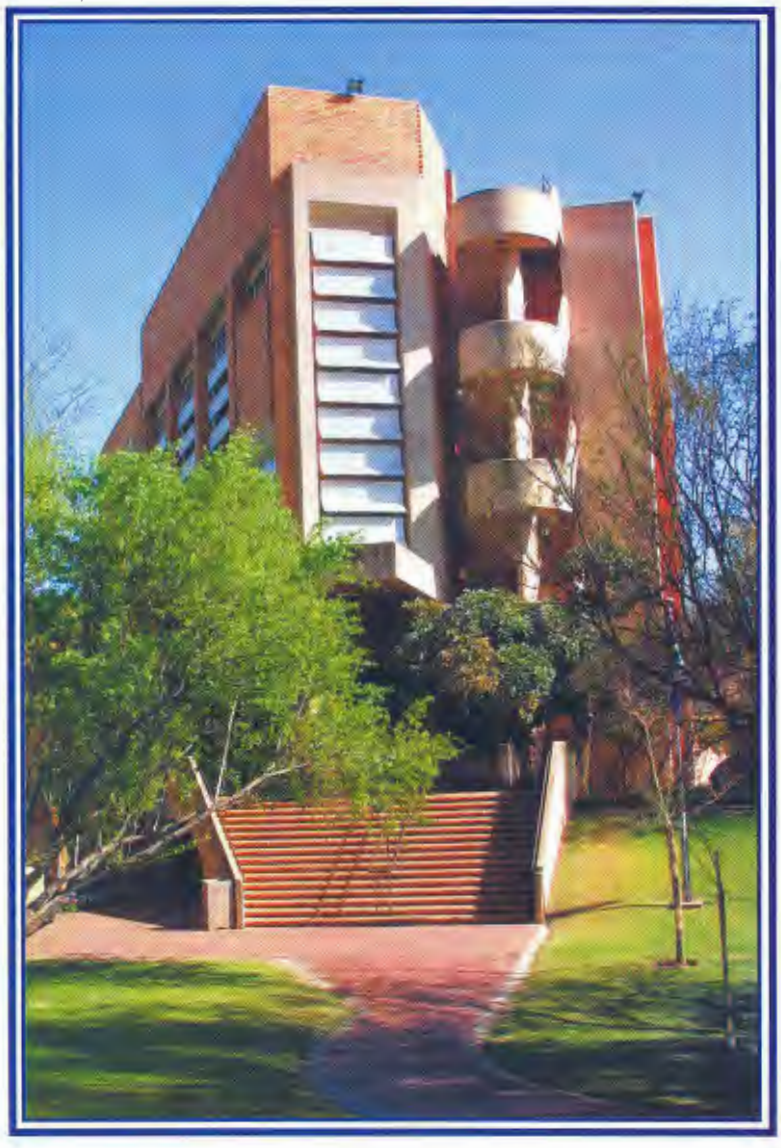
UNIVERSITY OF PRETORIA

FACULTY OF VETERINARY SCIENCE

FACULTY DAY SEPTEMBER 15, 2005

PROGRAMME AND SUMMARIES



FACULTY OF VETERINARY SCIENCE, UNIVERSITY OF PRETORIA

FACULTY DAY

15 SEPTEMBER 2005

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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 2005 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

It gives me pleasure to welcome you to this year's Faculty Day. This is one of the highlights of the Academic year at which some of the activities of the Faculty are showcased and recognition is given to excellence in teaching and in research

Excellence in research and in teaching is inextricably linked and the Faculty cannot be good in the one and lag in the other. We have international recognition for the quality of professional training here at Onderstepoort and we are working hard at obtaining the same level of recognition for our postgraduate and research programmes

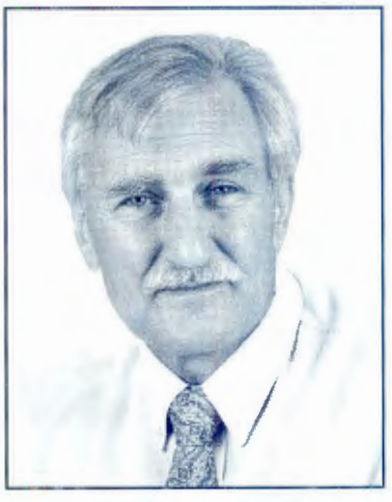
Marked progress has been made in various fields of research in the Faculty, and the volume of material has become such that competition is developing for the time available during the course of Faculty Day. This is a heartening development and should auger well for the Future

Research in the clinical fields is as important as that in the basic and preclinical disciplines. It is thus fitting that this year's Arnold Theiler lecture will be presented by Prof Sybrand van den Berg, who is one of the icons in the field of equine surgery in South Africa. He has shown that it is possible to combine a clinical career in academia with research and has established himself as one of the role models in the clinical field

I wish you a pleasant day here and trust that you will be surprised by the diversity of activities being pursued on the Onderstepoort Campus

Best wishes

NICK KRIEK
DEAN



Prof. S.S. van den Berg

PROFESSOR S S VAN DEN BERG

Sybrand Smit van den Berg was born in Lichtenburg in 1944. He matriculated from Lichtenburg High School in 1962. He received honorary colours in academics, rugby, and athletics and was head prefect. He was selected for the Western Transvaal Schools rugby team for two consecutive years and represented the Western Transvaal Athletics Team at the South African Championships in 1962.

After qualifying as a veterinarian in 1969, he practiced as a private practitioner for 8 years. He then joined the Department of Surgery at the Faculty of Veterinary Science at Onderstepoort in 1976 and was privileged to further his veterinary career as an academic. He obtained a BVSc (Hon) degree in 1981, his MMed Vet (Surg) in 1983, MMed Vet (Rad) in 1985 and DVSc in 1996. The Brahman Cattle Breeders Society acknowledged his research into the aetiology of *lamina interna* eversion and prolapse in the Brahman bull and this research achieved further acclaim from the American and Australian Breeders Societies. During his veterinary career, he received the following awards from The South African Veterinary Association: The Clinical award (1983), The President's award (1996) and Honorary Life President's award (2000). The Equine Practitioners Group Merit award (1998) and South African Farrier's Merit award (1999) followed after many years of service to the respective associations.

