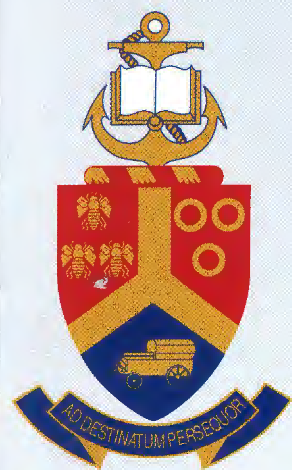

UNIVERSITY OF PRETORIA

FACULTY OF VETERINARY SCIENCE

Faculty Day

September 25, 2003

PROGRAMME AND SUMMARIES

FACULTY OF VETERINARY SCIENCE, UNIVERSITY OF PRETORIA

FACULTY DAY

25 SEPTEMBER 2003

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The Faculty of Veterinary Science wishes to express sincere thanks to the following sponsors for their very generous contribution towards the support of the 2003 Faculty Day programme:

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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. NPJ Kriek

The activities of Faculty Day reflect the quality, volume, and diversity of academic veterinary science, as it is practiced here on campus. The volume and quality of the research contained in the programme for the day this year reflect the increasing output of the Faculty in terms of research that is consistent with the strategic plan of the Faculty for the next few years.

I wish to acknowledge on this day, the motivation of the members of staff and students and their efforts in increasing the quality, relevance, and volume of research. This has put us on the road to being locally relevant and internationally competitive.

The output and relevance of the research conducted on campus also makes us an indispensable contributor to the pool of research solutions that is required to address the needs of the country. We are becoming role players that cannot be ignored.

The current philosophy of the University and the limited resources at our disposal forced research away from that which was driven by the concept of 'academic freedom', to more directed research. Thus we have focused on specific areas of research that relate to the needs of the country and that which is within the context of the main research objectives nationally.

My sincerest thanks go to those people who participate actively in today's proceedings and to those who have come to support them. I trust that you will all have a most enjoyable and enriching day.

NPJ KRIEK
DEAN

Prof. WFO Marasas

PROFESSOR WALTER MARASAS

Professor Wally Marasas was born in Boksburg in 1941 and matriculated from Voortrekker High School in 1958. He obtained his BSc (Agric) degree in Plant Pathology at the University of Pretoria in 1962 and three years later his MSc in the same field of study. In 1969 he was awarded his PhD in Plant Pathology at the University of Wisconsin, Madison, Wisconsin, USA for the thesis entitled "Moldy corn: Nutritive value, toxicity and mycoflora with special reference to *Fusarium tricinctum* (Corda) Snyder & Hansen". Professor Marasas began his professional career as a mycologist at the Plant Protection Research Institute of the Department of Agriculture in Pretoria in 1969. In 1975 he was appointed Chief Specialist Scientist at the Medical Research Council in Tygerberg and in 1992 became Director of the PROMEC (Programme on Mycotoxins and Experimental Carcinogenesis) Unit of the MRC, a position which he currently holds. In addition he serves as Adjunct Professor in the Department of Plant Pathology at the Pennsylvania State University (1986-Present) and Kansas State University, USA (2001 - Present). He is also an Extraordinary Professor in the Department of Microbiology & Biochemistry at the University of the Free State (1989 - Present) and FABI in the Faculty of Natural and Agricultural Sciences of the University of Pretoria (1998 - Present).

His research interests include mycology and mycotoxicology, particularly *Fusarium* taxonomy and human and animal diseases caused by *Fusarium* toxins in foods and feeds. Professor Marasas has received numerous awards and honours including the Christiaan Hendrik Persoon Gold Medal for Scientific Excellence and Outstanding Achievements in Plant Pathology, Southern African Society for Plant Pathology (1987); the Wellcome Gold Medal and Award for Medical Research (1993); the African Academy of Sciences/CIBA Prize for Agricultural Biosciences (1995); a D.Sc honoris causa, Faculty of Natural Sciences, University of the Free State (1996); the MRC Silver Medal for Excellence in Research (1997); the MT Steyn Gold Medal for Natural Sciences, SA Academy for Science and Art (1998) and the Distinguished Service Award for International Agriculture, Kansas State University (2003). He is widely travelled and has published extensively with three books to his credit, 51 chapters in books and 253 Journal Publications. He has presented 161 papers at International Conferences and promoted or examined 61 theses.

FACULTY OF VETERINARY SCIENCE, UNIVERSITY OF PRETORIA

FACULTY DAY

THURSDAY 25TH SEPTEMBER 2003

PROGRAMME

07:45-08:15 **Registration and Coffee**
Master of Ceremonies: *ProfessorH B Groenewald*

08:15-08:30 **Welcome and Opening Address**
Dean: Professor N P J Kriek

08:30-09:30 **RESEARCH PROGRAMME: ORAL PRESENTATIONS**
SESSION CHAIRMAN: *Professor B L Penzhorn*

Monitoring of stress in cheetahs
H J Bertschinger, L Köster, A Human

The use of probiotics in cheetahs (*Acinonyx jubatus*)
K N Koeppel, J Picard, M van Vuuren, H Bertschinger

Control of testosterone secretion, musth and aggressive behaviour in African elephant (*Loxodonta africana*) bulls using a GnRH vaccine
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Fixation of the uterus of African buffalo (*Syncerus caffer*) by glutaraldehyde perfusion under field conditions for histological evaluation by light- and electron microscopy
S Schmidt, D Gerber, J T Soley, T A Aire

Serum corticosterone concentrations in response to ACTH stimulation in African penguins (*Spheniscus demersus*)
F Lampen, H Bertschinger, N Parsons, A Human

09:30-10:20 **Sir Arnold Theiler Memorial Lecture: "Fumonisins: Historical Perspectives and Future Objectives"**
Professor W F O Marasas

10:20-11:00 **TEA** and Viewing of Posters, Commercial Exhibits and Photographic Exhibition

11:00-12:00 **RESEARCH PROGRAMME: ORAL PRESENTATIONS**
SESSION CHAIRMAN: *Professor A J Guthrie*

The development of an interferon-gamma (IFNγ) assay for the diagnosis of *Mycobacterium bovis* in African elephants (*Loxodonta africana*) and rhinoceros (*Diceros bicornis*)
D Morar, E Tijhaar, A L Michel, E H Venter, V P M G Rutten

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A case of severe combined immunodeficiency disease in an Arabian horse foal in South Africa
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Using scenario tree analysis to rank the causes of the low calving percentage in communally-grazed cattle
E Mokantla, C M E McCrindle

Comparative morphology of the crocodylian kidney: A preliminary report
H B Groenewald, J T Soley, F W Huchzermeyer

12:00-13:00 **RESEARCH PROGRAMME: PRESENTATION OF POSTERS**
SESSION CHAIRMAN: *Professor C M E McCrindle*

P1. **Innervation of the tusk pulp of the African elephant (*Loxodonta africana*)**
S C Boy, G Steenkamp, M N Bester

P2. **Bone density of the giraffe (*Giraffa camelopardalis*) and buffalo (*Syncerus caffer*) skeletons**
O L van Schalkwyk, J D Skinner, G Mitchell

P3. **Basic macroscopic features of the arterial supply to the reproductive system of the male ostrich (*Struthio camelus*)**
M Z J Elias, T A Aire, J T Soley

P4. **A unique modification of the smooth endoplasmic reticulum in Leydig cells of the sexually mature male ostrich**
J T Soley, E van Wilpe

P5. **Knowledge versus application of extension messages: internal and external parasites of cattle in the Moretele District of North West Province**
M J Sekokotla, C M E McCrindle

P6. **Economic analysis of a small-scale goat production system on communal grazing**
P J Sebei, C M E McCrindle

P7. **Domestic dog microsatellite markers used for parentage verification in the African wild dog (*Lycaon pictus*)**
J Kim, A J Guthrie, A Nel, C Harper, H J Bertschinger

P8. **Applying horse genetic markers to endangered Cape mountain zebra (*Equus zebra zebra*) populations affected with sarcoid tumour**
S P Sasidharan, A Nel, C Harper, A Guthrie, H J Bertschinger, Y Moodley, E Harley

P9. **The development of a DNA probe to detect *Babesia felis***
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P10. **Prevalence of Severe Combined Immunodeficiency Disease in Arabian horses in South Africa**
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P11. **Screening for two genetic diseases affecting calf survival in South African and Australian Brahman cattle**
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P12. **Towards a DNA vaccine for heartwater**
N E Collins, A Pretorius, MF van Strijp, M van Kleef, K A Brayton, B A Allsopp

P13. **The *Ehrlichia ruminantium* genome sequencing project**
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P14. **Basic electron microscopy: a picture is worth a thousand words**
E van Wilpe, D Meyer

13:00-14:00 **LUNCH**

14:00-15:00 **RESEARCH PROGRAMME: ORAL PRESENTATIONS**
SESSION CHAIRMAN: *Professor G E Swan*

Extraction of bioactive compounds from fresh and dried parts of *Urginea sanguinea* used in ethno-veterinary medicine
V Naidoo, D R Katerere, G E Swan, J N Eloff

Screening of several plants used in ethnoveterinary medicine in the northern parts of South Africa
J N Eloff, L J McGaw, D van der Merwe

Antibacterial activity of grasses – towards a natural antibiotic?
D R Katerere, E A Boomker, J N Eloff

***In vitro* antimicrobial activity of phytomedicine against acidogenic oral bacteria**
S Beukes, F Botha, J N Eloff

Antioxidant and antibacterial compounds in *Peltophorum africanum* (Fabaceae)?
E S Bizimenyera, G E Swan, J N Eloff

15:00-16:00 **RESEARCH PROGRAMME: ORAL PRESENTATIONS**
SESSION CHAIRMAN: *Professor F Reyers*

Diagnosis and treatment of leiomyosarcomas in the oral cavity of dogs
G Steenkamp, S C Boy

Effects of intravenous lidocaine on isoflurane concentration, physiological parameters, metabolic parameters and stress-related hormones in horses undergoing surgery
T B Dzikiti, P van Dijk, L J Hellebrekers

Diagnosis of feline haemoplasma infection using real-time PCR
R G Lobetti, S Tasker

Blood flow velocities and velocity waveform characteristics of some abdominal vessels in the dog
L M Koma, RM Kirberger

A retrospective look at snake envenomation in 155 dogs
R G Lobetti, K J Joubert

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

16:30-17:30 **RESEARCH PROGRAMME: PRESENTATION OF POSTERS**
SESSION CHAIRMAN: *Professor EH Venter*

P15. **Effect of collection method, time and transport medium on a PCR test for *Tritrichomonas foetus* in bulls**
P C Irons, N Mukhufhi, A Michel, F Peta, B Dungu-Kimbenga, H J Bertschinger

P16. **Comparison of three different media for freezing epididymal sperm from African buffalo (*Syncerus caffer*) and influence of equilibration time on the post-thaw sperm quality**
F C Herold, D Gerber, K de Haas, J O Nöthling, D Cooper, W Theunisen, B Spillings

P17. **Effect of bovine seminal plasma on the ability of buffalo (*Syncerus caffer*) spermatozoa to fertilise bovine oocytes in vitro**
D Gerber, K de Haas, J O Nöthling

P18. **Association of Foot and Mouth Disease virus with bovine oocytes during *in vitro* maturation**
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P22. **Can the use of *Ziziphus mucronata* extracts for treating bacterial infections be justified?**
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P23. **Efficacy of herbal extracts against pathogens of production animals**
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P24. **Screening of antifungal activity of members of the Combretaceae for selection of plants for detailed work**
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P26. **Control of ovine hepatic metabolism by cell volume**
J G van der Walt, A M Ali, H C Rossouw, H Engelbrecht

P27. **Early development of digestive function in the ostrich (*Struthio camelus*)**
J G van der Walt, P A Iji, T S Brand, E A Boomker, D Booyse

17:30- **COCKTAIL FUNCTION and PRIZE GIVING**
During the cocktail function the following awards will be presented:
Lecturer of the Year; Researcher of the Year; Young Researcher of the Year; Best Scientific Paper; Best Scientific Poster; Photography prizes

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

1. PHOTOGRAPHIC EXHIBITION
   An exhibition of photographs taken by staff and students. The photographs will be judged by Derek and Norma Pearman (Hon. FPSSA, FPSSA) who have extensive national and international judging experience.
   Organisor: Dr E van Dyk

2. EXHIBITS BY SPONSORS

3. SCIENTIFIC POSTERS

SIR ARNOLD THEILER MEMORIAL LECTURE

Fumonisins: Historical Perspectives and Future Objectives

WFO Marasas (wally.marasas@mrc.ac.za)

PROMEC Unit, Medical Research Council, Tygerberg, South Africa

Fumonisins were first isolated in South Africa in 1988 from cultures of *Fusarium verticillioides* (previously known as *F. moniliforme*) strain MRC 826 and the structures of fumonisin B_1 (FB_1) and B_2 (FB_2) were elucidated. During 1989/1990, maize screenings of the 1989 USA maize crop caused widespread outbreaks of leukoencephalomalacia (LEM) in horses and pulmonary oedema syndrome (POS) in pigs in the USA. Both of these syndromes were proven to be caused by FB_1 in 1990. Analytical methods for the detection of FB_1 and FB_2 in maize were developed in 1990 and naturally occurring levels reported in maize screenings associated with field outbreaks of LEM and POS. Fumonisins were also found to occur naturally in home-grown maize in a high-incidence area of oesophageal cancer (OC) in the Transkei region of South Africa. During 1991, FB_1 was shown to cause liver cancer in rats and to inhibit sphingolipid biosynthesis by researchers in South Africa and the USA, respectively. The latter finding indicated the use of changes in the sphinganine : sphingosine ratio as a biomarker of fumonisin exposure in animals. Initial studies on the toxicokineties of fumonisins in 1992 revealed that FB_1 is rapidly excreted in the faeces and urine of rats. It is still enigmatic why the fumonisins have such an array of pathological effects while they are excreted so rapidly, mostly unmetabolised.

Risk assessment parameters, ie tolerable daily intake (TDI) and probable daily intake (PDI), for fumonisins were proposed in 1996. Embryotoxicity of FB_1 in cultured rat embryos was demonstrated in 1996. In 1997, researchers in the USA reported that FB_1 inhibits folic acid transport by the folate receptor. Folic acid deficiency causes neural tube defects (NTD) and the authors postulated that some NTD in humans may be related to dietary exposure to FB_1. This hypothesis was taken a step further when the high incidence of NTD in Mexican American women along the Texas – Mexico border was associated with FB_1 in maize tortillas. Experimental evidence that FB_1 causes NTD in mouse embryos in whole embryo culture and that folic acid prevents FB_1 - induced NTD was published in 2002. Future objectives include the following:

- Mouse model: dose- effect and structure-activity relationships
- Primate model for NTD
- Fumonisins in OC and NTD
- Epidemiology and case-control studies
- Effect of folic acid supplementation
- Effect of implementation of maximal tolerable levels for fumonisins

In conclusion, the solution to the problem of fumonisins in maize is not regulation, but prevention of *Fusarium* infection and fumonisin contamination in the field.

Monitoring of stress in cheetahs

HJ Bertschinger[1], L Köster[1], A Human[1] (henk.bertschinger@up.ac.za)

[1] Veterinary Wildlife Unit, Faculty of Veterinary Science,
University of Pretoria, Onderstepoort 0110

Chronic stress is presumed to be a causative agent for a number of pathological conditions seen in captive cheetahs. These include gastritis, kidney disease and poor reproductive performance[1]. If stress is to be monitored in cheetahs the method of assessment itself may not cause a stress reaction. The only methods, which will allow this, are non-invasive monitoring of glucocorticoid metabolites in urine or faecal samples[2]. As urine is very difficult to obtain non-invasively from cheetahs, the only option left is to monitor faeces. This paper describes an attempt to validate faecal corticosterone assay as a means of non-invasive monitoring of stress in cheetahs and also draws attention to unforeseen problems in the planning of a research project.

