body mass can explain 92% of the variance in heart size in buffalo and 96% in giraffe, NL can explain 79% of Mh in giraffe and 64% of heart size in buffalo. The correlation between NL and Mb in giraffe is much higher than it is in buffalo indicating that increases in NL in giraffe are strongly linked to increases in Mb.

Correlation between tetracycline resistance in *Escherichia coli* isolated from impala (*Aepycerus melampus*) and *E. coli* isolated from their water source

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Although the acquisition of antimicrobial resistance (AMR) in common commensal and pathogenic bacteria is currently recognized as a worldwide problem in both human and veterinary medicine, there is little information available on the role that the environment and in particular drinking water plays in its spread. Thus a case control study was performed in the Kruger National Park, South Africa to find out whether the faeces of impala (*Aepycerus melampus*) were more likely to contain tetracycline resistant *Escherichia coli* (TR*EC*) when they drank from rivers that contained these bacteria compared to rivers that were uncontaminated with TR*EC*.

Five mainly perennial rivers, the Crocodile, Letaba, Olifants, Sabie and Sand Rivers were selected in the conservancy area of the Kruger National Park. A total of 11 points in these rivers were sampled on 3 separate occasions, namely March, April and June, and cultured for *E. coli*. The rivers were then grouped as containing TR*EC* or not. Between 5 to 10 fresh impala faeces was collected within 5 kilometres range of each water collection site for a total of 209 faecal specimens. Selective culturing of *E. coli* was done and the isolates were divided into resistant and sensitive to tetracycline using Ledenberg Replica Plating (LRP) method. Doxycycline was added at a concentration of 4 mg/*l* in the agar growth medium. Where present, a resistant and susceptible isolate was selected from each specimen, and subjected to the minimum inhibitory concentration (MIC) micro-broth dilution test for tetracyclines as prescribed by the Clinical and Laboratory Standards Institute, USA. The odds ratio (OR) was used to determine the comparative risk that impala exposed to TR*EC* in water would also carry TR*EC* in their faeces compared to that of unexposed impala.

Of the 33 water specimens examined, 63.63% were contaminated by TR*EC*. Among the 209 impala faeces sampled, 191 were positive for the presence of *E. coli* (91.38%). Within them, 35.6% had resistant *E. coli* based upon the LRP method, a relative high percentage if considered that these animals have never been treated with antibiotics. The OR was found to be 7.09 times greater in the faeces of impala drinking from TR*EC* contaminated rivers than unexposed impala.

It is concluded that antimicrobial resistance, specifically TREC, appears to be environmentally transmitted. This throws new light on the ecology of tetracycline resistance and the interaction between sewage of human origin and animals drinking from contaminated water sources, such as rivers.

Ultrastructure of the interstitial vasculature of the ostrich testis

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The macroscopic features of the arterial blood supply and venous drainage, as well as the distribution of the microvasculature (using light microscopy) of the testis of the ostrich, has been established. However, no information is available on the ultrastructural characteristics of these vessels and therefore of their function.

Testes samples from three sexually mature ostriches were processed for transmission electron microscopy using conventional techniques. Blood vessels located in the interstitial tissue were described and photographically recorded.

Numerous blood vessels were observed sandwiched between adjacent seminiferous tubules and at the interstices. Capillaries and larger vessels displayed similar morphological characteristics and it was difficult to distinguish between arterioles and venules as the endothelium of all vessels appeared to be surrounded by myofibroblasts and acellular elements of the interstitium. The endothelial cells rested on a prominent basal lamina. Very few pericytes (defined by their inclusion within the basal lamina of the blood vessel) were identified.

In most instances the endothelial cells were attenuated and formed complex interdigitations with neighbouring cells by means of slender cytoplasmic processes. Numerous similar processes also extended into the lumen of the blood vessels. The cytoplasm of the attenuated portions of the cells was moderately electron-dense and homogeneous in nature, with few organelles being observed. However, organelles were concentrated in widened parts of the cells, particulary in the vicinity of the cell nucleus. The most numerous organelles were free and poly-ribosomes, although short strands of rough endoplasmic reticulum, oval mitochondria, a small Golgi apparatus, lysosome-like structures and centrosomes were commonly encountered. Very few pinocytotic vesicles were observed and fenestrations could not be identified.

These results indicate that the interstitial blood vessels of the ostrich testes are of the continuous type and also confirm that the arrangement of the vessels is of the testicular type (adjacent seminiferous tubules share capillaries). The lack of fenestrations and the paucity of pinocytotic vesicles would also suggest that an active physiological exchange between the interstitial blood vessels and the seminiferous tubules is not as pronounced as that reported in the excurrent duct system (with the exception of the *rete testis*) of birds.

Present trends in udder health and emerging mastitogenic pathogens in South African dairy herds

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The aim of this study was to analyse results of milk samples obtained from mainly South African dairy herds from 1996 to April 2007, in order to identify possible trends in isolations of micro-organisms and their pathogenisity under field conditions.

A total of 379 000 milk samples were examined at the milk laboratory. Most of these samples were from herds rather than individual cases. Cytology and routine microbiology were performed on all samples. Results were analysed with the MSD program to assist veterinarians in applying these results in the field.

S*treptococcus agalactiae*, *S. uberis* and *Enterococcus canis* were isolated more frequently (33,03%; 15,03% and 41,89% respectively) from milk samples of lactating cows compared to dry cows, while *Enterococcus faecalis* was isolated more frequently (21,73%) from dry cow samples. Coagulase negative staphylococci (CNS) were by far the most frequently isolated pathogens from lactating cows, followed by *Staphylococcus aureus* and αß haemolytic *S. aureus*, and S*treptococcus agalactiae*. Although *S. aureus* remains the principle mastitogenic pathogen in South Africa due to its chronic nature and economic losses, most cases of mastitis (39,5%) were caused by CNS and not *S. aureus*. This conclusion increases the importance of CNS (formerly minor pathogens). Isolations of *S. agalactiae* peaked in 2003 at 9,82% and decreased since then to 3,29% by 2007. CNS isolates increased from 2002 to 2007.

Mastitis seems by no means under control in South African dairy herds. The apparent prevalence of mastitis and teat canal infection increased in laboratory samples from 19,2% in 2002 to 39,4% in 2006. The overall infection rate also increased. Mastitogenic pathogens isolated from cows over the years, paint a picture which is by no means static, due to the opportunistic nature of pathogens. Emerging pathogens which caused mastitis outbreaks over the past 11 years included αβ haemolytic *S. aureus,* S. *agalactiae* and *E. canis.*

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