In 1986 Sybrand became the first incumbent of the Abe Baily Professorship in Equine Surgery. He became Head of Department of Surgery in 1986 and retired at the end of 1999. During his academic career he was privileged to be part of the teams responsible for designing the new Veterinary Academic Hospital and for restructuring the veterinary curriculum based on a species approach. He is the author and co-author of 10 publications and presented 7 papers at international congresses. Respected as a specialist in large animal surgery and radiology, he acts as a consultant to practitioners, farriers, breeders and associations. Professor van den Berg is currently the President of the South African Veterinary Council. He has been an elected member of The South African Veterinary Council since 1992 and amongst others has acted as Chairperson of the Investigation Committee for 12 years, and chaired the Code of Conduct committee. He currently serves on the hospital sub-committee and has revised the rules for veterinary facilities, and also acts as an inspector.

He is currently serving a second three-year appointment as a locum in the Equine Section of the Department of Companion Animal Clinical Studies at the Faculty of Veterinary Science at Onderstepoort.

FACULTY OF VETERINARY SCIENCE, UNIVERSITY OF PRETORIA

FACULTY DAY

THURSDAY 15TH SEPTEMBER 2005

PROGRAMME

07:45-08:15 **Registration and Coffee**
Master of Ceremonies: *Professor J P Schoeman*

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

08:30-09:30 **RESEARCH PROGRAMME: ORAL PRESENTATIONS I**
SESSION CHAIRPERSON: *Doctor M-C Madekurozwa*

Comparative morphology of African buffalo (*Syncerus caffer*) and domestic cow (*Bos taurus*) placentomes and full term cotyledons
S Schmidt, D Gerber, J Soley, T Aire, A Boos

Mechanisms by which Lumpy Skin Disease Virus is shed in the semen of artificially infected bulls
C H Annandale, V P Bagla, E H Venter, P C Irons

Absence of Lumpy Skin Disease Virus in semen of vaccinated bulls
U I Osuagwuh, C H Annandale, V Bagla, E H Venter, P C Irons

Luteal and follicular count in bitches: assessment by means of magnetic resonance imaging
K G M de Cramer, J O Nöthling, D Gerber, V R Kammerl

Ultrastructural features of healthy and atretic ovarian follicles in the sexually immature ostrich (*Struthio camelus*)
W H Kimaro, M-C Madekurozwa

09:30-10:20 **Sir Arnold Theiler Memorial Lecture: "The Past, Present and Future of the Clinical Departments in the Faculty of Veterinary Science"**
Professor S S Van Den Berg

10:20-10:50 **TEA** and Viewing of Posters, Commercial Exhibits and Photographic Exhibition

10:50-11:50 **RESEARCH PROGRAMME: ORAL PRESENTATIONS II**
SESSION CHAIRPERSON: *Professor A L Leisewitz*

Seroprevalence of Feline Immunodeficiency Virus and Feline Leukaemia Virus infection and determination of FIV subtypes in sick domestic cats in South Africa
J P Schoeman, R Kahn, J Meers, J Seddon, T Schoeman, M van Vuuren

A study of the prognostic usefulness of blood leukocyte changes in canine parvoviral enteritis
A Goddard, A L Leisewitz, N Duncan, M Christopher

Ultrasonography of the liver, spleen and urinary tract of the cheetah (*Acinonyx jubatus*)
A Carstens, R M Kirberger, T Spotswood, W M Wagner, R J Grimbeek

Use of ultrasonography to determine incomplete ossification of the cuboidal bones in a premature equine foal
S M Higgerty, A Carstens

Diagnosis of suspected hypovitaminosis A using magnetic resonance imaging in African lions (*Panthera leo*)
M P Hartley, R M Kirberger, M Haagenson, L Sweers

11:50-12:30 **RESEARCH PROGRAMME: PRESENTATION OF POSTERS**
SESSION CHAIRPERSON: *Professor E H Venter*

P1. **Villous architecture and feto-maternal interdigitation in the African buffalo (*Syncerus caffer*) during different gestation stages**
S Schmidt, D Gerber, J Soley, T Aire, A Boos

P2. **Using the organic acid buffer MES to produce Foot and Mouth disease virus-free *in vitro* embryos**
F Jooste, D Gerber, W Vosloo, K de Haas

P3. **Effect of the acidic organic buffer MES on bovine *in vitro* embryo production**
K De Haas, I Luther, F Jooste, D Gerber

P4. **Effects of heterologous seminal plasma and semen extenders on progressive motility of frozen-thawed ram sperm**
G Mataveia, S J Terblanche, J O Nöthling, D Gerber

P5. **Validation of two different chromatographic methods for the analysis of urinary endogenous steroids in the Nile crocodile (*Crocodylus niloticus*)**
L C Bekker, J G Myburgh, G E Swan

P6. **The microvasculature of the testis, epididymis and proximal ductus deferens of the ostrich (*Struthio camelus*) as revealed by India ink injection**
M Z J Elias, T A Aire, J T Soley

P7. **Immunoreactivity of protein gene product 9.5, neurofilament protein and neuron specific enolase in the ovary of the sexually immature ostrich (*Struthio camelus*)**
W H Kimaro, M-C Madekurozwa

P8. **The ultrastructure of thecal gland cells in the ovary of the sexually immature ostrich (*Struthio camelus*)**
W H Kimaro, M-C Madekurozwa

P9. **Gross morphological features of the oro-pharyngeal cavity of the ostrich (*Struthio camelus*)**
C Tivane, J T Soley, H B Groenewald

P10. **Ultrastructural features of the *ductuli efferentes* of the ostrich (*Struthio camelus*)**
P C Ozegbe, T A Aire, J T Soley

P11. **The genome of the heartwater agent, *Ehrlichia ruminantium*, contains multiple tandem repeats of actively variable copy number**
N E Collins, J Liebenberg, E P de Villiers, K A Brayton, E Louw, A Pretorius, F E Faber, H van Heerden, A Josemans, M van Kleef, H C Steyn, M F van Strijp, E Zweygarth, F Jongejan, J C Maillard, D Berthier, M Botha, F Joubert, N R Thomson, M T Allsopp, B A Allsopp

P12. **Repetitive DNA in the complete genome sequence of *Ehrlichia ruminantium***
J Liebenberg, N E Collins, E Louw, F E Faber, M van Kleef, B A Allsopp