The 18 cheetahs for the study were anaesthetised with zoletil to allow easy frequent blood sampling. After either a saline (7 controls) or ACTH (11 treatment) injection, blood samples were collected 30 min before, just prior to treatment (baseline samples) and every 30 min for three hours thereafter. Faecal samples were collected from each individual daily from Days –1 to +6. Blood samples were assayed using a cortisol radioimmunoassy (RIA). Faecal samples were dried and extracted with 80 % methanol in water. The supernatant was assayed using a rat corticosterone RIA.

The results of the blood cortisol assays were as to be expected. Most placebo and ACTH animals showed higher concentrations at T –30 min than at T 0 min. This reflects the stress of immobilisation. The controls remained fairly constant after that. The ACTH Group showed a rapid rise in response to ACTH with most animals peaking at +150 min. The faecal results were rather nonsensical, mainly due to the fact that many animals only defecated on Days 3-5 after treatment.

The purpose of the project was to show the rise in faecal cortisol metabolites following ACTH injection. No one had considered that the cheetahs would most likely suffer from obstipation following anaesthesia. Food and water had been withdrawn the previous day and on the day the animals were anaesthetised water intake was probably limited. Feeding only commenced a day later. The combined affects of water and food deprivation resulted in obstipation. Post-treatment monitoring of faecal steroids was thus not possible. In the end, the results were not worth publishing. Subsequently we have overcome the problem by darting conscious cheetahs with ACTH. This time it was possible to obtain one to two faecal samples from each cheetah per day during the entire period.

References

1. Papendick RE, Munson L, O'Brien TD, Johnson KJ 1997 Systemic amyloidosis in captive cheetah (*Acinonyx jubatus*) *Veterinary Pathology* **34**: 549-556
2. Terio KA, Citino SB, Brown JL 1999 Fecal cortisol metabolite analysis for non-invasive monitoring of adrenocortical activity in the cheetah (*Acinonyx jubatus*) *Journal of Zoo and Wildlife Medicine* **30**: 484-491

The use of probiotics in cheetahs (*Acinonyx jubatus*)

KN Koeppel[1], J Picard[1], M van Vuuren[1], H Bertschinger[2] (katja_koeppel@gmx.net)

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Neonatal mortalities of up to 87 % and poor survival rates in cheetah are a problem in breeding facilities and zoos[1,2]. Enteritis has been a problem in juveniles and adults where cubs are very susceptible to enteric bacterial infection. Probiotics are documented to suppress neonatal scours and to improve the growth of young and stressed animals[3]. Probiotics consisting of lactobacilli, enterococci, bifidobacteria and yeasts have a beneficial effect on the host by adhering to the epithelial cells and thereby excluding or reducing pathogen adherence. Since these bacteria are very host specific only bacteria cultured from the intestines of healthy host species should be used[4]. The isolation of the potential probiotic bacterial species, namely *Lactobacillus*, *Enterococcus* and *Bifidobacterium* was attempted from eight faecal and two duodenal specimens obtained from healthy adult cheetah. These genera have in clinical trials been shown to be the most promising bacteria used in probiotics[4]. Bacterial counts were also done on these samples.

Twenty-eight juvenile cheetahs between eight and thirteen months old were divided into two groups A and B. For 28 days, group A received 10^9 CFU/day of probiotics (in nutrient broth) mixed into the feed while group B represented the control group. An equal mixture of Lactobacillus group II and *Enterococcus faecium* was used in the probiotics for the feeding trial. The animals were weighed and intestinal permeability measured at the beginning and end of the trial. Faecal appearance and percentage water content was recorded on a weekly basis. Haematology and biochemistry was done on blood and virus detection in faeces to detect any diseases.

The mean number of bacteria in the faeces was 2.24×10^7 CFU/g while in the proximal small intestines it was 85 CFU/ml with enterococci being predominant. The improvement in the faecal appearance of the probiotic-receiving group was statistically significant. No statistical differences in the percentage water content were noted. During the four weeks group A gained 10.4% ($P<0.05$) more weight compared to group B.

The results point towards an improvement in feed conversion and general health, and a decrease in diarrhoea in cheetah fed species-specific probiotics.

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Control of testosterone secretion, musth and aggressive behaviour in African elephant (*Loxodonta africana*) bulls using a GnRH vaccine

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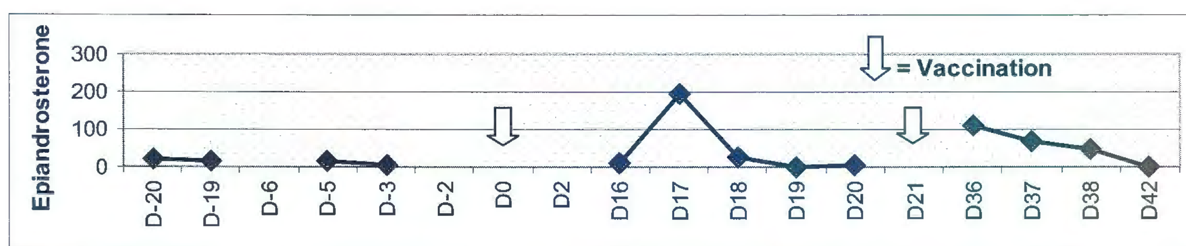
Captive and free-ranging elephant bulls can sometimes constitute a serious management problem when displaying aggressive behaviour with or without musth. They can endanger the lives of both other animals and people. Trained elephants usually have to be removed from work programs and necessitate restraint to such an extent that it becomes a welfare issue. As there is no practical way to control musth as yet, culling often constitutes the only resort for free-ranging problem bulls. Musth and aggressive behaviour seem to be related to high concentrations of testosterone. The aims of this project are to test the effects of a GnRH vaccine on testosterone secretion and behaviour, with a view to using the vaccine to control musth and aggressive behaviour in elephant bulls.

The vaccine (GnRH-tandem-dimer conjugated to ovalbumine, Pepscan Systems, Netherlands) will be used with either Montanide ISA 51 or Covaccine adjuvants[1]. Five elephant bulls will be vaccinated with 2 mg GnRH, 3 times at 3 weeks intervals by means of darting. Two control elephants will be darted with adjuvant only. Faecal epiandrosterone, using an enzyme immuno-assay (EIA), will be used to monitor testosterone secretion. Faecal samples will be collected during each week prior to vaccination and 4 months after the last vaccination. Furthermore, behaviour will be monitored during the same periods when faecal samples are collected.

The first elephant bull was vaccinated at the Johannesburg Zoo. This bull has been exhibiting aggressive behaviour with temporal gland secretion for some time now. Four vaccinations have been carried out using the ISA 51 adjuvant. The faecal epiandrosterone results following the first two vaccinations are shown below. No change was observed in the epiandrosterone faecal profile. As far as behaviour is concerned, the elephant has displayed calmer episodes after each vaccination and has also shown reduction in temporal gland secretions. The effect was more marked after the 4th vaccination. The use of the vaccine has shown no side effects whatsoever. The primary vaccination has just been administered to the second bull.



Faecal epiandrosterone concentrations (ng/g dry faeces) before and after the first two vaccinations

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Fixation of the uterus of African buffalo (*Syncerus caffer*) by glutaraldehyde perfusion under field conditions for histological evaluation by light- and electron microscopy

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The optimal preservation of tissues for histological examination requires the use of the correct fixative, the shortest possible interval between death and the onset of fixation, and the appropriate method of fixation (immersion fixation or vascular perfusion). This study describes the application of a convenient method of fixation under field conditions using glutaraldehyde for the ultrastructural and histological preservation of uterine and placental tissue *in toto* from African buffalo culled in the Hluhluwe/Umfolozi Game Reserve.

Four pregnant (first trimester) and two non-pregnant uteri were used in this study. Approximately 10 minutes after the animals were shot, the uteri were removed via ventral midline laparotomy. Suction catheters of appropriate size (6-12 G) were partly inserted into the uterine arteries and connected via plastic tubes (2.5 m long, 1 cm diameter) to a 20 l plastic bucket filled with buffered saline to flush blood from the vascular system. Following adequate flushing, the catheters were connected to a bucket filled with 2.5% glutaraldehyde in 0.013 M Millonig's buffer. To ensure a perfusion pressure approximately equivalent to physiological systolic blood pressure (≅150 mmHg), the buckets were fastened 1.5 m above the organ (≅0.15 bar). In non-pregnant animals the uterine lumen was additionally flushed/fixed via catheters inserted into the uterine horns. Free flow of fixative was achieved by making a uterine incision in the proximity of the cervix. In animals carrying a larger foetus, perfusion was also performed via both umbilical arteries after removal of the foetus in order to optimise fixation of the chorion from the foetal aspect. In animals with a smaller foetus it was impractical to perfuse via the umbilical arteries and fixative was injected directly into the allantoic and amniotic cavities. The organs were perfused until hardening of the tissue was apparent. The perfusion time varied between 15-25 minutes. The uteri were then opened longitudinally, placed in buckets filled with 2.5% glutaraldehyde for further fixation by immersion, prior to detailed sampling of tissue in the laboratory.

Perfusion of whole uteri through the uterine arteries and additionally into the uterine lumen or via the umbilical arteries resulted in excellent fixation of the entire organ. Preliminary light microscopic observations on uterine and placental material stained with H & E indicated that the foetal/maternal interface was undisturbed. Moreover, the morphological features of the cells and tissues showed minimal fixation dependent artefacts. Another advantage of the technique was that the uterine lumen remained open enabling a more precise and consistent selection of sampling sites to be made. In addition, flushing of the uterine lumen with saline not only gave excellent results on scanning electron microscopy (SEM), but a great deal of the mucous, which often covers the epithelial surface, was removed. The combination of perfusion and immersion fixation of the luminal surface provided excellent preservation of the ciliated and non-ciliated elements of the uterine epithelium when viewed by SEM. The firm nature of the uteri fixed *in toto* also eliminated the cell damage often caused when sampling fresh uteri.

The described method of organ fixation has proven successful for the histological and ultrastructural study of organs obtained under field conditions.

Serum corticosterone concentrations in response to ACTH stimulation in African penguins (*Spheniscus demersus*)

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According to the Penguin Conservation Assessment and Management Plan[1] the African Penguin (*Spheniscus demersus*) is listed in Appendix II of the Convention for the Conservation of Migratory Species of Wild Animals (Bonn Convention). In 2000, 18 000 of an estimated total world population of 180 000 adult African Penguins needed to be rehabilitated because of oil contamination. It is assumed that the high, chronic levels of stress to which birds become exposed in the rehabilitation process, will cause immunosuppression and pathology leading to an increased incidence of diseases such as avian malaria. The degree of involvement of stress in the pathogenesis of disease is unclear. Although serum corticosterone concentrations are generally accepted to be a measure of stress levels in birds[2], no one has measured the stress response in penguins undergoing rehabilitation. The purpose of this study was to test the blood corticosterone response of penguins to ACTH injection and to compare resting concentrations in wild and rehabilitating birds.

Serum samples were taken from 35 birds being rehabilitated at SANCCOB, and a further 20 samples were collected from wild birds breeding at the Boulder Beach, Simonstown, colony. Serum was assayed using a corticosterone radioimmunoassy (RIA). A pilot trial was first conducted to validate the effects of ACTH on blood corticosterone concentrations in penguins. ACTH was administered to four penguins while another four penguins served as controls and were injected with an equivalent volume of saline intravenously.

The means for the four control and four ACTH-treated penguins at T 0, 30, 60, 90 and 150 min were 55,2, 70,5, 24,3, 1,6 and 32,3 and 39,8, 158,2, 184,8, 195,1 and 195,7 nmol/*l* respectively. The decrease observed after 30 min in the controls, probably reflects habituation to handling. The other birds responded well to ACTH stimulation and peaked at approximately 90-150 min. The mean serum corticosterone concentrations of rehabilitating and wild penguins were 69,5 and 24,1 nmol/*l*, respectively.

These preliminary results indicate that the birds respond well to ACTH stimulation and that the rat corticosterone RIA is suitable to determine corticosterone concentrations in serum of African penguins. It also shows that the stress response brought about by the rehabilitation process can be measured using corticosterone serum concentrations.

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The development of an interferon-gamma (IFNγ) assay for the diagnosis of *Mycobacterium bovis* in African elephants *(Loxodonta africana)* and rhinoceros *(Diceros bicornis)*

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Tuberculosis is a chronic, debilitating disease caused by mycobacteria of the *Mycobacterium tuberculosis* complex. *Mycobacterium bovis* is commonly associated with tuberculosis in cattle and the disease is referred to as bovine tuberculosis (BTB). Over the past decade *M.bovis* has been found to have an exceptionally wide host range, which includes both captive and free-ranging wildlife. To avoid spread of the infection in relation to breeding or reallocation projects, procedures to diagnose infection in various wildlife species, including elephants, need to be developed. A variety of diagnostic tests are available for the detection of BTB in domesticated animals such as cattle and goats but these tests posses a number of disadvantages when implementing them for the diagnosis of BTB in wildlife, especially pachyderms.

The objective of this project was therefore to design a diagnostic test that will prove valuable in the detection of BTB in African elephants *(Loxodonta africana)* and hooked-lipped rhinoceros *(Diceros bicornis)* using the cytokine IFNγ as an indicator of *M.bovis* specific responsiveness.

Interferon-gamma is a type II interferon; a cytokine produced mainly by Thl cells and cytotoxic T-cells expressing surface markers CD4 and CD8, respectively, and natural killer cells. Apart from increasing the ability of macrophages to destroy ingested micro-organisms, this cytokine promotes antibody dependent phagocytosis. Interferon-gamma therefore plays a pivotal role in limiting both initial cellular recruitment and acquired lymphocytic responses to mycobaterial infection.

In order to develop such an assay, recombinant elephant and rhinoceros IFNγ was cloned, sequenced, expressed and purified. Subsequently a monoclonal antibody against IFNγ was produced. Monoclonal antibodies were selected via a number of ELISAs based on coated recombinant IFNγ. Preliminary results are promising and further tests are underway regarding the specificity and sensitivity of the assay before field trials can be performed.

The results of this study will determine the design of an IFNγ diagnostic kit for the diagnosis of *M.bovis* infections in African elephants and hooked-lipped rhinoceros, as well as in other wildlife affected by this debilitating disease.

Determination of the duration of lumpy skin disease virus in blood and skin of experimentally infected bulls using different diagnostic techniques

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Lumpy skin disease (LSD) is classified by the Office of International des Epizooties as a list A disease of cattle because it may cause great economic losses and impact negatively on trade. This disease is endemic in most African countries and outbreaks may have severe socio-economic consequences.

In order to gain a better understanding of the epidemiology of the disease, and in particular the duration of persistence of LSD virus in body tissues, six post-pubertal bulls (two Dexters, two Holstein / Friesian and two Dexter / Friesian cross-breeds) from a herd where vaccination against LSD was not practised, were purchased and infected with a virulent, South African field isolate of LSD virus at a titre of 5 log $TCID_{50}$. In each animal clinical signs were monitored, and blood samples and skin biopsies were collected at regular intervals after infection for laboratory analysis.