P13. **18S sequence data reveals genetic diversity in *Theileria equi* and *Babesia caballi***
R Bhoora, N E Collins

P14. **Phylogenetic analysis of *Babesia* parasites in cheetahs in South Africa**
A-M Bosman, M C Oosthuizen, E H Venter, B L Penzhorn

P15. **Piroplasmosis as a possible cause of mortality in giraffes**
M C Oosthuizen, N E Collins, B L Penzhorn

P16. **Verification of parentage in African wild dog (*Lycaon pictus*) packs shows multiple paternity**
C Moueix, C Harper, A J Guthrie, H J Bertschinger, M L Schulman

P17. **Identification of anti-babesial activity for four ethnoveterinary plants *in vitro***
V Naidoo, E Zweygarth, J N Eloff, G E Swan

12:30-13:00 **RESEARCH PROGRAMME: ORAL PRESENTATION III**
SESSION CHAIRPERSON: *Professor C J Botha*

Is Meloxicam the saviour of the Asian White-Backed Vulture?
V Naidoo, G E Swan, E Kilian, K Wolter, M Dietman, D J Pain, R Cuthbert

Is diclofenac sodium toxic to African White-Backed Vultures (*Gyps africanus*)?
G E Swan, V Naidoo, N Duncan, E Kilian, K Wolter, G Verdoorn, P Bartells, D J Pain

Evaluation of the extent to which grape seed extract induces or depresses expression of the p-glycoprotein pump
H Chikoto, J N Eloff, M Oosthuizen, V Naidoo, A L Leisewitz, G E Swan

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

13:45-15:00 **RESEARCH PROGRAMME: ORAL PRESENTATIONS IV**
SESSION CHAIRPERSON: *Doctor S P R Bisschop*

The role of *Mycoplasma* species in bovine respiratory disease complex in feedlot cattle in South Africa
C A P Carrington

Evaluation of a method to detect *Mycobacterium bovis* in formalin-fixed paraffin-embedded tissues of domestic and wild animals
M T Sethusa, E H Venter, A Michel, M Williams

Demonstration of equine encephalosis virus in tissue culture using immunoperoxidase staining with ultrastructural controls
A D Pardini, G Goosen, M M E Smit, E van Wilpe, P G Howell, A J Guthrie, L Prozesky

Development of an ELISA for the detection of interferon-gamma as a diagnostic tool for tuberculosis in black rhinoceros (*Diceros bicornis*) and white rhinoceros (*Ceratotherium simum*)
D Morar, E Tijhaar, A L Michel, A S Negre, J Godfroid, J A W Coetzer, V P M G Rutten

Isolation of lumpy skin disease virus (LSDV) from experimentally infected semen samples using different diagnostic techniques
V P Bagla, E H Venter

The efficacy of a thermostable Newcastle disease vaccine in village chickens when administered by community volunteers – a South African experience
C K Modise, S P R Bisschop, C M E McCrindle, B L Mogoje

The oral application of the Onderstepoort Biological Products fowl typhoid vaccine, its safety, efficacy and duration of protection in commercial laying hens
C Purchase, S P R Bisschop, J Picard, L Mogoje

A survey of *Campylobacter jejuni/coli* in a high throughput poultry abattoir using PCR
A J Bartkowiak-Higgo, C M Veary, E H Venter, A-M Bosman

15:00-16:00 **RESEARCH PROGRAMME: ORAL PRESENTATIONS V**
SESSION CHAIRPERSON: *Dr V Naidoo*

***Athrixia phylicoides* (bush tea): possibilities for a new health-promoting beverage**
L J McGaw, J N Eloff

Antifungal activity of *Markhamia* species
F Nchu, J N Eloff

The diversity of antifungal compounds of six South African *Terminalia* species (Combretaceae) determined by bioautography
P Masoko, J Picard, J N Eloff

Anthelmintic activity of *Peltophorum africanum* extracts against parasitic gastrointestinal nematodes of livestock
E S Bizimenyera, J B Githiori, J N Eloff, G E Swan

Isolation and identification of antioxidants from *Bauhinia tomentosa* Linn (Fabaceae)
M A Aderogba, A O Ogundaini, J N Eloff

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

16:30-17:30 **RESEARCH PROGRAMME: ORAL PRESENTATION VI**
SESSION CHAIRPERSON: *Professor B A Allsopp*

Characterization of South African *Theileria parva* isolates
K P Sibeko, N Collins, D Geysen

Molecular characterization and treatment of a chronic *Babesia gibsoni* infection in South Africa in an imported pit-bull puppy
T P Matjila, B L Penzhorn, A Leisewitz, R Bhoora, R Barker

Central and peripheral *Babesia canis rossi* parasitaemias and their association with outcome of infection
M Böhm, A L Leisewitz, P N Thompson, J P Schoeman

The elevated serum urea: creatinine ratio in canine babesiosis is not of renal origin
M P de Scally, A L Leisewitz, R G Lobetti, P N Thompson

The impact of two dipping systems on endemic stability to bovine babesiosis and anaplasmosis in cattle at four communally grazed areas in Limpopo Province, South Africa
B O Rikhotso, W H Stoltsz, N R Bryson, J E M Sommerville

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; Nursing Lecturer 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

The past, present and future of the clinical departments in the Faculty of Veterinary Science

S S van den Berg (ssvdberg@op.up.ac.za)

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

The essence of Veterinary Science can be encapsulated in three categories, namely: investigation or research, cure or treatment and prevention. The ideal is a balance between these three categories to enable any continent to attend holistically to animal and human health. In the history of veterinary science in South Africa the focus moved slowly from investigation or research to cure and treatment. The Arnold Theiler and co-workers era focused on investigation or research. Tropical diseases that wiped out large populations of production animals and horses were investigated, identified and described. Once the causative agents were identified the next logical step was to cure and prevent. This led to the embodiment of the Onderstepoort Research Institute and in particular the development of effective vaccines.

Highly gifted intellectuals, the likes of Theiler, Weiss, Bigalke, du Toit, Jansen, Adelaar and de Boom, to mention but a few, elevated the pre- and paraclinical departments into world renowned centres of excellence.

In the latter part of the sixties through to the middle nineties the clinical departments grew exponentially mainly fueled by demands from the first economic sector. Then, just before the change of government in 1994, it became clear that our partnership with the rest of Africa in a new dispensation would demand a refocus on the paraclinical sector. A grim reminder that a balance must always be maintained between these three categories was the severe animal losses experienced recently due to so-called "old diseases".