Virus isolation was performed on blood and skin samples using bovine dermis cell cultures and viral DNA was detected by the polymerase chain reaction (PCR). The virus was identified in skin lesions by transmission electron microscopy and by immunoperoxidase staining of tissue sections. The serum viral neutralization and indirect fluorescent antibody tests were used to determine antibody titres of the infected animals.

Preliminary results indicated that the incubation period after intravenous inoculation of the virus was 4 to 6 days. The febrile reaction lasted for 3 to 14 days. No biphasic fever response was observed. Viraemia was detected in each of the six animals for 1 to 12 days. The PCR was able to detect virus in skin samples for at least three months after infection indicating that viral DNA can persist in the skin for extended periods.

Virus isolation in cell cultures as a diagnostic method is labour intensive and time consuming but is still needed to determine virus infectivity. However, PCR is a fast and sensitive method whereby lumpy skin disease virus can be detected in blood and skin samples.

A case of severe combined immunodeficiency disease in an Arabian horse foal in South Africa

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Subacute to chronic bronchopneumonia and osteomyelitis was diagnosed in a seven week – old Arabian filly by ultrasound and radiography and subsequently confirmed at necropsy. Haematology revealed a severe lymphopenia and a marginal total leucocyte count. There was also aplasia of the thymus, as well as a severe generalized hypoplasia of lymphnodes. The spleen was small with inconspicuous lymphoid tissue. In addition, a moderate fibrinopurulent inflammation of the mandibular salivary glands was apparent. The histopathological changes confirmed the macroscopic changes. These included severe diffuse lymphoid hypoplasia and a total absence of lymphoid tissue in the periarteriolar lymphoid follicles of the spleen, as well as numerous large intranuclear inclusion bodies within the epithelial cells of the mandibular salivary glands. On transmission electron microscopy of salivary gland sections, characteristic adenovirus particles could be identified within the intranuclear inclusion bodies. A smooth enteropathogenic isolate of *E. coli and Klebsciella pneumoniae* were isolated from several organs, including the lungs. *Nocardia asteroides* was isolated from the purulent osteomyelitis.

Using PCR amplification with specific DNA primers, DNA from wax-embedded blocks of spleen tissue from this foal displayed the 158 base pair alleles characteristic of severe combined immunodeficiency disease (SCID) gene mutation. DNA extracted from blood samples collected from the sire and dam of the foal were heterozygous for the 158 and 163 base pair alleles typical of carriers of SCID.

SCID, expressed as severe B and T lymphocyte deficiency and dysfunction, occurs in approximately 0.2 – 2% of Arabian foals of both sexes. Affected foals are normal at birth, but frequently die at a young age due to bacterial and adenovirus infections associated with diarrhoea and pneumonia. The breed and the history of death from multiple infections at the early age of seven weeks, in conjunction with severe lymphopenia, lymphoid hypoplasia and positive PCR results are consistent with a diagnosis of SCID in Arabian and part-arabian horses. The presence of the intranuclear inclusions within the mandibular salivary glands was unusual since they are often reported to occur within the bronchial and alveolar epithelial cells of the lungs.

This represents the first genetically confirmed case of SCID in an Arabian or part-arabian horse in the Republic of South Africa.

Using scenario tree analysis to rank the causes of the low calving percentage in communally-grazed cattle

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Management is an important aspect of cattle farming. The communal grazing system is generally known for its low input, low output type of management. The actual inputs and outputs of the farmers are not known and the farmers are often unaware of problems that lower the calving percentage. Although the causes of low calving percentage are well understood in commercial beef farming enterprise, in South Africa, the same is not true for communal farming systems. In order to improve calving percentages through veterinary extension, the causes must be well understood and their importance ranked. The aim of this study was to determine the impact of diseases, management and bull fertility on the calving percentage of cows in a communal farming system.

Fourteen farmers from six villages in Jericho, North West Province, with a total of 330 cows and 17 bulls, were purposively selected using two-stage cluster sampling. The criterion for selection was that each farmer should have a minimum of 10 breeding cows. Demographics of farmers' herd composition and management were assessed using structured and informal interviews. This was followed by a 12-month observational study of 10 of these herds with 265 cows and 13 bulls that were visited monthly and evaluated for fertility.

The calving percentage was found to be 37.7%. This is lower than the calving percentage for commercial beef cattle on extensive grazing, consequently, there is much room for improvement. The factors playing a role in low calving percentage were ranked using scenario tree analysis of field data. From this it appeared that failure of cows to become pregnant, rather than pregnancy loss, was the main cause of poor calving percentage. Sub-fertility of the bulls and poor body condition scores of cows were considered to play a major role in causing low conception and pregnancy rates. Management factors probably were the main reason for poor body condition scores of cows (the second most frequent cause of low conception rate). Pregnancy loss was the second most frequent cause of low calving percentage. It was found that infectious diseases (trichomonosis, campylobacteriosis and brucellosis) played a much lower role than anticipated.

Comparative morphology of the crocodylian kidney: A preliminary report

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Crocodylids possess metanephric kidneys which are characterised macroscopically by a complex pattern of convoluted lobules. In view of observed species differences in the pattern and arrangement of the lobules, this preliminary study compares the morphology of the kidneys of three crocodilian species, namely, *Crocodylus niloticus, Crocodylus acutus* and *Alligator mississippiensis*.

Kidneys fixed whole in 10% buffered formalin were received from commercial operations. Seven kidneys of each species were utilised for a description of the gross anatomy while three kidneys of each species were sampled and routinely processed for light microscopy (LM).

Convoluted lobules were apparent in all three species on gross examination, with those of *C. niloticus* and *C. acutus* appearing finer than those of *A. mississippiensis*. In cross-section the lobules of *C. niloticus* and *C. acutus* presented as U-shaped structures with the arms of the U being directed peripherally. The arrangement of kidney lobules of *A. mississippiensis* followed a more complex pattern.

On LM the kidney lobule of *C. niloticus* consisted of two rows of renal corpuscles situated on either side of a connective tissue tract containing large intralobular blood vessels. The renal corpuscle was composed of Bowman's capsule and a glomerulus and a structure resembling a juxtaglomerular apparatus was sometimes observed. Proximal and distal components of the nephron could be identified. The latter merged with a peripherally situated system of collecting ducts. A similar basic pattern was obvious in both *C. acutus* and *A. mississippiensis* although in A. mississippiensis the renal corpuscles were fewer and larger than those present in the other two species.

These preliminary findings would seem to indicate that species differences do in fact exist amongst crocodylids regarding kidney morphology, particularly at the macroscopic level, and that further research in this field is warranted.

Extraction of bioactive compounds from fresh and dried parts of *Urginea sanguinea* used in ethno-veterinary medicine

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Urginea sanguinea (Hyacinthaceae) is a commonly found invader, distributed throughout South Africa. This entire plant is toxic to animals mainly due to the high level of cardiac glycosides, yet it continues to be used medicinally, either alone or in combination with other plants, for the management of a variety of animal diseases such as redwater, heartwater and gallsickness. The objective of this study was to investigate the effect of freezing on extraction and chemical composition, and to determine biological activity.

Bulbs of *Urginea sanguinea* were extracted with acetone after one of four pretreatments: Freshly harvested (F), dried fresh material (FD), material defrosted overnight (L), and defrosted material dried (LD). Freezing was carried out in a commercial freezer unit. Material to be tested as dried was held at 37 °C, to constant weight. The yield from frozen material exceeded those from non-frozen material by up to 29%. The chemical composition of treated samples, as analysed by thin layer chromatography, differed. This was quantified by the summation of all bands visible with vanillin spray reagent, after development in a four solvent systems. (F= 10, FD= 17, L= 10, LD= 25)

The minimum inhibitory concentration (MIC) as determined by a serial dilution method was 1.25 mg/ml for *Staphylococcus aureus*, 0.63 mg/ml for *Enterococcus faecalis*, 0.63 mg/ml for *Escherichia coli* and 0.31mg/ml for *Pseudomonas aeruginosa*. None of the extracts had free radical scavenging (anti-oxidant) activity.

Anti-protozoal activity was determined using *Babesia caballi* and *Theileria equi* cell cultures grown by the Onderstepoort Veterinary Institute. The assay made use of non-infected equine erythrocytes, infected *in vitro*. *U. sanguinea* reduced the degree of parasitaemia for *B. caballi* by 40% and showed no activity against *T. equi*. Anti-richettsial activity was determined using calf endothelial cell cultures infected with *E. ruminantium*. The extracts were toxic to the cell cultures to a concentration of 1 ug/ml (equivalent to approximately 200 to 40 ng/ml of transvaalin). At 0.1 ug/ml, cell cultures were minimally affected, and parasitaemia was reduced by 77%.

Freezing does appear to be a viable option, when extracting bulbous material. Although the plant extracts had some anti-bacterial and anti-babesial activity, the toxic principle transvaalin makes it unsuitable as a medicinal plant, as both active and toxic principles appear to be extracted together. However, the reported high degree of activity against actively growing endothelial cells may possibly make this plant a useful adjunct during cancer therapy due to cardiac glycoside induced endothelial cell apoptosis.

Screening of several plants used in ethnoveterinary medicine in the northern parts of South Africa

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Structured interviews were conducted with rural stock farmers in the northern part of South Africa. From these a list was drawn up of plants used for several animal diseases. A qualified veterinarian surgeon confirmed the diseases. The identities of the plants were determined from voucher specimens in the National Herbarium of the National Botanical Institute [PRE].

Different parts of 16 plant species were collected, dried, ground and extracted with hexane, methanol and water. The following plants were investigated: *Berchemia zeyheri, Cissus quadrangularis, Cussonia spicata, Dicerocaryum eriocarpum, Dombeya rotundifolia, Gnidia capitata, Hippobromus pauciflorus, Pouzolzia mixta, Pterocarpus angolensis, Rhus lancea, Ricinus communis, Schkuhria pinnata, Schotia brachypetala, Sclerocarya birrea, Secamone filiformis, Synadenium cupulare and Ziziphus mucronata.* In some cases the same species from more than one locality was investigated.

The extracts were dried and reconstituted with solvents that would not affect the subsequent bioassay. Minimum inhibitory concentrations (MIC) for antibacterial activity were determined by a serial microplate dilution method using *Staphylococcus aureus* (Gram-positive) [American Type Culture Collection number 29213] *Pseudomonas aeruginosa* (Gram-negative) [ATCC 27853], *Escherichia coli* (Gram-negative) [ATCC 25922] and *Enterococcus faecalis* (Gram-positive) [ATCC 21212] as test organisms. Anthelminthic activity was by using a free-living nematode (*Caenorhabditis elegans*) and toxicity was determined by using the brine shrimp mortality test.

P. aeruginosa was the most resistant to the extracts with only 4 of the 70 extracts tested having MIC values of 12.5 mg/ml or lower. This was followed by *E. coli* with 10/70, *E. faecalis* with 39/70 and *S. aureus* with 48/70 extracts having MIC values of 12.5 mg/ml or lower. The best value found was 0.1 mg/ml. At a concentration of 2 mg/ml 15 of the 70 extracts had some anthelminthic activity, 6 had reasonable activity, 2 had good activity and 2 had very good activity. Only 24 of the 70 extracts killed more than 10% of the nauplii at the highest concentration of 5 mg/ml tested.

Post-graduate students are currently investigating some of the plants that displayed promising activity. In general the results confirmed the rationale for the ethnoveterinary use of these plants.

Antibacterial activity of grasses – towards a natural antibiotic?

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In our work investigating the antimicrobial properties of South African plants, we discovered that, despite the ubiquitous occurrence of Poaceae species, apparently only *Cynodon* species has been documented in ethnomedicine. *Cynodon dactylon* is used as a lucky charm and to arouse the spirits. There is also a dearth of phytochemical information on grasses. Animals are known to be infected by certain bacteria which reduce their meat-conversion efficiency and general well-being. The widespread use of pharmaceuticals in intensive husbandry practices for preventive (growth promotion) and curative therapy are of major concern due to the possibility of their appearance in meat products and the consequences of these residues in the consumer population. Little work appears to have gone into assaying for the anti-microbial activity of grasses. Screening of grasses for antibacterial and anti-oxidant activity could therefore be seen as of academic and commercial interest with potential for use in animal husbandry and nutrition. The aim of this study was to carry out a preliminary investigation on the antibacterial properties of certain fodder grasses.

Five species of grasses were chosen for this study. These were *Eragrostis curvula* (weeping love grass), *Panicum maxima* (guinea grass), a hybrid between *Pennisetum purpureum* and *P. americanum* (bana grass / elephant grass / napier grass), *Phalaris* species (reed canary grass) and *Avena sativum* (oat). The samples were each split into two batches of 16 g. One batch was extracted fresh while the other was dried for two weeks in the shade before extraction. The extraction was done with 50% dichloromethane/acetone which was then dried off leaving the extract to be reconstituted into a 20 mg/ml stock solution in an appropriate solvent. Thin layer chromatography was used to analyse the chemical composition of the extracts. The antimicrobial activity was investigated by bioautography using *Staphylococcus aureus* (ATCC 29213) and microtitre plates using *Staphylococcus aureus* (ATCC 29213), *Pseudomonas aeruginosa* (ATCC 25922), *Escherichia coli* (ATCC 27853) or *Enterococcus faecalis* (ATCC21212) as test organisms. Ampicillin and neomycin were used as positive controls.

The extracts of dried *Phalaris* were the most active with a minimum inhibitory concentration of 154 µg/ml against both *S. aureus* and *E. faecalis*. In all the other grass species investigated, drying did not appear to influence the antibacterial activity. All the extracts had substantial antioxidant activity in the DPPH assay used.

Because antimicrobial compounds may influence the rumen flora and may partially explain the effect of different fodder plants on animal productivity, further work will use rumen fluid to investigate the effect of these extracts on the resident organisms. An attempt on phytochemical analyses of the grasses will also be made.

In vitro antimicrobial activity of phytomedicine against acidogenic oral bacteria

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Patients receiving orthodontic treatment have an increased risk of plaque accumulation because of the increased difficulty of plaque removal associated with the use of fixed appliances. Bacteria causing decalcification such as *Streptococcus mutans, Streptococcus sobrinus, Lactobacillus casei* and *Actinomyces naeslundii* become active when the environment becomes more favourable, such as in cases of poor oral hygiene. The use of herbal medicines in dentistry has led to the development of some toothpastes and oral rinses.

In this study the *in vitro* activity of ethanol, acetone and hexane extracts of eight medicinal plants (*Hydrastis canadensis*, *Cyclopia intermedia*, *Hypericum perforatum*, *Ginkgo biloba*, *Passiflora incarnata*, *Achillea millefolium*, *Arciosiaphylos uva-ursi* and *Artemisia absinthium*) were tested against *S. mutans, S. sobrinus, L. casei* and *A. naeslundii* which have been implicated in dental demineralisation. The selected plant extracts were analysed by thin layer chromatography (TLC), the chromatograms were tested for antimicrobial activity and the minimum inhibitory concentration (MIC) of the extracts was determined by serial dilution of the extracts using a microplate method.

Acetone extracts had the highest activity followed by hexane and ethanol extracts, indicating that non-polar and intermediate polarity compounds are the most important. *S. mutans* was more sensitive to the plant extracts than *S. sobrinus, L. casei* and *A. naeslundii*. *Achillea millefolium*, *Passiflora incarnata*, *Arciosiaphylos uva-ursi* and *Hypericum perforatum* showed high activity against the bacteria tested and MIC values ranging from 0.04 – 0.3 mg/ml were achieved. The acetone extracts showed inhibition zones with 5 of the selected herbs and the hexane extracts with 6 of the selected herbs. MIC values compared well with control treatments using fluoride and chlorhexidine.