Does the slight shift in emphasis away from the clinical departments pose a threat to be dealt with? It will be a wise decision if a balance between the pre-, para-, and clinical studies are maintained in order to benefit all the sectors in this country. These studies are interdependent and active relationships are necessary to ensure our excellent veterinary education and work maintains the respect and reputation it has earned internationally.

Spending time in a clinical department is a unique and exciting privilege. The value of the exposure to the clinical or final stage of disease will give the student with an investigative mind a much better means to prevent and manage the causative agents and factors. The skill of interpretation, which is cultured in a clinical environment, has tremendous value in allowing the prevention of disease at an early stage. Further to this there can be few pleasures greater than the knowledge that through one's efforts nature is helped on its way.

The future role of the clinical departments will amongst others be determined by intrinsic and extrinsic factors such as visionary leadership, a strong and stable economy, political wisdom and the ability to retain and attract personnel that is willing and able to make a difference.

"You can do what I cannot do. I can do what you cannot do. Together we can do great things."- Mother Theresa

Comparative morphology of African buffalo (*Syncerus caffer*) and domestic cow (*Bos taurus*) placentomes and full term cotyledons

S Schmidt[1,2], *D Gerber*[1], *J Soley*[3], *T Aire*[3], *A Boos*[2] (susanne.schmidt@up.ac.za)

[1] Department of Production Animal Studies, Faculty of Veterinary Science, University of Pretoria, Private Bag X04, Onderstepoort 0110, South Africa
[2] Department of Veterinary Anatomy, University of Zurich
[3] Department of Anatomy and Physiology, Faculty of Veterinary Science, University of Pretoria, Private Bag X04, Onderstepoort 0110, South Africa

Anchorage of the ruminant placenta is achieved by complementary indentation of fetal chorionic villi into maternal endometrial crypts within numerous placentomes. Placentome morphology and geometrical patterns of interdigitation of feto-maternal surfaces vary greatly, even among closely related members of the bovid family.

Study material included placentomes at different stages of development and cotyledonary tissue from normally delivered bovine and buffalo fetal membranes. Samples were routinely prepared for light- and scanning electron microscopy.

During early pregnancy, defined as crown-rump-length of up to 8 cm, placentomes were generally dome-shaped in both species. Thereafter, divergent placentomal development became apparent. While buffalo placentomes remained dome-shaped until term, bovine placentomes developed a characteristic mushroom shape with a distinct caruncular stalk. Cotyledonary villous architecture reflected these differences in respect of the organisation and shape of the villous trees. In the buffalo the primary (stem) villi emerged vertically from the chorionic plate and were arranged in parallel formation. Each stem villus was relatively broad along much of its length and from which emerged numerous leaf-shaped secondary and tertiary villi. These relatively stubby villi resembled the surface projections of a dried pinecone. In appearance, each stem villus with its branches resembled a Tuscany cypress. In the domestic cow the stem villi radiated at approximately right angles from the chorionic plate towards the tip of the narrow caruncular stalk. The stem villus was a relatively thin, tapered structure, appearing fairly broad at its base and narrowing towards the tip. Secondary villi bearing complex tertiary branches emerged from the stem villus, those at its base being longer than those more distally positioned. Due to the progressive distal decrease in length of the secondary villi, each individual villous unit resembled a Christmas tree.

Based on differing characteristic morphological features, the *Syncerus caffer* placenta would appear to be less effective with regard to feto-maternal transfer of nutrients and oxygen by diffusion compared to the *Bos taurus* placenta. This may explain the approximately two months longer gestation period of the African buffalo. Further, the less reticulate morphology of the buffalo placenta might be the explanation of the short involution period as assessed by the extremely short interval from calving to conception in this species.

Mechanisms by which Lumpy Skin Disease Virus is shed in the semen of artificially infected bulls

C H Annandale[1], *V P Bagla*[2], *E H Venter*[2], *P C Irons*[1] (henry.annandale@up.ac.za)

[1] Section of Reproduction, Department of Production Animal Studies and
[2] Department of Veterinary Tropical Diseases, Faculty of Veterinary Science, University of Pretoria, Private Bag X04, Onderstepoort 0110, South Africa

Lumpy skin disease (LSD) has significant economic importance in Africa. It causes considerable production losses and its presence in semen is a constraint to international trade. Recent findings have shown that LSD virus particles can be found in the semen of artificially infected bulls for up to five months, while viable virus could be isolated 42 days after infection. There is clearly a need for studies into the mechanism by which this protracted shedding occurs.

Six healthy, seronegative, post-pubertal Dexter bulls were housed in vector-free stables and challenged with LSD virus by intravenous injection. Sheath washes, vesicular fluid and semen collection was performed every other day and samples subjected to virus isolation and PCR. Blood was also collected for serum neutralization tests and virus isolation, and ultrasonography of the reproductive tract performed. Semen was centrifuged to separate cell-rich and seminal plasma fractions, and tested by PCR. Clinical parameters were recorded twice daily. Bulls shedding virus particles 28 days after challenge regarded as period of prolonged shedding were slaughtered, their reproductive tracts were harvested and diagnostic post mortem was performed. Histopathology, immunoperoxidase staining and electron microscopy were done on tissue samples.

Of the six bulls, two showed no clinical signs, two showed mild and two showed severe clinical signs. Fever appeared five to seven days and lesions eight to ten days post challenge. Bulls were viraemic and febrile during the same time. Virus particles were isolated from all semen fractions of all bulls, but more consistently from the cell-rich fraction and from the bulls showing the most severe clinical signs. Ultrasonography showed infarction in the testes and epididymides of the two most severally affected bulls. The spermiograms of these two bulls showed severe morphological defects and oligospermia from 18 days post challenge. Post mortem of the two bulls that were still shedding after 28 days showed testicular degeneration and infarction, as well as epididymal granuloma formation. None of the accessory sex organs showed obvious pathology. Electron microscopy of negative-stained preparations of testicular material revealed virus particles.