This is the first time that some of the above techniques have been tested on facultative aerobic organisms. We conclude from these findings that plant extracts definitely have a place in the prevention of demineralisation and could perhaps even outperform some of the currently used products.

Antioxidant and antibacterial compounds in *Peltophorum africanum* (Fabaceae)?

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Peltophorum africanum (Weeping wattle; African wattle) is a deciduous tree of the family Fabaceae. This acacia–like tree is widespread in Southern Africa and grows to about 15m high with a wide canopy. Traditionally, the bark and roots are used to treat diarrhoea, dysentery, wounds, sore throat, toothache, ascites, abdominal and joint pains, infertility and HIV-AIDS. Rural farmers and pastoralists use the root and bark to treat diarrhoea and dysentery, and also use it as a general tonic to improve fertility and resistance to disease in cattle. There are indications that the plant could have antibacterial, anti-inflammatory and antioxidant properties.

The leaf, bark and roots were collected from mature plants naturally growing at Onderstepoort. Dried plant parts were extracted with acetone, ethanol, dichloromethane and hexane. Thin layer chromatography (TLC) chromatograms were sprayed with 0.2% DPPH to screen for antioxidant activity. Total antioxidant activity was determined using gallic acid as standard. Bioautographic procedures using 2mg/ml p-iodonitrotetrazolium were performed to determine the activity of extracts against *S.aureus* (ATCC 29213), *E.coli* (ATCC 25922), *P.aeruginosa* (ATCC 27853) and *E.feacalis* (ATCC 29219). Minimum inhibitory concentration (MIC) values and total activity were determined using gentamycin as standard antibiotic.

Acetone and ethanol extracted the largest quantity of the compounds. Antioxidant and antibacterial activity was demonstrated by the extracts. The root, bark and leaf had antioxidant activity values of 49.2%, 46.7% and 23.4% respectively. Antibacterial activity was higher against gram-positive than against gram-negative bacteria, as seen by MIC values of 0.16mg/ml for *S.aureus* and 0.63mg/ml for *E.coli*. The total activity (ml/g) determinations showed that the extracts had high antibacterial activity (1262.5 for ethanol extract of root against *S.aureus* and 800 for acetone extract of root against *P.aeruginosa)*.

This preliminary screening of *P.africanum* revealed that this plant contained antioxidant and antibacterial compounds, a finding that could validate its traditional use in both ethnoveterinary and ethnomedical healthcare practices.

Diagnosis and treatment of leiomyosarcomas in the oral cavity of dogs

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The clinico-pathological features, diagnosis and management of primary intraoral leiomyosarcomas (LMS) have not been described in the oral cavity of dogs. These tumours are smooth muscle neoplasms rarely encountered in the oral cavity of humans.

In this paper we describe four cases of LMS. Case 1 was an intact 2-year-old Great Dane dog with a mass previously diagnosed as a fibrosarcoma on the labial mucosa. Case 2 was an 11-year-old spayed Border Collie bitch that presented with an ulcerative bone-destroying mass previously diagnosed as fibrous hyperplasia in the left mandibular area caudal to the canine. Case 3 was an intact 6-year-old Bull Mastiff dog with complaints of dysphagia and stridor. On clinical examination a large mass was found on the soft palate, extending to and partially obstructing the oropharynx. The fourth case was an 11-year-old intact German shepherd dog with an ulcerating mass on the gingiva and palatal mucosa extending from the left canine to the 4th premolar and medially to the midline of the palate.

Histological examination of all four tumours showed spindle-shaped cell tumours with varying growth patterns and mitotic indices. In association with diffuse positive-staining with smooth muscle actin, focal positivity with desmin and PAS-positive, PAS-negative cytoplasmic granules, the diagnosis of leiomyosarcoma was made.

Cases 1 and 2 responded well to surgical treatment and are respectively 23 and 22 months tumour-free post-operatively. Case 3 died following airway obstruction caused by a large palatal tumour and the fourth case was lost to follow-up after its owners declined treatment.

In conclusion, this report demonstrated that primary LMS should be included in the clinical and pathological differential diagnoses of intraoral tumours in dogs of various age groups, especially older adult males. The diagnosis should be established on histological examination in association with immunohistochemical analysis with SMA. Radical surgical excision with adequate margins seemed to be the treatment modality of choice in operable intraoral tumours. The long-term prognosis seems to depend on the size and anatomical location of the tumour with successful surgical excision. It is possible, however, that these neoplasms may not have an aggressive biological behaviour. Additional prognostic indicators need to be determined and confirmed in larger follow-up studies.

Effects of intravenous lidocaine on isoflurane concentration, physiological parameters, metabolic parameters and stress-related hormones in horses undergoing surgery

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Effects of intravenous lidocaine on required isoflurane concentration, physiological parameters, metabolic parameters, muscle enzymes and stress-related hormones were evaluated in 12 adult horses anaesthetised with isoflurane in oxygen. Two groups (six horses in each group) were studied: a lidocaine group (IL), which received intravenous lidocaine, and a control group(C), which received intravenous saline.

Horses in both groups were premedicated with detomidine intravenously, and anaesthesia was induced with midazolam-ketamine intravenously. Ventilation and blood pressure were controlled. The lidocaine group received intravenous lidocaine starting at 15 minutes after induction to anaesthesia as loading dosage of 2.5 mg/kg followed by a maintenance dosage of 50 μg/kg/min, while the control group received an equal volume of saline. End-tidal isoflurane and standard physiological parameters were measured. Blood was sampled for measurement of lidocaine, stress hormones and physiologic parameters.

The end-tidal isoflurane concentration in the control group was $1.28 \pm 0.06\%$ versus $0.96 \pm 0.06\%$ (mean ± SD) in the lidocaine group, a reduction of 25%, which was significant ($P<0.05$). No significant differences were found regarding stress-related hormones, metabolic parameters and physiological parameters.

This study suggests that the use of lidocaine (in the dosages used here) to decrease the concentration of isoflurane to obtain sufficient surgical anaesthesia has no subsequent effects on physiological and metabolic parameters or stress-related hormones.

Diagnosis of feline haemoplasma infection using real-time PCR

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Haemobartonella felis has been reclassified within the genus *Mycoplasma* as *Mycoplasma haemofelis* and *M. haemominutum*. Both species are collectively referred to as the feline haemoplasmas. The purpose of this study was to evaluate the prevalence of infection with both *M. haemofelis* and *M. haemominutum* in blood samples collected from cats in the Johannesburg area, using a real-time PCR assay and to document associations between feline haemoplasma infection, patient characteristics and haematological values.

Surplus EDTA-anticoagulated blood from 78 feline samples submitted to Golden Veterinary Laboratories in Johannesburg for routine haematological testing that had been diagnosed with Mycoplasmosis (*Haemobartonella*) on blood smear evaluation was stored at -20ºC. The samples were from cats with a variety of disease conditions. Genomic DNA was prepared from 100µl blood samples using the DNeasy 96 Tissue Kit. A feline haemoplasma real-time PCR assay was performed on all samples.

Cats were divided into four groups based on the results of the real-time haemoplasma PCR assay: negative (43 cats, 55%), *M. haemominutum* only positive (25 cats, 32.1%), *M. haemofelis* only positive (5 cats, 6.4%), and dual positive (5 cats, 6.4%). Overall, of the 78 samples, 35 (44.9%) were positive for one or both feline haemoplasma species. Group 4 were negative for both *M. haemofelis* and *M. haemominutum*. Of the haematological variables evaluated by ANOVA or Kruskal-Wallis ANOVA, six were significantly different between the four groups. These were red cell count ($P<0.001$), haemoglobin ($P=0.001$), haemotocrit ($P=0.001$), mean cell volume ($P<0.001$), monocyte count ($P=0.04$) and platelet count ($P=0.02$).

The results of the study showed that cats that were positive for *M. haemofelis* showed macrocytic regenerative anaemia, monocytosis and thrombocytopaenia. This report documents the existence of both haemoplasma species in cats in South Africa.

The study concluded that the diagnosis of feline mycoplasmosis could be difficult without the use of PCR. However, a diagnosis can be assumed on the basis of haematological changes and blood smear evaluation.

Blood flow velocities and velocity waveform characteristics of some abdominal vessels in the dog

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Blood circulation is an essential physiological function concerned with the delivery of oxygen, nutrients and hormones required for tissue metabolism and coordination of body activities, and removal of harmful substances from the body. Blood flow responds rapidly to physiological or pathological changes within the organs and tissues perfused. The ability to measure and characterise haemodynamics provides an important potential tool for closer evaluation of body function, clinical diagnosis, monitoring and management of diseases, e.g. chronic diarrhoeas. The aim of this study was to measure blood flow velocities, and characterise flow patterns of abdominal vessels in the dog, using Doppler ultrasonography.

The abdominal aorta (AAo), coeliac artery (CA), hilar splenic artery (HSA), cranial mesenteric artery (CMA), left renal artery (LRA), interlobar artery (ILA), interlobar vein (ILV), left renal vein (LRV), hilar splenic vein (HSV), main portal vein (MPV) and caudal vena cava (CVC) were identified ultrasonographically in 11 healthy beagles. Velocity waveforms of each blood vessel were obtained, and peak systolic velocity (PSV) or peak velocity (PV) was measured in fasted dogs by Doppler ultrasonography. The waveforms were characterised to define the patterns of blood flow within each vessel.

Arterial blood flows were identified by pulsatile, and venous flows by non-pulsatile velocity profiles. Arterial flows were further subdivided into low resistance, e.g. LRA, ILA and HSA, medium resistance (CA) and high resistance (AAo and CMA) flows. Venous flows were generally monophasic, except that biphasic flow was occasionally seen in the CVC. In addition, the pattern of blood flow in each vessel had unique characteristics. The peak velocities of the various vessels are given in the table below:

Blood vessel	PSV/PV (Cm/s)	SD (±)
AAo	127.1	33.7
CA	115.2	27.8
CMA	103.4	20.2
LRA	85.0	38.8
LRV	38.8	14.8
MPV	22.8	10.3
CVC	38.5	20.0

Doppler ultrasonography is a non-invasive technique that provides information on blood flow. Peak velocities and velocity waveforms are characteristic of each blood vessel, and reflect the physiological status of the organ that is served. Assessment of changes in flow patterns of these vessels can be a valuable tool for clinical evaluation of abdominal organs.

A retrospective look at snake envenomation in 155 dogs

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The 2 most common snakes that cause snake envenomation in dogs are puffadders and cobras. The purpose of this retrospective study was to evaluate the incidence, signalment, haematological changes, therapy, and outcome of dogs presented to the Outpatients section of the Onderstepoort Veterinary Academic Hospital for confirmed snake envenomation.

Records of dogs presented for snake envenomation from 1998 to 2002 were reviewed and analysed by means of descriptive statistics. In total, 155 cases were identified and analysed.

The majority of cases (56%) occurred in the autumn – March to May. Dogs were 3 to 168 months old with a median of 36 months. No sex predilection was identified. Ten percent of cases died because of the snake envenomation, whereas 90% survived. Fifty seven percent and 43% of snakes were puffadders and cobras, respectively. There was no difference in mortality between the 2 groups of snakes. Of the cobras 60% were the snouted cobra, 14% Mozambique spitting cobra, and 15% rhinkals. Swelling in the area of the bite, usually the face and forequarters was the primary clinical abnormality. Significant haematological findings were leukocytosis (median 17.3 x 10^9/l; range 0.4-44), neutrophilia (median 13.6 x 10^9/l; range 0.3-39.9), band neutrophilia (median 0.4 x 10^9/l; range 0-5.32), and thrombocytopaenia (median 124 x 10^9/l; range 3-555). Dog's envenomated by a puffadder had a greater degree of thrombocytopaenia: median of 68 versus 205 for the cobra group. The most commonly used treatments were intravenous fluids, antibiotics, and glucocorticoids. Thirty-eight dogs were treated with polyvalent antiserum: 9 for puffadder envenomation and 29 for cobra envenomation. Only 2 of the dogs that received antisera died, both of them of cobra envenomation.

The study concluded that snake envenomation in dogs is associated with high morbidity but low mortality rate and that the most significant haematological abnormality is thrombocytopaenia.

Innervation of the tusk pulp of the African elephant (*Loxodonta africana*)

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The innervation of the dentin-pulp complex of human and certain animal teeth has been investigated and described in great detail. Various authors have referred to the pulp innervation of tusks of Indian and African elephants. In 1999, however, Fagan and co-workers analysed the dental pulp tissue of African and Asian elephants using immunohistochemical staining techniques developed to show the presence of neural tissue. They concluded that no nerve fibres, either myelinated or non-myelinated could be demonstrated in any of the numerous sections taken from the pulp tissue of these elephants. Due to the clinical implications of their results, it was decided to examine the pulp tissue of an African elephant tusk for the presence of nerve tissue.

Formalin-fixed extracted pulp of a 2-year-old male African elephant was used for this study. Longitudinal and transverse sections were cut in the proximal-, mid-, and distal parts of the pulp. All sections were then embedded in paraffin wax after which the blocks were sectioned at 4 µm and stained with haematoxylin and eosin (H&E). To search for the presence of any neural components immunohistochemical examination with antibodies directed against S-100 protein (DAKO Corporation, Carpinteria, CA 93013) was performed on each group of sections.

Macroscopically the pulp tissue consisted of a firm, gelatinous, grey-white soft tissue mass with a slightly curved long axis, 23cm in length. Light-microscopical examination of H&E-stained sections revealed numerous endothelium-lined, thin-walled veins and lymphatic channels as well as small and medium-sized muscular arteries in a loosely arranged connective tissue stroma. Positive S-100 staining was encountered in various nerve bundles accompanying vascular structures as well as in small bundles distant from vascular structures.

Macroscopical, microscopical, and immunohistochemical examination of the tusk pulp in this study confirmed the presence of various nerve bundles, both autonomic fibres accompanying and innervating vascular vessel walls and ramifying sensory fibres distant from blood vessels. The findings of this study would suggest that clinicians should take definite precautions against causing pain when performing any surgical procedure on the pulp of an elephant tusk.

Bone density of the giraffe (*Giraffa camelopardalis*) and buffalo (*Syncerus caffer*) skeletons

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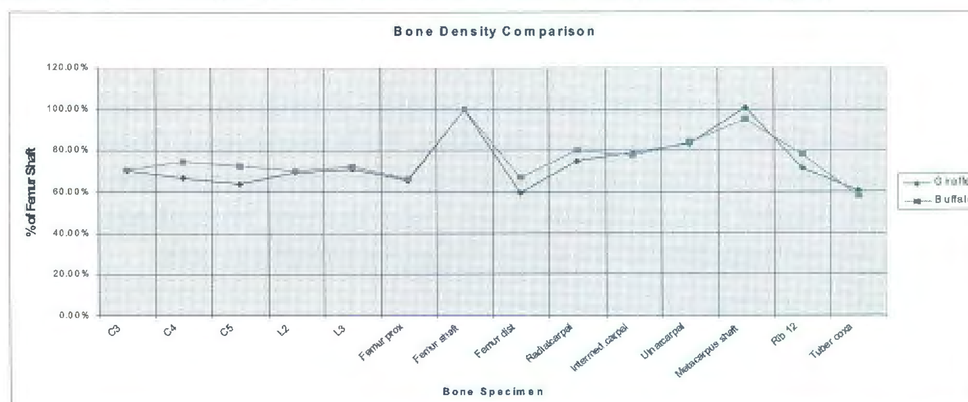
The unique features of a giraffe's anatomy are its long neck and slender long legs. Its growth rate and the proportion of its body mass that is skeleton are also high[1,2]. Neck length depends on elongation of the cervical vertebrae and leg length on elongation mainly of the metapodial bones. To establish whether cervical vertebrae and metapodial elongation are associated with changes in bone density, we have compared the density of some marker bones in giraffe with those of a non-browsing megaherbivore of equivalent size, but short legs and neck, the African Buffalo (*Syncerus caffer*).

Bones were collected from carcasses, cleaned, air-dried, weighed, and their volume determined by water displacement. Absolute density (g/cm^3) was calculated by dividing mass by volume. Relative density (%) was obtained by comparing the density of each bone with that of a common marker bone, the femur shaft[3]. (Absolute density ($x \pm SD$) = 1.85 ± 0.10 g/cm^3 in giraffes and 1.95 ± 0.08 g/cm^3 in buffalo respectively)

Our results are the first obtained for the bone density of any wild Pecoran. Analysis of absolute density shows that the cervical and lumbar vertebrae, and Rib 12 of giraffes are significantly less dense than their counterparts in buffalo ($t=3.2$ and 3.0, $P<0.05$). No significant differences in absolute density between the two species could be found for any of the other bones analysed. Small, not significant ($P>0.05$) differences in relative densities exist between the skeletons of these two species of megaherbivores (see graph). The data show that in both species the absolute density of the cervical and lumbar vertebrae, the carpal bones, Rib 12 and T. coxa are significantly less dense than their femur, while their metapodials are as dense as the femur.

These findings are consistent with the idea that the long neck of giraffes is not supported by vertebrae of increased density and strength. On the other hand their long slender metapodial bones are as heavily mineralised as the femur and are as dense as those of African buffalo.



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Basic macroscopic features of the arterial supply to the reproductive system of the male ostrich (*Struthio camelus*)

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The increasing economic importance of the ostrich as a farmed bird makes it imperative to fully understand the reproductive biology of this species, particularly in view of the low fertility and hatchability which is reported to be prevalent in the industry. The basic structure of the vasculature of the reproductive organs in the male bird has been studied more fully only in the domestic fowl and pigeon, but not in the ostrich. This report is a preliminary study of the pattern of arterial supply to the reproductive organs of the male ostrich, using departmental collections of abattoir ostrich carcasses from which both the skin and hind-limbs had been removed.

The blood vessels of each torso were flushed free of blood by means of normal saline injected through the descending aorta, using a 50 ml syringe. Using the same technique, red latex was injected into the blood vessels through the same route. A total of 8 birds were prepared in this fashion after which the latex-filled blood vessels were exposed by careful dissection and described.

The aorta gives off, on either side, the cranial renal artery (*A. renalis cranialis*) from which arises the testicular artery (*A. testicularis*) supplying the testis. In addition, each cranial renal artery sends a cranial ureterodeferential artery (*A. ureterodeferential cranialis*) to the cranial part of the ductus deferens and ureter. The sciatic artery, which arises from the aorta at the level of the acetabulum, immediately gives rise to a ventral branch, the middle renal artery (*A. renalis media*) that supplies blood to the middle lobe of the kidney, and via a branch, the middle ureterodeferential artery (*A. ureterodeferentialis media*), to the middle part of the deferent duct and ureter. The aorta divides terminally, at about the middle of the pelvis, into the left and right internal iliac arteries (*Aa. iliacae internae dextra et sinistra*) and continues as the thin caudal median artery (*A. mediana caudae*). The internal iliac artery divides into the internal and external pudendal arteries (*Aa. pudenda interna et externa*). The internal pudendal artery supplies several branches to the distal part of the deferent duct and ureter, and to the cloaca. The external pudendal artery also supplies several branches to the caudal part of the deferent duct before terminating in the cloaca and phallus.

The general pattern of vascularisation of the reproductive organs of the male ostrich is similar to that described for the domestic fowl and pigeon. However, an accessory testicular artery or arteries were observed in 60% of the birds examined and the cranial ureterodeferential artery was seen to arise from this vessel in 12.5% of birds. In 25% of the specimens, an extra arterial branch supplying the right deferent duct arises from the aorta, between the two caudal lobes of the kidney. An accurate description of these variations in more birds will constitute a significant part of further survey studies of the arteries of the reproductive organs of the male ostrich.

A unique modification of the smooth endoplasmic reticulum in Leydig cells of the sexually mature male ostrich

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The Leydig cells of the mammalian testis are characterised by an abundance of smooth endoplasmic reticulum (SER) and in some species modifications of this organelle are a consistent feature. In contrast, the Leydig cells of birds are reported to be generally poor in SER, with only the guinea fowl displaying elaborations of the SER in the form of membranous whorls. This paper reports on the occurrence and structure of a unique modification of the SER in Leydig cells of sexually mature ostriches during the breeding season.

Samples of testes were obtained from five sexually mature birds slaughtered during the breeding season at the abattoir of the Klein Karoo Landboukoöperasie, Oudtshoorn, Western Province, South Africa. Small blocks of testicular tissue were immersion-fixed in 4% glutaraldehyde in Millonig's phosphate buffer for 24 hours at 4°C after which they were routinely processed for transmission electron microscopy. Thin sections were stained with uranyl acetate and lead citrate and examined with a Philips CM 10 transmission electron microscope operated at 80kV.

In addition to the typical cytoplasmic organelles generally found in avian and mammalian Leydig cells, some cell profiles in the ostrich revealed collections of relatively long, membrane-bound, rod-shaped structures with moderately electron-dense, homogeneous contents. These arrays were observed in all five birds and were generally localised in a specific part of the cell cytoplasm together with concentrations of homogeneous dense bodies and mitochondria. In longitudinal profile the rod-shaped structures were aligned in parallel formation, were generally separated from each other, but showed a degree of branching and anastomosing. Longitudinal linearities were discernable in some of the profiles. In transverse section individual units revealed a circular profile with a membranous sub-structure characterised by one or more concentric membranous rings. The rod-shaped bodies were approximately 50 – 80 nm in diameter, although a certain degree of variability was observed, particularly in transverse sections of the cytoplasmic arrays. Occasional large multiple bodies bound by a common membrane were also seen. Profiles of SER were intimately associated with the cytoplasmic arrays and higher magnification appeared to indicate that the rod-shaped structures were modifications of the SER cisternae. The limiting membrane at the tips of some SER cisternae was observed to have thickened and to enclose additional concentrically arranged thickened membranes. This process resulted in widening of the original SER cisterna, with the increase in density resulting more from the accumulation of thickened membranes than to the concentration of luminal contents.

The rod-shaped elements identified in Leydig cells of the ostrich are the first reported modifications of the SER found in non-passerine birds, with the exception of occasional membranous whorls seen in guinea fowl Leydig cells. However, structures with similar morphological features termed cylindrical bodies have been described in rat and mouse Leydig cells. Whether these structures in the ostrich are designed to increase the production capacity of the SER or simply represent a seasonal developmental stage of the organelle, could not be determined.

Knowledge versus application of extension messages: internal and external parasites of cattle in the Moretele District of North West Province

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The main aim of agriculture should be to improve productivity. Resource-poor farmers in marginal areas are faced with constraints to achieving this. These include lack of appropriate knowledge and skills. Extension methodology requires that knowledge and skills imparted to farmers should be measurable in terms of improving productivity as well as improving knowledge levels. However, it is recognised that farmers seldom implement the extension messages. The purpose of this study was to appraise the need for extension on internal and external parasites of cattle, design an extension message to meet these needs, implement extension to farmers and evaluate the impact of the extension on farmer's knowledge and application of that knowledge.

This participatory research was undertaken in the Moretele District of North West Province, using a structured interview with 90 farmers as well as assessing their cattle for the control of internal and external parasites. External parasites were assessed by doing tick counts on five sentinel animals per herd and internal parasites were assessed by evaluating pooled samples of faeces for nematode eggs, monthly. The data were compared before and after extension to evaluate whether extension had an impact on parasite control. Knowledge was measured by personal interviews before and after a farmer's information day and implementation of a visit and training method of extension over a 12 month period.

It was found that, prior to extension, only 53% of the farmers said they knew something about ticks and tick-borne diseases. Of those that said they had knowledge of the internal and external parasites of cattle, less than a third could recognise ticks correctly or knew which diseases they caused. Only 35% of respondents said they knew about internal parasites. However, when questioned in detail about types of worms found and the symptoms caused, less than one fifth of these farmers actually had the correct knowledge.

It was found that, after extension, there was a significant improvement in the knowledge level. There was, however, no significant change in the level of knowledge of internal and external parasites.

Economic analysis of a small-scale goat production system on communal grazing

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In communal systems there can be an improvement in land use only with a thorough understanding of all socio-economic and ecological factors that influence productivity, together with the institutional and the political framework in which communal systems have to operate. Indigenous goats on communal grazing are utilised for savings, ritual slaughter and meat with perhaps a little being left over for sale. The majority are kept by part-time small-scale farmers using low-input : low-output scavenging systems, with little or no feed supplementation. These farmers do not implement extension messages to improve the outputs from the goats. This is important as, in 1987, only 315 of the estimated 700,000 goats in North West Province were slaughtered at abattoirs. Thus the great majority are utilised in the informal sector. The aim of this study was to investigate the economic aspects of the above production system using partial farm (enterprise) budgeting. This is a tool usually used for planning and decisionmaking in commercial farming systems. The parameter selected for evaluation was weaning percentage of kids.

A two-stage cluster sampling was done where goat herds (n=13) were the primary units and individual ewes (n=155) the secondary units. The selection method was purposive – only communally-grazed goats in herds with >5 adult ewes were included. Data used for the economic evaluation was obtained from structured interviews (n=20), field data on the number of kids born and weaned (monthly visits over a 14 month period), informal interviews with farmers (n=13) and the literature.

The number of kids that survived to weaning (n=83) was calculated as 53% during the field visits. Goats were sold alive and the average price was R300 per adult and R150 for a weanling. Weaning was natural (observed as +/- 180 days). Prices of feed and water were recorded from informal interviews. The farmers did not treat for internal parasites, treat sick goats or vaccinate against heartwater. Goats were treated approximately twice a year with diluted Jeyes Fluid, to control ticks. Costs were calculated from retail prices for suggested remedies and formal housing structures. The weaning percentage observed during this study was lower than the 88.3% survival to weaning recorded for indigenous goats "on station". However, the return on capital investment of 13.84% was comparable to the 12.5% return offered by commercial banks on savings accounts over the same period. The main reason was because it was found that the price of communal goats was not related to their mass. Better feeding would probably have resulted in a heavier kid. However, it was established that this would not have influenced the price received as a majority of the goats were slaughtered for ritual purposes where age, colour and gender were more important to the purchaser than body mass.

Domestic dog microsatellite markers used for parentage verification in the African wild dog (*Lycaon pictus*)

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Microsatellites are short tandem repeats of 2 – 4 base pairs in length, which are found in almost all eukaryotic organisms. They are usually highly polymorphic and abundantly dispersed throughout the genome. They have distinctive flanking sequences that are generally conserved across closely related species. These features make microsatellites useful as genetic markers for gene mapping, population studies, genetic diversity, parentage, social structure, and various conservation biology studies_.

Whole blood of 20 African wild dogs from de Wildt Cheetah and Wildlife Centre were stored on FTA® (Whatman Bioscience, Cambridge, UK) paper. The samples included one known set of parents and our aim was to determine which of the offspring were related to these parent animals. Twelve microsatellite markers used routinely in the domestic dog were applied to the wild dog samples. PCR was performed in two multiplexes of six loci with no observed overlap in allele range. Eight loci out of 12 provided valuable information for the study. Genotyping of each sample was done using a 310 Genetic Analyser (Applied Biosystems) and the resulting data analysed using *STRand* (Board of Regents, University of California) software programme. Based on the genotypes of the parents and the offspring, we were able to confirm the parentage of animals. Eight individuals were confirmed as offspring of the pair while the rest were excluded. The results were consistent with the breeding records at de Wildt.

The study clearly indicated the efficiency of parentage testing using microsatellite markers from the domestic dog and the possible use in evaluating the actual reproductive success of a dominant pair as well as the potential extra-pair mating in a pack of wild dogs.

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Applying horse genetic markers to endangered Cape mountain zebra (*Equus zebra zebra*) populations affected with sarcoid tumour

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Recently, sarcoid tumours have been reported in a few populations of the rare and endangered Cape mountain zebra (CMZ). The CMZ populations are considered to have been established from a very small genetic pool and are possibly very inbred. Reports of associations between the major histocompatibility complex (MHC) genes and sarcoid tumours in the horse[1] led us to investigate possible associations between the levels of inbreeding in the affected CMZ populations and the expression of the sarcoid tumours by using equine genetic markers. Microsatellites are short repetitive stretches of nucleotides, distributed throughout the genome and have been widely used in conservation genetics, population genetics and forensics[2]. A total of 13 such microsatellite markers, routinely used for parentage analysis in horses by the Veterinary Genetics Laboratory, Onderstepoort, were employed for investigations into the affected Gariep and Bontebok CMZ populations.

Polymerase chain reaction (PCR) was performed on DNA extracted from zebra blood using the GeneAmp 9700 system™ (Applied Biosystems). Out of the 13 horse markers, 11 amplified in the zebra. Fragment separation was performed on an ABI PRISM™ 310 Genetic analyser and the output data analyzed using *STRand* software (Board of Regents, University of California) on a PC. Allele frequency data, heterozygosities and inbreeding coefficients of the affected populations were calculated using CERVUS[1] and GENEPOP[3]. These were then compared with the equine research centre Thoroughbred database for the same loci.

The results indicate that the CMZ populations expressing the sarcoid tumour have extremely reduced mean number of alleles per locus and show significantly reduced heterozygosity. Allele frequency-based correlation and heterozygosity analysis based on Weir and Cockerham's *f*-statistics estimates (F_{IS}) reveal high levels of within-population inbreeding[4].

It was thus proved that horse genetic markers could be applied for genetic studies on zebra populations. The data generated from these markers demonstrated that the sarcoid tumour- affected CMZ populations are highly inbred.

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The development of a DNA probe to detect *Babesia felis*

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The identification of *Babesia felis* is generally done by morphology and serology. These methods are not reliable especially in the detection of parasites with similar morphology. This was clearly illustrated with the isolation of a small piroplasm that was isolated from lions (*Panthera leo*). Although it was morphologically similar to *B. felis* it was serologically and phylogenetically distinct. A need for the development of more specific and sensitive methods for the identification of parasites therefore exists.

The Reverse Line Blot (RLB) hybridization assay is a technique that can simultaneously detect the genus and differentiate between the species of haemoparasites in blood, organs and ticks. This assay is based on the PCR amplification of DNA of related parasite species and the hybridization of the PCR amplicons to a membrane that contains species-specific oligonucleotides (probes). Sequences of the 18S ribosomal RNA (rRNA) of *B. felis* as well as *Babesia leo* were used to develop a *B. felis*-specific diagnostic DNA probe for use on the RLB.

Blood samples of various felid species were collected in EDTA, and stored at -20 °C. Filter paper with blood spots was also collected and stored in a dry place at room temperature. DNA was extracted from whole blood using the commercially available QIAamp DNA Mini Kit (Qiagen, Southern Cross Biotechnologies), and from blood collected on filter paper using the FTA extraction reagent (Whatman® Bioscience, Laboratory Specialist Services). The PCR was performed on these samples and analysed using the RLB hybridization technique.