Our results show that LSD virus is not limited to specific fractions of the ejaculate and that the testes and epididymides are most profoundly affected. Results suggest that the ejaculate is contaminated with virus particles as they are shed from lesions in the testis and epididymis. Results from immuno-peroxidase (IMP) staining are still outstanding but could indicate whether the virus has tropism for certain cell types. Further research will focus on the ability of infected semen to produce disease as well as treatment protocols that could render semen free of virus particles.

Absence of Lumpy Skin Disease Virus in semen of vaccinated bulls

U I Osuagwuh[1], *C H Annandale*[1], *V Bagla*[2], *E H Venter*[2], *P C Irons*[1] (uche4us@yahoo.com)

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

Lumpy skin disease virus (LSDV) is reported to be present in the semen and to cause a decline in semen quality of susceptible bulls for up to 5 months following experimental infection. The objective of this study was to determine the efficacy of vaccination in preventing LSDV excretion in semen and subsequent negative effects on semen quality.

Six serologically LSDV-negative bulls 11-16 months of age were vaccinated and on confirmation of seroconversion were experimentally challenged by intravenous injection with a virulent field strain (V248/93) of LSDV. Six unvaccinated bulls were similarly challenged to act as controls. All animals were observed for clinical signs and blood and semen was collected and evaluated twice a week until day 43 post-vaccination and thereafter every two days until day 28 post-infection when the trial was terminated. Serology was performed using serum neutralization test and viraemia was determined by virus isolation. Semen was examined macroscopically and microscopically and PCR was used to detect the presence of virus particles.

No clinical signs were detected following vaccination, and were limited to mild lymph node enlargement in four bulls following challenge of the vaccinated bulls. Two of the unvaccinated controls developed severe LSD, two showed mild symptoms and two were asymptomatic. Vaccinated bulls showed no change in semen quality following vaccination and challenge. Three of the vaccinated bulls were serologically positive at the time of experimental infection and four at the end of the trial. No vaccinated bulls were found to be viraemic at any stage. Viral DNA was not detected in any semen samples following vaccination or challenge in vaccinated bulls. All unvaccinated bulls excreted the virus in semen following challenge although only four became viraemic.

Vaccination prevented the excretion of viral particles in semen. LSD vaccination prevented the effect of experimental infection with virulent virus on semen quality.

Luteal and follicular count in bitches: assessment by means of magnetic resonance imaging

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[2] Section of Reproduction; Department of Production Animal Studies, Faculty of Veterinary Science, University of Pretoria, Private Bag X04, Onderstepoort 0110, South Africa
[3] Radiology Department, Krugersdorp Private Hospital, P.O.Box 1074, Krugersdorp, 1739, South Africa

Researchers require an accurate measurement of fertility in the bitch for the comparison of insemination methods, assessing the effects of procedures on fertility and assessing the effects of drugs on fertility. In clinical practice, a prospective measurement would enable the practitioner to select bitches with the largest numbers of follicles for breeding by artificial means or natural mating with valuable semen or studs. Currently the ovaries of a bitch have to be removed before the corpora lutea can be counted accurately. This results in spoiling the opportunity to use the same animal in future fertility trials. The ratio between the numbers of corpora lutea or follicles and litter size is a more sensitive measurement of fertility than is litter size, as the latter does not reflect the total number of oocytes that were available for fertilization. Magnetic resonance imaging (MRI) has proven to be of value in diagnostic imagery of particularly soft tissues. This is the first study that evaluated the use of MRI in canine reproduction and was done to determine whether the numbers of corpora lutea or follicles can be counted accurately by means of MRI.

The study was divided into two experiments. In experiment 1, 16 bitches were used. They were either in the follicular, early, or late luteal phase. Their ovaries were collected via ovariohysterectomy and placed into a phantom for MRI. The phantom was then scanned in three planes with a slice interval and slice thickness of 1 mm, using a 1.5 Tesla SIEMENS Magnetom Symphony scanner. A circular polarized head array coil was also used around the phantom. The T_2-weighted images were considered better than T_1-weighted images. This study showed that although the corpora lutea or follicles could be identified in most but not all images, it was not possible to accurately count the number of corpora lutea or follicles in the ovaries of bitches using MRI techniques and apparatus commonly used in human MRI units. Follicles were commonly confused with corpora lutea on the images. Although the study was not designed to detect ovarian pathology, MRI of the ovary in the phantom study, showed promise in detecting cystic ovarian disease.

In Experiment 2, it was attempted to find the ovaries, and count the structures in them, by MRI on two live, sedated bitches that were in the late follicular phase. Thereafter the bitches were spayed and the ovarian structures counted. The effect of motion was so great that no image could be generated in which any ovary or its structures could be identified.

There still exists no reliable, non-invasive means by which the number of corpora lutea or follicles can be counted in the bitch. Further studies are required to evaluate more advanced MRI techniques and apparatus.

Ultrastructural features of healthy and atretic ovarian follicles in the sexually immature ostrich (*Struthio camelus*)

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The ultrastructure of the ovarian follicular wall has been extensively studied in the domestic fowl[1] and the goose[2]. The present study was undertaken to investigate ultrastructural changes during folliculo-genesis and atresia in sexually immature ostriches, which have been reported to exhibit cyclic ovarian changes.

A total of 26 sexually immature female ostriches, aged between 12 and 14 months were used in the present study. Ovarian tissue was fixed by immersion in 2.5% glutaraldehyde in 0.1M cacodylate buffer. The samples were then processed and prepared for transmission electron microscopy, using standard methods.

In healthy primordial follicles the oocyte contained a spherical Balbiani's vitelline body, which was composed of a dense accumulation of mitochondria, rough endoplasmic reticulum and smooth endoplasmic reticulum. Balbiani's vitelline body was fragmented in previtellogenic follicles. Yolk granules were evident in the oocyte of vitellogenic follicles. The granulosa cell layer in primordial follicles was composed of a single layer of cuboidal cells. The granulosa cell layer was pseudostratified columnar in previtellogenic follicles and simple columnar in vitellogenic follicles. In all follicular sizes, transosomes were observed at the apical regions of cytoplasmic processes from the granulosa cells. A basal lamina separated the granulosa cell layer from the thecal layer. The basal lamina closest to the granulosa cell layer was more electron-dense than that adjacent to the thecal layer.

Major ultrastructural changes were observed in the organelles of both the oocyte and the surrounding follicular wall of atretic follicles. Atretic primordial follicles lacked a Balbiani's vitelline body. The oocyte in atretic follicles was characterized by the presence of vacuoles, lipid droplets, electron-dense bodies and swollen mitochondria. The granulosa cell layer in atretic primordial and previtellogenic follicles was multilayered. The granulosa cells contained numerous lipid droplets and electron-dense bodies. Very few transosomes were observed in atretic follicles. The theca interna cells of atretic vitellogenic follicles differentiated into interstitial thecal gland cells, which contained numerous lipid droplets.

The results of this study indicate that the ultrastructure of healthy and atretic follicles in the sexually immature ostrich is similar to that of other avian species.

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Seroprevalence of Feline Immunodeficiency Virus and Feline Leukaemia Virus infection and determination of FIV subtypes in sick domestic cats in South Africa

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The prevalences of Feline leukaemia virus (FeLV) and Feline immunodeficiency virus (FIV) infections have been shown across the world to be higher in sick than in healthy cats and to vary widely from study to study and from country to country. The prevalence of FIV and FeLV infections, as well as the subtypes of FIV infecting domestic cats in South Africa is currently unknown. The aim of this study was to determine the prevalence of these viral infections in sick domestic cats and to determine the subtype(s) of FIV virus that exist in South Africa.

Serum was collected from 454 sick cats presenting to the Onderstepoort Veterinary Academic Hospital over a 7-year period from 1998 to 2004. All serum samples were submitted to the Department of Veterinary Tropical Diseases, University of Pretoria for detection of specific antibodies directed to FIV *gag* (group antigen) and *env* (envelope) gp40 proteins and the group-specific p27 core antigen of FeLV by ELISA. In addition, heparinised whole blood was collected from FIV positive cats, consisting of 11 of the above cats and a further 20 cats from 3 different centres in the country, viz Cape Town (12), Durban (4) and Johannesburg (4). The whole blood samples were sent to the University of Queensland and subjected to polymerase chain reaction (PCR) amplification and sequences were determined for the V3-V5 region of the *env* gene of FIV.

Fifty-six out of 454 (12.3%) samples were positive for FeLV antigen and 101 out of 454 (22.2%) samples were positive for FIV antibody. Sixteen out of 454 cats (3.5%) were co-infected with both viruses. Twenty-two out of 31 (71%) samples revealed FIV subtype A and 9 out of 31 (29%) samples revealed FIV subtype C.

The prevalence rates of these two viruses in sick cats in South Africa are in line with prevalences encountered in the rest of the world. The FIV prevalence is amongst the highest in the world, closely resembling prevalences found in two separate studies of cats in Australia and Italy and is only slightly higher than prevalences from the UK and France. The prevalence of FIV is approximately twice that of FeLV in sick cats in South Africa. Although early literature suggested that each FIV subtype was limited in its geographical distribution, the data presented here add to growing evidence that many FIV subtypes are widely distributed around the world. This study thus provides supportive data that the introduction of the FIV vaccine containing subtype A, should protect the majority of cats in South Africa.

A study of the prognostic usefulness of blood leukocyte changes in canine parvoviral enteritis

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Canine parvoviral enteritis is an economically important disease in South Africa and globally. Although treatment of dogs with parvoviral enteritis is often successful, many dogs die of complications related to septicaemia or are euthanised because of anticipated high costs. Over the past 6 years (1999-2004) an annual average of 522 dogs with clinical signs of parvovirus enteritis have been hospitalized at the OVAH (not all of them were confirmed as being positive for parvovirus on electron microscopy). Of these, 444 (85%) survived and 78 (15%) died. The average cost per case was R350-00, but the lowest admission quote was R600-00. More effective prediction of the outcome of this disease will have an economic impact if a prognosis can be determined early in the course of the disease. Although leukocyte responses seldom are pathognomonic for a specific disease, they can provide clinical information to establish a fairly reliable prognosis.

A prospective study was performed on 62 puppies presented to the OVAH with typical clinical signs of canine parvovirus enteritis that subsequently was confirmed on electron microscopy. An EDTA blood sample was collected at admission for full haematology, as well as every consecutive day until death or discharge. Of the 11 puppies that died (18%), nine died due to complications of the disease and two were euthanised due to financial restrictions and a poor prognosis. The puppies that died due to the disease died within the first three days of hospitalization. All the puppies that died were sent for a full post mortem examination and histopathological evaluation.

Provisional statistical analysis has so far shown that there is a definite difference between the puppies that died and those that survived in several of the leukocyte parameters. These parameters include the total leukocyte, lymphocyte, and monocyte counts. In none of the puppies that died from the disease did the total leukocyte count rise above 4.5 x 10^9/l (normal reference range: 6.0-15.0 x 10^9/l). In fact the total leukocyte count did not even rise above 1.0 x 10^9/l. In the puppies that survived, the total leukocyte count started rising within 24-48 hours after admission and often resulted in a rebound leukocytosis. The puppies that died did not show a lymphocytosis to indicate an immune response, whereas the surviving puppies showed a lymphocytosis within 24-48 hours after admission. The puppies that died also did not develop a monocytosis. Evidence of impaired leukocyte production was found on histopathology. Most of the puppies that died from the disease showed marked to severe thymic and lymphoid atrophy and marked to severe bone marrow hypocellularity.

Provisionally, these results do show that a reliable prognosis can be obtained within 24–48 hours after admission by studying leukocyte parameters, specifically the total leukocyte-, lymphocyte- and monocyte counts.

Ultrasonography of the liver, spleen and urinary tract of the cheetah (*Acinonyx jubatus*)

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Diseases of the abdomen of the cheetah (*Acinonyx jubatus*) include those affecting the liver, spleen and urinary tract. The most common diseases of captive-bred cheetah are gastritis, gastric ulceration, glomerulosclerosis and hepatic veno-occlusive disease, and are the most frequent causes of mortality in these animals.