The *B. felis* probe was used to screen samples from domestic and wild cats using the RLB with both newly developed and existing probes. This led to the identification and characterization of not only the known *Babesia* spp. of domestic and wild cats, but also indicated the presence of undescribed species. The probe did not cross-react with other organisms in the *Babesia* and *Theileria* group of organisms and proved to be specific for *B. felis*.

Prevalence of Severe Combined Immunodeficiency Disease in Arabian horses in South Africa

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Severe combined immunodeficiency disease (SCID) is an autosomal recessive hereditary disease affecting Arabian horses. The genetic defect responsible for this disease was recently identified as a 5 base pair deletion that causes a frameshift in the gene for DNA-dependent kinase, catalytic subunit (DNA-PK_{CS})[1]. Carriers for this condition are heterozygous having alleles of 158 and 163 base pairs (bp) while unaffected horses are homozygous having only the 163 bp allele. SCID has recently been genetically confirmed for the first time in South Africa. The aim of this study was to estimate the prevalence of SCID heterozygotes in Arabian horses in South Africa.

Specific primers for SCID were fluorescently labeled for inclusion in the standard genotyping panel performed by the Veterinary Genetics Laboratory for parentage verification of Arabian horses. Briefly, DNA obtained from blood or hair samples of the horses was subjected to a polymerase chain reaction (PCR) to amplify DNA fragments using fluorescently labeled primers. The products were sized using a genetic analyzer (ABI 310, Applied Biosystems). Samples from 398 Arabian horses were randomly and anonymously selected for testing.

Of the 398 samples examined, a total of 33 animals (8.3%) were identified as heterozygous carriers of SCID. Assuming random mating, the prevalence of homozygous affected SCID cases was calculated to be 0.2% (1:500).

The prevalence of SCID carriers in South Africa is similar to that reported in the United States of America (8.4%) but is substantially greater than that of 2.8% reported in the United Kingdom. The DNA test incorporated into the standard genotyping panel for Arabian horses allows breeders of Arabians to test their stock for the SCID mutation. An advantage that the DNA SCID test provides is that carriers bred only with normal horses can be confidently used in breeding programmes with no fear of producing an affected foal.

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Screening for two genetic diseases affecting calf survival in South African and Australian Brahman cattle

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South African and Australian Brahman cattle are both derived from American Brahmans, which themselves are a composite of four *Bos indicus* breeds from the Indian subcontinent. The mutations responsible for two autosomal recessively inherited diseases affecting calf survival in Brahmans have been described and screening tests for them have been developed. A congenital myasthenic syndrome (CMS), recently described in South African Brahmans, results in progressive skeletal muscle weakness beginning between birth and one month of age. The condition results from homozygosity for a deletion (470del20) in the gene encoding the epsilon subunit of the nicotinic acetylcholine receptor. Generalised glycogenosis (Pompe's disease) has been described in Australian Brahmans and is caused by one of two mutations in the acidic alpha-glucosidase gene, the most common being 1057delTA. The usual clinical manifestation is progressive skeletal muscle weakness and death soon after weaning.

Screening for the 470del20 and 1057delTA mutations is currently being conducted on South African Brahman herd sires using PCR with fluorescently labelled primers and sizing by capillary electrophoresis. Screening for the 470del20 mutation in Australian Brahmans was conducted on a random selection of stored DNA samples that had previously been screened for the 1057delTA mutation, as well as samples from 126 Brahmans intended for importation to Australia from the USA between 1995 and 1997, and 300 samples submitted from the Americas and South Africa between 1997 and 2003. Genotypes were determined by PCR followed by electrophoresis in ethidium bromide-stained agarose gels.

To date, approximately 600 South African and 1 000 Australian registered Brahmans, predominantly herd sires, have been genotyped. The results so far indicate that the prevalence of 470del20 (CMS) heterozygotes is approximately 2% in South Africa, while only two carriers have been found in Australia. The prevalence of 1057delTA (Pompe's disease) heterozygotes in South Africa appears to be approximately 6%, compared with the estimated 12% in Australia. Several carriers of the 1057delTA mutation, but none of the 470del20 mutation, were found amongst the animals intended for importation from the USA. Examination of pedigrees indicates that both mutations were probably introduced into South Africa and Australia by imported animals, most likely from the USA.

Under an assumption of random breeding, out of every 10 000 pedigree Brahman calves born in South Africa, approximately 200 will be carriers of the 470del20 mutation and one will be affected with CMS; approximately 580 calves will carry the 1057delTA mutation while 9 will be affected with Pompe's disease. However, this may be a conservative estimate as the practice of line breeding tends to increase inbreeding, with an associated increase in the frequency of homozygous recessive alleles. Although the overall economic impact of the two conditions is not likely to be large, individual herds with a higher prevalence of carriers may be adversely affected. The availability of screening tests for the two mutations now enables accurate selection against the diseases.

Towards a DNA vaccine for heartwater

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Heartwater is a tick-borne disease of ruminants that is caused by the intracellular rickettsia, *Ehrlichia* (formerly *Cowdria*) *ruminantium*. The disease occurs throughout Africa south of the Sahara and is present in the Caribbean where it poses a threat of spreading to the American mainland. The current "vaccine" involves infection of the animals to be vaccinated with live organisms followed by antibiotic treatment. There are a number of drawbacks associated with such a vaccine strategy and there is an urgent need for a new and improved vaccine.

Protective immunity to heartwater is engendered by a cellular immune response. At present there are no effective tools for identifying the genes that code for *E. ruminantium* proteins that induce cellular immunity. We have initiated a genome sequencing project which will identify all *E. ruminantium* genes; when the genome is complete, we intend to mine it for genes that may code for useful candidate vaccine antigens. In the initial stages of the project, a number of large clones were sequenced, including a 20 kb clone from an *E. ruminantium* library constructed in a cosmid vector. Eleven open reading frames (ORFs) were identified and cloned into a DNA vaccine vector, pCMViUBs. The cloned genes were inoculated into mice and four potentially protective ORFs were identified. These four genes are associated with a locus that is involved in transport of an essential nutrient required by *E. ruminantium*.

A cocktail of these four ORFs was used to immunise five sheep. Five negative control sheep were inoculated with empty vector, and a positive control group was infected and treated. The sheep were boosted twice and lymphocyte proliferation assays were performed weekly after the last boost. No lymphocyte proliferation was observed in negative control sheep, while four out of the five positive control sheep showed lymphocyte proliferation. Lymphocyte proliferation was found in four out of the five sheep inoculated with the ORF cocktail. Five weeks after the final boost, the sheep received a lethal intravenous *E. ruminantium* challenge. All of the positive control sheep were solidly immune. All negative control sheep developed symptoms of heartwater and intervention with liquamycin was necessary. Despite antibiotic treatment, one of the negative control sheep died. Sheep inoculated with the ORF cocktail exhibited mild heartwater symptoms. Their temperatures were elevated, but none was over 41.7°C. None of these sheep stopped eating and antibiotic intervention was not necessary. All five sheep survived a lethal needle challenge. A second trial in sheep indicated that the results were repeatable, and sheep were protected against both homologous and heterologous needle challenge with five other strains of *E. ruminantium*. Five sheep immunised with the ORF cocktail were subjected to a virulent heterologous tick challenge, but only one animal survived. This may indicate that tick challenge is more virulent than needle challenge and a protein boost may be required to improve the ability of the DNA vaccine to protect against field challenge.

The *Ehrlichia ruminantium* genome sequencing project

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Heartwater is a tick-borne disease of ruminants caused by the obligate intracellular rickettsia, *Ehrlichia ruminantium*. The disease leads to serious economic losses of livestock throughout sub-Saharan Africa and in the Caribbean. All domestic ruminants are affected, and 80% - 95% of naïve animals die within three weeks. Animals which do recover are immune to subsequent challenge with the homologous genotype, and the problem of how to stimulate this immunity by means of a vaccine has been studied at Onderstepoort for nearly 80 years.

The protective immunity is T cell mediated, and there are currently no directed strategies which allow the identification of the genes coding for the proteins which stimulate this immunity. DNA vaccination, however, allows us to test genes *in vivo* for their ability to stimulate protection against the organism. The question of which genes to test then leads inexorably to the realisation that one needs the full genome sequence of the organism.

The *Ehrlichia ruminantium* genome project was first proposed in August 1995. At that time there were no completely known bacterial genome sequences, while today there are 112. Funding was obtained from the Department of Science and Technology in 1999 and a consortium was formed, led by the Onderstepoort Veterinary Institute (OVI), and including the University of Stellenbosch, CIRAD (France) and the University of Utrecht (the Netherlands).

The technical challenges posed by this organism were not trivial. It is a fragile bacterium with exacting culture requirements, it can only be grown in mammalian or tick cell lines, and is difficult to separate from the eukaryotic nuclei after cell disruption. There is a very high AT content in the genome, and clones in cosmid and BAC vectors are unstable. All these difficulties were overcome, but this inevitably slowed down the project and only about 20% of the sequence had been obtained when the first grant ended. The overseas partners then withdrew from the effort, but the DST grant was renewed, and the Onderstepoort team continued with the project, culminating in the completion of the raw sequence in August 2003.

This is the first genome to be completely sequenced in Africa, and only the second in the entire southern hemisphere. The final sequence database contains 25,156 readings, averaging 570 bases per reading, to give a genome coverage of 9.46 times over the total 1,516,263 bases. The task of annotating these data, to identify and classify all the genes and other genomic features, is now underway. The annotation team consists of scientists from the Department of Veterinary Tropical Diseases, Faculty of Veterinary Science and the Bioinformatics Unit, Faculty of Natural and Agricultural Sciences, University of Pretoria, and the Onderstepoort Veterinary Institute. We expect this work eventually to lead to the identification of effective vaccine candidate genes, which will finally enable us to market an efficient vaccine against heartwater in South Africa.

Basic electron microscopy: a picture is worth a thousand words

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Routine electron microscopy has become an under-appreciated investigative tool in the fast developing field of electron microscopy where immuno-electron microscopy, cryogenic techniques and environmental scanning electron microscopy are currently the techniques of choice. The advent of the confocal microscope, new fluorescent microscopy techniques, atomic force microscopy and immunocytochemistry at the light microscopical level also further compound the problem. This paper refutes the question whether basic electron microscopy has a right to exist or whether it is redundant. The use of routine transmission electron microscopy as a basic diagnostic tool in the veterinary context is illustrated by means of two recent case studies where transmission electron microscopy proved indispensable in confirming the initial diagnosis. In all instances samples were prepared for transmission electron microscopy using standard techniques

In the first instance two suspected cases of rabies in dogs were studied by routine light microscopy and immunocytochemical staining of tissue sections. Haematoxylin and eosin staining of the cerebellum revealed identical pale-pink intracytoplasmic inclusions in the Purkinje cells of both dogs. Immunocytochemistry identified one of the dogs as positive and the other as negative for rabies. Morphological confirmation with the transmission electron microscope was necessary to rule out rabies in the other case due to the very nature of the immunocytochemical technique where an undetected aberrant chemical or immunological reaction will produce misleading information. Electron microscopy clearly demonstrated rabies virus particles within Negri bodies in the positive case and a normally occurring intracytoplasmic structure, namely the confronting cisternae complex, in the other.

The second case concerned canine oral biopsies originating from a suspected blistering disease called epidermolysis bullosa. In this disease it is of prognostic importance to determine the exact location where the blister splits the epidermis and dermis, that is, whether it occurs within the basal cell layer above the basal lamina, between the basal cells and the basal lamina or below the basal lamina in the dermis. The basal lamina zone often appears hazy with the PAS stain and it is therefore not always effective in determining the exact level of the cleavage. In this instance electron microscopy revealed that the basal lamina was attached to the sloughed-off epidermis with the dermis devoid of any basal lamina material, pointing to the dystrophic form of epidermolysis bullosa, which has a poor prognosis. It should also be emphasised that there are numerous other examples where routine electron microscopy can be applied to obtain definite morphological information, for example sperm abnormalities, ultrastructural pathology, viral inclusions from wax embedded samples, parasite morphology and negative stain viral detection.

Routine electron microscopy can be applied to a wide variety of samples and provides important and unique clues regarding abnormal cell structure. It remains the most accurate technique for the descriptive morphology of biological material. There are also indications that ultrastructural review is superior to other techniques for diagnostic purposes and can provide timely, cost-effective and accurate information.

Effect of collection method, time and transport medium on a PCR test for *Tritrichomonas foetus* in bulls

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Tritrichomonas foetus is a flagellated protozoon causing significant reproductive losses in susceptible females. Infection in bulls is inapparent. Semen donors must be confirmed free of the disease. Samples collected for culture are susceptible to the effects of time delay, sample handling and contamination by other organisms. The objectives of this study were to investigate the effect of sampling method, the addition of guanidinium thiocyanate (GuSCN), and sample storage on the sensitivity and specificity of a PCR diagnostic test for *T. foetus*.

Five infected and eight control bulls were sampled six and ≥ three times respectively by both sheath washing and scraping. A portion of the sample was cultured, and a GuSCN solution was added to half of each sample. The GuSCN-treated and GuSCN-free samples were subjected to DNA extraction within 6 hours, 30 hours and 5 days of storage at 4 °C. DNA was isolated with GuSCN and silica. PCR was performed using primers TFR3 and TFR4 with an annealing temperature of 60°C. PCR products were analysed by electrophoresis on a 1.5% agarose gel containing ethidium bromide. A two-tailed Chi square test was used to test for differences.

Results for infected bulls are given in the table, showing the sensitivity of PCR testing of GuSCN-free samples from infected bulls. The sensitivity of culture was 83% for both sampling methods. The sensitivity of the PCR ranged from 0.9 in sheath washing samples extracted within 6 h to 0.41 in sheath scrape samples with GuSCN extracted after 5 d. Holding time reduced sensitivity for samples collected by both methods at 5 d, but there was no significant effect at 30 h. Sampling method had no effect with the exception of samples held for 5 d with GuSCN, where sheath washing gave higher sensitivity than sheath scraping. The addition of GuSCN had no effect on test sensitivity. No samples from the eight control animals subjected to any of the twelve treatments gave a positive PCR result.

The current PCR is as sensitive as culture when performed within 6 h and is highly specific. PCR tests done after 30 h will render adequate sensitivity, if serial sampling is done. The addition of the chaotropic agent guanidinium thiocyanate has no effect. PCR is more rapid than culture.

	Culture		PCR after 6 h		PCR after 30 h		PCR after 5 d	
	Wash	Scrape	Wash	Scrape	Wash	Scrape	Wash	Scrape
Positives	24	24	26	24	20	18	18	12
Number tested	29	29	29	29	29	29	29	29
Sensitivity[#]	0.83	0.83	0.9[a]	0.83[b]	0.69	0.62[a']	0.62[a']	0.41[a'b']

[#] Treatments with superscripts a and a' and b and b' differ in sensitivity $P < 0.05$

Comparison of three different media for freezing epididymal sperm from African buffalo (*Syncerus caffer*) and influence of equilibration time on the post-thaw sperm quality

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Epididymal sperm is commonly collected under field conditions and frozen as a method of genetic resource banking. Little information exists with respect to the freezing of epididymal sperm from the African buffalo. The aim of this study was to compare the effects of three extenders and equilibration times of 2 to 9 h, on the post-thaw motility and acrosomal integrity of epididymal sperm of the African buffalo. The extenders are commercially available and easy to use in the field.