The purpose of this study was to describe the ultrasonographic anatomy of the normal liver, spleen, kidney and bladder of the anesthetized captive-bred cheetah. The animals were anesthetized using four recognised different anesthestic protocols, namely: a combination of medetomidine and ketamine (MK); a combination of propofol and fluothane (PF); alphaxalone/alphadolone alone (S) and a combination medetomidine and alphaxalone/alphadolone. Twenty one cheetahs were examined. Eight of the 21 animals had sub-clinical evidence of either gastritis or chronic renal disease. The ultrasonographic appearance of the liver, gall bladder, common bile duct, and spleen were evaluated and various measurements made. Statistical analyses of the measurements were performed on all the healthy and subclinically ill animals taking sex, age, mass and anesthetic protocol into account. There were no significant differences in any parameters between the healthy and subclinically ill animals ($P>0.25$) and data were combined for statistical analyses. The mean mass was 41.1 kg (±8.8) and the mean age was 5.0 years (±2.2).

The mean thickness of the liver medial to the gall bladder was 67.0 mm (±14.8) and the liver was within the left costal arch in 75% of animals, extended caudal to the right costal arch in 50% of animal for an average of 30mm and extended caudal to the sternum in 63% of animals for an average of 32.5 mm. The maximum mean hepatic vein diameter at the entrance to the caudal vena cava was 8.6 mm ±2.8; the mean diameters of the portal vein at the hilus and that of the caudal vena cava as it entered the liver were 7.5 mm ±1.6 and 9.9 mm ±4.1 respectively. The mean diameter of the caudal vena cava was significantly affected by the type of anesthetic used ($P=0.09$), being greater in the MK anesthetic combination (12.85 mm ±3.46 n=8) versus both the S (6.67 mm ±2.8 n=6) and the PF (7.45 mm ±2.05 n=2) anesthetic regimens. The mass of the animals was significant in explaining the variance in maximum portal vein diameters ($P<0.10$). The mean maximum velocities of the hepatic vein flow at the entrance to the caudal vena cava was 25.3 cm/s ± 2.8 (n=4), the hilar portal vein was 11.7 cm/s ±3.3 (n=7) and the caudal vena cava was 33.8 cm/s ± 19.8 (n=5). The mean maximum gall bladder length and width, and mean common bile duct diameters were 44.6 mm (±10.4), 23.3 (±5.0) and 8.1 mm (±2.4), respectively. Age was significant in explaining the variance in gall bladder lengths ($P<0.10$).

Urinary tract ultrasonography was performed only in animals that had normal urea and creatinine levels (n = 13). Renal corticomedullary distinction was present in all kidneys and a corticomedullary rim sign was seen in 21/26 kidneys. Mean kidney length, height and width were 63.9 mm ±5.7, 38.1 mm ±5.2 and 42.1 mm ± 5, respectively. The average resistivity index was 0.58 (n=5). Mean urinary bladder length, height and width was 57.0 mm, 19.2 mm and 34.9 mm, respectively.

Use of ultrasonography to determine incomplete ossification of the cuboidal bones in a premature equine foal

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Incomplete ossification of the cuboidal bones of the carpus and tarsus in premature and twin foals has prognostic significance for the future development of the foal and its athletic career. The cuboidal bones ossify late in gestation. These foals generally require intensive care if they are to survive, and a quick accessible method to determine whether ossification is incomplete will have an impact on prognosis and treatment options.

A 303-day gestational age premature twin foal was compared to 2 other full term foals and a donkey foal, using a portable real-time scanner (Aloka SSD-500) with a 7.5MHz linear array transducer to obtain ultrasound images of the dorsal carpus and distal radial physis and epiphysis. Radiographs were taken on the same days as ultrasound images were taken, to determine the ossification of the carpal bones using the Skeletal Ossification Index (SOI) [1]. The ultrasonographic distal radial physis width was measured, and compared with radiographic measurements. The average ultrasonographic width of the physis of term foals was 0.39 cm (±0.03), and radiographically 0.4 cm compared to the premature foal, which had a physeal width of 0.63 cm ultrasonographically, and 0.6 cm radiographically respectively. The intermediate carpal (Ci) bone was chosen for the ultrasound images, due to its relative flatness, and repeatability of images. Full term foals were chosen due to their availability, closeness in size to the premature foal and having normal carpi on radiographs for neonates according to the SOI (1). The Ci bone was measured transversely and sagitally both ultrasonographically and radiologically, and the average measurements of the term foals compared with those of the premature foal. At 7 days of age the average height of the term foals Ci bone (excluding the donkey) ultrasonographically was 1.28cm (± 0.22), compared to that of the premature foal of 0.93cm.

Marked differences were seen on the ultrasound images between the premature and full term foals. The most striking was the 'plateau' appearance of the full term foals Ci bone silhouette compared to the 'hill' silhouette of the premature foal. The distal radial physis on ultrasound image of the premature foal had a step down to the epiphysis with a widened rounded epiphyseal edge, whereas the full term foals had a triangular physis with right-angled edges and no step between physis and epiphysis.

The differences between the premature foal and the full term foals dorsal carpal ultrasound images are very evident and are comparable to radiographic evaluation. Portable real time ultrasound scanners are generally readily available in most practices. To evaluate measurements more accurately, additional numbers of foals are required for further study.

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Diagnosis of suspected hypovitaminosis A using magnetic resonance imaging in African lions (*Panthera leo*)

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Vitamin A deficiency has been described in captive lions with exclusive feeding of lean red meat. Ante-mortem diagnosis can either be made by serum analysis or liver biopsy, both of which are problematic. The use of imaging techniques to diagnose hypovitaminosis A has not been described to date. This study utilized magnetic resonance imaging (MRI) as a non-invasive, reliable diagnostic tool to identify vitamin A deficiency in lions with relatively mild clinical signs, which could otherwise be attributed to numerous other neurological conditions.

Four clinically affected lion cubs aged between 6 and 9 months and weighing between 20 - 46 kg were investigated over a period of 18 months. A clinically normal cub also underwent MRI examination as comparison. Sagittal and/or transverse and/or dorsal slices of the brain and upper cervical region were made using T1 and T2 spin echo and FLAIR sequences. Multiple 3-5 mm slices with 0.3-2 mm slice intervals were obtained. Additional T1-weighted images were made after intravenous administration of gadoteric acid paramagnetic contrast agent at 1 mmol/kg. To accommodate varying lion ages and sizes, a number of cranium and brain measurements were compared to that of the maximum diameter of the ocular vitreous humor.