The epididymides of six adult bulls were removed and flushed with air within 10 minutes of culling. Samples were split in three aliquots and diluted with either Triladyl™ AndroMed® (both Minitüb, Germany) or Red Ovine Freezing Buffer (ROFB) (IMV, France). Sperm was loaded into 0.25 mL French straws, cooled to 4°C over 1 h and equilibrated for a total time of 2 to 9 h before being frozen. Sperm motility was evaluated immediately after flushing, and 0, 1 and 2 h after thawing. Post-thaw acrosomal integrity was assessed on FITC-PNA-stained wet preparations. A one-way-repeated-measures-ANOVA with bulls as subjects was used to test for differences among extender-time combinations. Interactions between extender and time were not considered. Tukey's test was used to identify different groups. $P<0.05$ denoted significant differences.

When extenders were viewed separately, post-thaw motility was similar among equilibration times of 4 to 9 h, whereas the motility was occasionally lower for shorter times. Data for shorter equilibration times were thus ignored and averages for times 4 to 9 h used for further analysis. Progressive motility immediately after thawing was higher for sperm frozen with ROFB (28.9±15.17) than for sperm frozen with Triladyl™(19.3±12.34) or AndroMed® (14.1±13.85). Progressive motility 1 and 2 h after thawing was higher for Triladyl™(21.0±14.96 and 15.8±11.32) than for the other extenders. Neither equilibration time nor extender influenced the acrosomal integrity.

Medium	Fresh		Time after thawing						Intact acrosomes
			0 hours		1 hour		2 hours		
	T	P	T	P	T	P	T	P	
AndroMed	57.2	31.0	46.1^{a}	14.1^{a}	48.3^{a}	7.1^{a}	44.7	2.5^{a}	53.0
	(17.47)	(21.43)	(13.15)	(13.85)	(12.07)	(10.86)	(12.07)	(5.79)	(6.83)
ROFB	58.0	24.0	56.9^{b}	28,9^{b}	58.3^{b}	10.0^{a}	51.4	2,4^{a}	55.4
	(13.92)	(15.05)	(11.91)	(15.17)	(15.40)	(12,56)	(15.70)	(6.38)	(5.91)
Triladyl	55.7	19.2	53.9^{a,b}	19.3^{a}	55,6^{a,b}	21.0^{b}	49.2	15.8^{b}	56.5
	(13.37)	(10.85)	(13.37)	(12,34)	(12.75)	(14.96)	(12.04)	(11.32)	(6.50)

Note: T = total motility, P = progressive motility. ROFB = Red Ovine Freezing Buffer. Values in a column with different superscripts differ ($P<0.05$).

This study shows that any equilibration time between 4 and 9 h may be used before freezing epididymal sperm from African buffalo and that Triladyl™is more suitable than AndroMed® or ROFB for the freezing of such sperm because Triladyl™results in higher percentages of progressively motile sperm 1 or 2 h after thawing. Others have shown that glycerol for more than 4 h is detrimental to ejaculated bull semen, which contrasts with the findings of the present study on epididymal buffalo sperm.

Effect of bovine seminal plasma on the ability of buffalo (*Syncerus caffer*) spermatozoa to fertilise bovine oocytes in vitro

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Oocytes from African buffalos (*Syncerus caffer*) are scarce and an attempt was therefore made to use bovine oocytes to characterise buffalo semen for the use in an in vitro fertilisation system. Our previous work showed that the fertilisation rate was between 0% and 3%. The aim of this study was to test if bovine seminal plasma could improve the fertilisation rate of bovine oocytes by buffalo spermatozoa in vitro.

Oocytes with an intact corona radiata and at least 3 layers of compact cumulus cells were selected from ovaries from a local abattoir and matured for 22 h under mineral oil in 50 µL droplets (10 per droplet) of TCM 199 (Earle's salts) supplemented with 25 mg/ml gentamycin, 100 µM 2-mercaptoethylamine, 25 mM hydroxymethyl piperazine-N-2-ethane sulphonic acid (HEPES) and 5% steer serum at 39°C in 5% CO_2 in air with 100% humidity. Matured oocytes were fertilised with $1x10^6$ spermatozoa per droplet in a modified tyrode-albumin-lactate-pyruvate (TALP) medium including 25 µg/ml gentamycin, 0.6% essentially fatty acid free bovine serum albumin, PHE (2.0 mM penicillamine, 1.0 mM hypotaurine and 25 µM epinephrine) and 30 µg/ml heparin. Epididymal and ejaculated sperm, each from 3 different African buffalo bulls (*Syncerus caffer*), frozen-thawed in 0.25 ml straws, was swim-up separated in either 1 mL fert-wash (same as the fertilization medium without PHE and heparin) or in 0.5 mL fert-wash and 0.5 mL seminal plasma from one of 10 dairy or beef bulls. Sixty oocytes were used per treatment. A control bull was used with each run. After 20 h the oocytes were fixed for 48 h in 1:3 acetic acid:methanol, stained with 1% aceto orcein and viewed at 20 to 40x using Nomarski optics. Oocytes were classified as degenerated (Deg); arrested at metaphase II (Met II); one pro-nucleus (PN); fertilised (2PN) or polyspermic (Pol). The effect of the seminal plasma was tested by repeated measure ANOVA with semen source (ejaculated or epididymal) and seminal plasma donor as main effects and buffalos as subjects (nested in semen source). $P<0.05$ denoted significant differences).

There was no difference between ejaculated and epididymal sperm. The addition of seminal plasma had a significant effect on Deg, Met II, 2PN and Pol. Without and with the addition of seminal plasma there were 5.5±2.39 (mean±SD) and 10.2±4.40 Deg, 50.8±3.19 and 10.5±5.00 Met II, 1.0±1.30 and 35.2±5.78 2PN and 0±0.00 and 2.1±1.55 Pol respectively. For the control bull there were 13.8%±3.13% Met II and 72.3%±4.17% 2PN. The plasma donor had a significant effect on MII and Pol and the buffalo bull on Deg, 2PN and Pol.

There appears to be a "fertility factor" in bovine seminal plasma, which is absent in the tested buffalo bulls. The concentration and or the structure of this "fertility factor" may be different in individual beef and dairy bulls' seminal plasma. Buffalo appear unable to fertilize bovine oocytes without this "fertility factor". Further studies are needed to identify the "fertility factor" and to assess the mechanism by which it allows the fertilisation of bovine oocytes by buffalo spermatozoa. This "fertility factor" could be responsible for the variable ability of individual bulls' spermatozoa to fertilise oocytes *in vitro* and it could further contribute to the difference in fertility in general.

Association of Foot and Mouth Disease virus with bovine oocytes during *in vitro* maturation

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The risk of Foot and Mouth Disease virus (FMDV) transmission by *in vivo* produced embryos is extremely small. *In vitro* produced (IVP) embryos carry a greater risk of transmitting FMDV. IVP day 7 embryos, exposed to FMDV, could not be freed of the virus by washing. Exposing oocytes to FMDV during *in vitro* maturation (IVM) and *in vitro* fertilization (IVF) would give a more realistic model to judge if oocytes collected from infected donor cattle pose a risk of transmitting FMDV. This is of importance, since high virus concentrations were found in follicular fluid of cows in the viraemic stage of the disease. The aim of this study was to test if bovine oocytes, which are co-incubated with FMDV during IVM, can be washed free of the virus after maturation.

Five batches of 200 oocytes each, with an intact corona radiata and at least three layers of compact cumulus cells (COC) were obtained from ovaries from a local abattoir. Each batch was matured for 22 h in TCM 199 (Earle's salts) supplemented with 25 µg/ml gentamycin, 100 µM 2-mercaptoethylamine, 25 mM hydroxymethyl piperazine-N-2-ethane sulphonic acid (HEPES), 5% steer serum and $2x10^6$ tissue culture infective dose 50 ($TCID_{50}$) FMDV at 39°C in 5% CO_2 in air with 100% humidity. Half the mature oocytes from each batch were kept as entire cumulus oocyte complexes (COC's) and the other half were denuded of cumulus cells by vortexing. Each group was further divided into two equal groups, 50 being used for virus detection by pig kidney monolayers (PKM) and 50 for PCR should the PKM result be negative. All COC's were washed 10 times in 300 µL IVF medium; i.e. modified tyrode-albumin-lactate-pyruvate (TALP) including 25 µg/ml gentamycin, 0.6% essentially fatty acid free bovine serum albumin (BSA) and PHE (2.0 mM penicillamine, 1.0 mM hypotaurine and 25 µM epinephrine). One group of 50 COC's was put in 1 mL of phosphate buffered saline (PBS) for virus detection on PKM and the other group washed and put in 200 µL PBS to be analysed by PCR. The denuded oocytes were treated in the same way. The IVM fluid of each batch, containing the cumulus cells, was also analysed for the presence of FMDV.

FMDV was detected in all samples on PKM; therefore no PCR tests were run. After 24 h of incubation the cytopathic effect was, subjectively assessed, distinctly less severe for the denuded oocytes than for the other samples.

This trial shows that in vitro matured bovine COC's, cumulus cells and denuded oocytes, which are matured in the presence of FMDV are not free of the virus after washing them 10 times. The results do not indicate whether the FMDV infects the cumulus cells and the oocytes, if it does only adhere to their surface or if 10 washes are not enough to remove all the IVM fluid containing the virus. Low pH is used to destroy viral particles in cell culture media without affecting internalised virus. In a follow up trial, COC's, cumulus cells and denuded oocytes should be exposed to an acidic environment for a few seconds. At pH 5 the FMDV is inactivated at a rate of 90% per second. Assuming that FMDV does not penetrate the zona pellucida it is likely that exposing oocytes to an acidic environment would render denuded oocytes or embryos free of the virus without destroying them.

The use of plasma progesterone concentration to predict the optimal breeding time in bitches six days in advance

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Practicing veterinarians and owners need a method whereby they may find out in advance when a bitch should best be inseminated. Such predictability has various advantages: it will facilitate the use of chilled semen because it provides an early warning when the semen has to be collected, chilled and shipped; it also provides owners with an early indication when they should transport animals for mating. Such predictability may also reduce the number of visits to a veterinarian during the heat period of a bitch. The sooner one can predict when to inseminate a bitch, the greater these benefits. The identification of the LH peak may provide such an advanced warning but LH assays are not generally available. In contrast, sensitive and accurate assays to determine the concentration of progesterone in serum or plasma (PPC) are more generally available. It has been shown that PPC starts rising concomitantly with the onset of the LH surge in bitches. The aim of this study was to determine whether the first rise above 6 ηmol/L of PPC can be used to predict when to best inseminate bitches.

Fourteen bitches of nine breeds of a variety of sizes were each used during one estrous cycle. The heat periods were monitored daily throughout pro-estrus and estrus by means of vaginal cytology and vaginoscopy. PPC was usually measured daily but sometimes with two-day intervals starting during late pro-estrus and ending in late estrus. The first decrease in edema of the vaginal mucosa, characterized by shrinking rounded folds, was evaluated as a predictor of the period during which PPC was likely to start rising. One bitch that was inseminated once only, 6 d after the rise in PPC and the others were inseminated twice with a one-day interval between inseminations. The first insemination was done four (one bitch), five (3 bitches), six (nine bitches) or seven days (one bitch) after the initial rise of PPC. Frozen-thawed semen was deposited into the uterus in all cases. Semen quality and source varied.

Eight bitches were inseminated 6 and 7 days after the onset of the rise and all conceived with a mean litter size of 5.6 (SD 3.11). Thirteen bitches were inseminated 5 days or more after the onset of the rise and all conceived with a mean litter size of 6.0 (SD 2.71). In the 10 bitches where all data are available, the edema in the vagina started to decrease 1.3 (SD 1.75) days before the rise in PPC, with a range of three days before to 2 days after the first decrease in edema. In these 10 bitches the edema in the vagina had sufficiently decreased to cause angularity of the mucosal folds 3.0 (SD 1.17) d after the rise in PPC. Bitches whelped 65.0 (SD 2.00) d after the rise in PPC, 59.3 (SD 2.07) d after the first insemination and 55.9 (SD 1.40) d after the onset of cytological dioestrus. The interval between the first decrease in edema and whelping was 63.6 (SD 5.10) d whereas the interval between the onset of angularity of the vaginal folds and whelping was 60.8 (SD 3.96) d.

This study indicates that it may be suitable to inseminate bitches six and seven days after PPC first exceeds 6 ηmol/L. Vaginal oedema starts to decrease at least four days before the first insemination is due, making vaginoscopy a suitable tool with which to determine when to start measuring PPC.

The significance of serial exhaustive extraction in isolating antibacterial compounds from *Combretum imberbe*

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Hypocrateropsis is a section of the genus Combretum with four taxa in Southern Africa (*Combretum imberbe, Combretum padoides, Combretum celestroides* subsp *orientale* and *Combretum celestroide* subsp *celestroides*). Only *Combretum imberbe* and *Combretum padoides* had significant antibacterial activity (minimum inhibitory concentrations of 0.04 mg/ml and 0.08 mg/ml respectively). As a first important step in a bioassay guided fractionation we used *Combretum imberbe* leaf powder to compare two extraction methods; the direct extraction of leaves with acetone (DEA) and serial exhaustive extraction (SEE) using attractants of increasing polarity.

Leaf material of plants was collected from the Pretoria and Lowveld National Botanic Gardens. Dried, powdered leaf materials were extracted directly with acetone and serially with hexane, dichloromethane, acetone and methanol. Solvent-solvent fractionation process was used to fractionate the active extracts. Bioautographic procedures using 2 mg/ml p-iodonitrotetrazolium salt on thin layer chromatography (TLC) chromatograms of extracts was performed at each level of fractionation to ascertain the presence and the activity of antibacterial compound(s) using *S. aureus* (ATCC 29213), *P. aeruginosa* (ATCC 27853), *E. coli* (ATCC 25922) and *E. faecalis* (ATCC 29219). The minimum inhibitory concentration (MIC) values of active fractions and isolated compounds were determined using a serial dilution microplate assay with gentamycin as positive control.

Most of the activity was present in the dichloromethane (DCM) extract. Subsequent solvent-solvent fractionation of the DCM extract from SEE gave better results than that obtained with acetone from DEA based on TLC chemical profiles and antibacterial compounds present.

A known pentacyclic triterpenoid (imberbic acid) with MIC values on *S. aureus* and *E. faecalis* (>0.63mg/ml) and 0.08mg/ml) greater than that of the crude extract (0.039 mg/ml and 0.078mg/ml) was isolated with relative ease using the SEE approach. The prior defatting step by hexane in the SEE process may have made antibacterial compounds more extractable by DCM leading to a less complex extract, and allowing the easy isolation of active compounds on the first Silica Gel60 column. SEE may be a better initial step for the isolation of antibacterial compounds than DEA.

Isolation and biological activty of three antibacterial flavanoids from *Combretum apiculatum* subsp. *apiculatum*

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In a systematic investigation of the antibacterial activity of members of the Combretaceae, C*ombretum apiculatum* subsp. *apiculatum* was the first member of the Section Ciliatipetala of the genus *Combretum* to be investigated. This plant is a tree of up to 10 m high, that occurs widely in the northern parts of South Africa and has been used for treating snakebites, diarrhoea, conjunctivitis and abdominal disorders.

To determine the best extractant, the efficacy of several extractants for the extraction of antibacterial compounds from dried ground *C. apiculatum* subsp. *apiculatum* leaves was determined. Ten different solvents with varying polarity were investigated. Extractants with intermediate polarity were the most effective. After examining the antibacterial activity, chemical complexity of different extracts, as well as the number of antibacterial compounds detected by bioautography, acetone was selected as the best extractant.

Bioassay guided fractionation was used to isolate the antibacterial compounds. An acetone extract of dried leaves was fractionated by solvent-solvent extraction, silica gel column chromatography and fractional crystallisation. Three compounds were isolated by bioassay-guided fractionation. The chemical structures were determined by nuclear magnetic resonance spectroscopy and mass spectroscopy. The compounds isolated were flavonoids previously isolated from other plants but not from the Combretaceae. The minimum inhibitory concentration (MIC) of the three compounds alpinetin, pinocembrin and flavokawain-A were determined by a serial dilution microplate method and varied from 40 to 600 µg/ml for S*taphylococcus aureus, Enterococcus faecalis, Pseudomonas aeruginosa* and *Escherichia coli.* The MIC values of the isolated compounds were not much lower than the MIC values of crude extracts. Because the relative to the front (Rf) values of the isolated compounds coincided with the Rf values of bioactive compounds present in the crude extract, this may mean that the main antibacterial compounds were isolated, but that a synergistic effect may be present in the crude extracts.

The antibacterial compounds isolated from this plant varied substantially from those isolated from other sections of the genus *Combretum*. The chemical data and biological activity supports the taxonomic delineation based on morphological characters and supports the approach to investigate different members of the Combretaceae.

Can the use of *Ziziphus mucronata* extracts for treating bacterial infections be justified?

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Ziziphus mucronata is widely distributed in southern Africa and appears to be widely used for the treatment of infectious diseases. The root infusions are used for the treatment of diarrhoea, dysentery, lumbago and snakebite. Leaf paste is used for the treatment of boils and other skin infections. The plant is also used for treating animals in Northern Province.

In an effort to discover possible useful antibacterial compounds from plants, dried ground leaves of *Ziziphus mucronata* were serially extracted with solvents of increasing polarity (hexane, dichloromethane, acetone and methanol). To determine the number of antibacterial compounds and the relative to the front (Rf) values, extracts were separated by thin layer chromatography. The chromatograms were dried overnight and sprayed with a dense culture of *Staphylococcus aureus*. After overnight incubation chromatograms were sprayed with tetrazolium violet. Clear bands indicated inhibition of growth. For quantitative data minimum inhibitory concentrations [MIC] were determined with a serial dilution microplate method.

There were two major and at least five minor antibacterial compounds present in the leaf extract. The crude extracts from the hexane and dichloromethane (DCM) inhibited the growth of *Staphylococcus aureus,* with the highest activity being observed for the DCM extract. Minimum inhibitory concentration (MIC) values varied from 0.04 to 2.5 mg/ml for the different test organisms. The Gram-positive pathogens (*S. aureus and Enterococcus faecalis*) were more sensitive than the Gram-negative organisms (*Pseudomonas aeruginosa and Escherichia coli*). No antibacterial activity was observed for the subsequent acetone and methanol extracts.

These preliminary antibacterial activity studies confirm the rationale of using *Z. mucronata* extracts topically for boils and skin infections, although the bioactive compounds are non-polar and not likely to be available in aqueous extracts. We are in the process of determining the chemical structure of the antibacterial compounds isolated from leaf extracts.

Efficacy of herbal extracts against pathogens of production animals

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There is an increased concern about the prophylactic use of antibiotics in food producing animals such as cattle, pigs, and chickens. In some cases the antibiotics used for meat producing animals are the same class of antimicrobial agents that are used for humans. The major issue here is the increase in cross-resistance of antibiotics used for both animals and humans. The problem that we might be facing after administering antibiotics as a feed additive is that there might be some residues in the meat. This may be consumed by humans and may lead to antimicrobial resistance in human pathogens. The European Union intends to ban the use of all antibiotic feed additives in 2006. Worldwide, scientists are working on finding replacements for antibiotic feed additives.

We have previously screened many herbal medicines that are non-toxic to humans for antibacterial activity. The objective of the project is to determine the antibacterial activity of the plant extracts using different extractants against standard bacterial isolates (American Type Culture Collection strains) and bacteria isolated directly from poultry. Eight bacterial isolates of clinical significance were used, employing a serial plate microdilution assay and bioautography. Six plants were used to test for antibacterial activity, comparing different method of extraction and extractants of varying polarity. The identities of the plants and the extraction procedures have to be kept confidential for intellectual property and possible subsequent commercialisation reasons.

The antibacterial activity results indicate, that for all the plants tested, the more polar extracts showed the highest activity against both Gram-positive and Gram-negative organisms. When comparing the *E. coli* strains (1 ATCC strain and 2 strains isolated from poultry), the ATCC strain was more resistant to most of the plant extracts tested, while the chicken isolates were more susceptible. The *Salmonella* isolates were more susceptible to the non-polar extractants. *Pseudomonas aeruginosa* was more resistant to the plant extracts than any of the other bacterial strains tested.

After developing the most active extracts against the clinical pathogens, toxicity trials and feeding trials in animals will be considered.

Screening of antifungal activity of members of the Combretaceae for selection of plants for detailed work

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Members of the Combretaceae are used for many medicinal purposes by traditional healers. They include treating abdominal disorders, abdominal pains, backache, bilharziasis, chest coughs, colds, diarrhoea, dysmenorrhoea, fever, headache, hookworm, leprosy, pneumonia, scorpion bite, snake bite, syphilis, toothache, gastric ulcer, venereal diseases, heart diseases, cleanse the urinary system, sore throats, nosebleeds and general weakness. The Combretaceae consists of 18 genera, the largest of which are *Combretum*, with about 370 species, and *Terminalia*, with about 200 species. Species from the genus *Combretum* and to a lesser extent *Terminalia* are most widely used for medicinal purposes. These genera are widespread in part of Africa, and they are also distributed over southern Africa. With the increasing acceptance of traditional medicine as an alternate form of health care, the screening of medicinal plants for bioactive compounds has become important. There have been reports of antifungal activity in members of the Combretaceae, but there have been no follow up studies. The aim of this project was to expand our research in the Combretaceae family by also investigating antifungal activity.

As a first step in the isolation of antifungal compounds from *Combretum* and *Terminalia* species, twenty four *Combretum* species and six *Terminalia* species were screened for antifungal activity. Dried powdered leaves were extracted with acetone, hexane, dichloromethane and methanol. Chemical constituents of the extract were analysed by thin layer chromatography (TLC) using three eluents. For detection of chemical compounds chromatograms were sprayed with a vanillin sulphuric acid spray reagent and 0.2 % DPPH to screen for antioxidant activity. Bioautography procedures using 2 mg/ml p-iodonitrotetrazolium (INT) were performed to determine the number of antifungal compounds of extracts against *Candida albicans*, *Aspergillus fumigatus*, *Cryptococcus neoformans*, *Sporothrix schenkii* and two dermatophytes, *Trichophyton mentagrophytes* and *Microsporum canis*. Minimum inhibitory concentrations (MIC) were determined by a serial dilution microplate method using amphotericin B as a standard antibiotic control. As a first step extracts of the following plants were examined: *C. celastroides, C. imberbe, C. padoides, C. caffrum, C. erythrophyllum, C. krausii, C. woodii* and *C. collinum*.

Initially there were complications with the bioautography, as the time of incubation appears to be more critical than with bacterial cultures especially with *A. fumigatus*. Acetone and methanol were the best extractants indicating that in the Combretaceae more polar compounds are antifungal whereas more non-polar compounds appear to be antibacterial. Most of the extracts had some antioxidant activity (TLC chromatograms sprayed with 0.2 % DPPH). Many extracts had in the order of three antifungal compounds based on bioautography results. The antifungal activity was much higher than the antibacterial activity with many extracts having MIC values of 0.08 mg/ml.

The results show the potential value of bioprospecting for antifungal compounds in the Combretaceae.

Isolation and characterization of antibacterial compounds from *Cussonia* species (Araliaceae)

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The genus *Cussonia*, called "umbrella tree" (Araliaceae), which is represented by 40 species, has its center of distribution in tropical areas in southern Africa and Madagascar. Some species have been used to treat acne, diarrhea, syphilis, mental diseases, malaria and stomach ulcers. *Cussonia paniculata* has been reported to provide good fodder for stock and the Zulu name (Umsenge) refers to this as goat's food. The thick root can be peeled and eaten raw as an emergency food or source of water and the Vhavhenda use the bark to treat stomach ulcers and for magical purposes. It has also been used for treating animals in the northern parts of South Africa for infection-related diseases. A preliminary survey indicated that extracts have some antibacterial activity. However, there is little background information on the antibacterial activity of this plant in the literature. In this paper the antibacterial activity of *Cussonia spicata* and *C. nicholsonii* was examined.

Ground dried leaves were extracted using acetone, hexane and methanol. The chemical composition was determined by thin layer chromatography using ethyl acetate-methanol-water, chloroform-ethyl acetate-formic acid and benzene-ethanol-ammonium hydroxide as eluents and vanillin-sulphuric acid as detecting agent. Antibacterial activities were determined by bioautography and minimum inhibitory concentration (MIC) values were determined using a microplate serial dilution technique with *Escherichia coli* (ATCC 25922), *Staphylococcus aureus* (ATCC 29213), *Pseudomonas aeruginosa* (ATCC 27853) and *Enterococcus faecalis* (ATCC 29219) as test organisms. Gentamycin was used as a control for MIC tests. Antioxidant activity was evaluated by spraying the developed TLC plates with DPPH.

According to the TLC chromatograms, there was a large difference in the chemical composition between the same species collected from different places. One or two bands indicated slight antioxidant activity. In the order of six bands from each extract showed antibacterial activity against the standard indicator organisms by the presence of clear zones on the chromatograms. *Cussonia spicata* extracts were more active against *E. coli* and *P. auruginosa* than *C. nicholsonii* extracts while *C. spicata* collected from the Onderstepoort campus had higher antibacterial activity than *C. spicata* collected from the main campus of the University of Pretoria. In contrast to the situation with most other plant extracts, the MIC values for Gram-negative organisms was lower and ranged from 0.078 to 1.25 mg/ml, whereas the values for Gram-positive bacteria ranged from 0.312 to 1.25 mg/ml. The total activity of the acetone extracts of *Cussonia spicata* collected from Onderstepoort campus was 186 ml against *E. coli* and *P. aeruginosa*.

In the next phase of this project different *Cussonia* spp. will be evaluated quantitatively and qualitatively for antibacterial activity before selecting the best species from which to isolate the most important antibacterial compounds.

Control of ovine hepatic metabolism by cell volume

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The ruminant liver must balance the nitrogen requirements for the entire animal. Furthermore, the rate of gluconeogenesis from amino acids is greatest after a meal and declines during fasting, inverse to that in monogastric animals. This may be due partly to osmolarity changes in portal blood induced by the uptake of nutrients. The isolated, perfused ovine liver has been validated as a model to investigate hepatic metabolism. We therefore investigated the effect of changing the osmolarity of the perfusion medium on the uptake of ammonia, and the output of urea and glutamine using this model.

South African Mutton Merino wethers (24-35 kg live weight) fed a mixed diet of lucerne and tef hays served as organ donors. Each caudal lobe was removed under full surgical anaesthesia, after perfusion (Krebs-Henseleit medium) was initiated, and transferred to the tissue bath for perfusion. The buffer osmolarity was raised from 300 to 330 mOsm/l by the addition of sucrose. The concentrations of ammonia, urea, glucose, and glutamine and glutamate were determined by spectrophotometric analysis. The data were statistically analysed using Student's t test or an analysis of variance.

At 300 mOsm/l, the capacity of the liver to take up ammonia saturated at a rate of about 1200 nmol/g/min, similar to *in vivo* data. However, at 330 mOsm/l, the maximum rate increased significantly to almost 1900 nmol/g/min. This implies that the raised osmolarity of portal blood (absorbed nutrients at peak fermentation) will assist the liver in clearing excess ammonia from the circulation. There was an obligatory production of urea by the liver (120 nmol/min/g), even when no exogenous ammonia was added, increasing linearly thereafter with further addition of ammonia. This production rate did not plateaux, even though ammonia uptake was saturated at the highest ammonia concentration. Raising the buffer osmolarity initially suppressed obligatory urea production, which nevertheless responded to increased ammonia uptake by the lobe. Once again, there was no sign of saturation. Glutamine production was considerably lower than that of urea, although similar in pattern. At 300 mOsm/l, the rate increased only 50% over the basal rate at the highest ammonia concentration tested. However, when osmolarity was increased to 330 mOsm/l, the glutamine production rate increased dramatically (48 to 177 nmol/g/min).

This model can be used to examine the role of cell hydration in mediating hormone and amino acid effects on the metabolism of the hepatocyte, free from systemic interference. These results suggest a prime role for cell volume in the regulation of nitrogen metabolism in the sheep.

Early development of digestive function in the ostrich (*Struthio camelus*)

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The gastrointestinal tract of the adult ostrich resembles that of the ruminant. The effects of adverse nutrition are most pronounced in early life and may partly cause the high mortality rate in the neonatal ostrich. The objective of the present study was to evaluate changes in body growth in relation to changes in the development of the various regions of the digestive tract of the young ostrich.

Ostrich chicks (3 to 72 days old) were fed pellets (Starter, followed by Grower) as a group in a heated room (slatted plastic floor) for 2 weeks, after which they were allowed outside daylight access. From the 27th day, lucerne hay was supplied *ad libitum*. The birds were slaughtered in batches of 6, and samples were collected 3, 27, 41, 55 and 72 days after hatch. The combined empty weight as well as that of the contents of the proventriculus and gizzard, small intestine, colon, caeca, pancreas and the liver was recorded. Sub-samples were taken for the preparation of homogenate and brush-border membrane vesicles, which were used to determine maltase activity. Protein concentration was measured in all tissues. The supernatant from pancreatic homogenate was analysed for lipase, trypsin and chymotrypsin activities. Liver homogenate was prepared and tested for urea and arginase activity.

Body weight increased 11-fold up to Day 72. The relative weights of the proventriculus/gizzard, small intestine and pancreas increased till Day 27, and remained constant thereafter. The weight of the yolk sac was 5 to 36 % of total body weight at 3 days of age and was absent by Day 27. The relative weights of the caeca and colon started to increase after Day 55. Gas from digesta fermentation was only produced in the hindgut segments, and then only after Day 55. The activities of the enzymes tested started high (with the exception of trypsin) and declined rapidly after Day 27. There were no significant changes in protein content over the period of investigation. Although maltase activity was present throughout the small intestine, it's relative activity declined rapidly from Day 3 to 27, remaining constant thereafter. The relative weight of the liver and its protein content increased up to Day 41, remaining constant thereafter. Arginase activity was not detected in the liver of 3-day old chicks, but was present thereafter. Urea was not detected in the liver homogenate.

The pattern of development in the present study is similar to that in broiler chickens. However, the development of the ostrich gastrointestinal tract switches dramatically from the typical bird neonate (i.e. large post-natal yolk sac) through that resembling the typical monogastric animal to that of a hindgut fermenter, all in the space of about 70 days. This radical developmental pattern may well contribute to the high mortality rate associated with commercially farmed ostrich chicks.

Faculty of Veterinary Science
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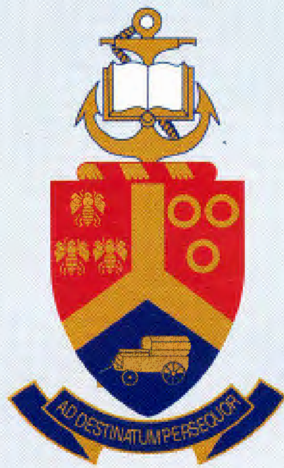
Photographic Exhibition	:	Dr E van Dyk
Marketing	:	Mrs L Euthemiou


AD DESTINATUM PERSEQUOR