In the four affected cubs the *tentorium cerebelli osseum* and associated cranial bones were markedly thickened resulting in a small caudal fossa with cerebellar herniation, loss of sulci visibility and compression of the third and fourth ventricles with mild to moderate secondary hydrocephalus of the lateral ventricles in all four cubs. The prominent dens caused cord compression. Ocular ratios of the *tentorium cerebelli osseum* and occipital bone were most reliable in diagnosing the thickened osseous structures. The ratio maximum : minimum dorsoventral diameter of the C1 spinal cord was also of value.

The authors recommend that T1- and T2-weighted spin echo sequences be made in sagittal (from eye to C2) and transverse (olfactory lobe to C2) planes and that the following measurements be taken: vitreous humor and cervical spinal cord diameter, and thickness of the *tentorium cerebelli osseum* and occipital bone. Increased values and ratios supports the diagnosis and allows for early treatment of the condition which to date has resulted in recovery of the patients.

Is Meloxicam the saviour of the Asian White-Backed Vulture?

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Three *Gyps* species (*G. bengalensis; G. indicus; G. tenuirostris*) are on the verge of extinction due to the widespread use of diclofenac in cattle and livestock populations in India and Pakistan. These mortalities have been linked with the vultures feeding on livestock carcasses containing diclofenac residues[1] left in the open. Studies in South Africa showed that the African white-backed vulture (AWBV) was equally or more sensitive to the toxic effects of diclofenac than the Oriental white-backed vulture. To facilitate and expedite a ban on diclofenac use in livestock, a survey was initiated by the RSPB to identify a non-steroidal anti-inflammatory drug (NSAID), of relatively low toxicity to vultures, which could be used in its place. A questionnaire on the use of NSAIDs by veterinarians at zoos and wildlife centres identified meloxicam as the potential alternative to diclofenac. Studies were subsequently conducted in South Africa to establish the tolerance of the AWBV to meloxicam.

A four-phased study approach was used. The tolerance of meloxicam was initially titrated in 24 captive, non-releasable AWBV under experimental housing conditions using a sequential 3-phased, unbalanced, two-armed, parallel study design. During phases I through III 8 vultures each were randomly allocated to meloxicam (n=5) and water treated groups (n=3). A single dose of meloxicam was administered orally by gavage and increased step-wise, starting at 0.5 mg/kg (recommended clinical dose) to 1 mg/kg and 2 mg/kg during each phase, respectively. The highest dose was based on the worst-case scenario of a bird being exposed to the maximum possible residues present in meat or offal. In residue analysis this maximum was found to be in the liver of calves, slaughtered 8 hours after a 5 day treatment course (at 0.7 mg/kg o.i.d. i.m.)[2] and equates to a maximum exposure in vultures of 8.54 mg meloxicam per kg meal or a potential dose of 1.55 - 1.89 mg/kg for an average sized AWBV.

No signs of toxicity were observed in any vultures treated with meloxicam at all dose levels during phases I to III. The results were subsequently confirmed in Phase IV in 28 wild-caught and 14 non-releasable birds, with no history of exposure to NSAIDs, at the highest dose of 2 mg/kg. All vultures were monitored frequently for clinical signs of toxicity and blood was collected at various intervals to determine the haematological profiles, thromboxane, uric acid and meloxicam concentrations. Serum uric acid levels were within the range of the normal birds for the entire study. It was concluded that meloxicam is safe to AWBV at a single dose up to 2 mg/kg. From this promising result the Indian government has subsequently placed a ban on the animal use of diclofenac with the complete phase out of the drug expected by the end of the year.

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Is diclofenac sodium toxic to African White-Backed Vultures (*Gyps africanus*)?

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Diclofenac residues in carcasses of dead cows and livestock left in the open or taken to carcass dumps were found to be the cause of catastrophic declines of three *Gyps* species (*G. bengalensis; G. indicus; G. tenuirostris*) in India and Pakistan[1]. Studies to identify alternative non-steroidal anti-inflammatory drugs (NSAID) that are of relatively low toxicity to vultures are required to facilitate and expedite a ban on diclofenac use in livestock. Due to the impending extinction of the vulture populations in India the use of the African white-backed vulture (*G. africanus*) (AWBV), a close relative to the Oriental white-backed vulture (*G. bengalensis*) (OWBV), as a surrogate model, was explored.

Toxicity of diclofenac was examined in 4 captive, non-releasable AWBVs in a randomised, two-arm, parallel study design under experimental housing conditions. Two 2 vultures were treated with a single oral dose of 0.8 mg/kg diclofenac sodium and two kept as water-treated controls. The design was based on a fitted probit model for generating the dose-response[2]. The birds were frequently monitored for clinical signs of toxicity. Blood samples were collected at regular intervals after treatment for haematology, serum uric acid, alanine transferase, creatinine kinase, urea and serum diclofenac concentrations.

Other than an apparent reduced feed intake, noted soon after treatment the first signs of acute toxicity occurred at 36 h and 39 h in the two diclofenac-treated vultures, respectively. Severe depression, closing of the eyes, drooping of the neck, abrupt recumbency and apparent coma, from which the vultures could initially be aroused, were observed. These signs persisted and appeared to increase with intensity until mortality a few hours later. The corresponding plasma uric acid concentrations at this time were ten-fold increased in comparison to the untreated controls. Post-mortem lesions confirmed the presence of visceral gout as seen in the OWBV with corresponding severe necrosis of the proximal convoluted tubules of the kidneys on histopathology.

It was concluded that diclofenac at 0.8 mg/kg was as least or more toxic to the AWBV as the OWBV and that it could be used as a suitable surrogate model for further studies.

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Evaluation of the extent to which grape seed extract induces or depresses expression of the p-glycoprotein pump

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The use of herbal medicines is gaining widespread acceptance in both the human and veterinary fields. However little information is provided on safety or potential interactions. Herb-drug interactions can occur at the level of the p-glycoprotein (p-gp) pump, where its inhibition or induction can lead to an unintended increase or decrease in bioavailability of drugs taken concurrently. The aim of this study was to determine the degree to which grape seed extract as an herbal supplement has the ability to increase or decrease the expression of the dog MDR1-gene, which encodes for intestinal p-gp pump.

Five, healthy German Shepherds were used for the study. Duodenal biopsies were collected 6 days before administration of the test product, grape seed extract, or the control product, rifampicin. Three dogs received 70 mg of grape seed extract twice daily for 14 days and two dogs 10 mg/kg of rifampicin daily for the 14 days. Post treatment biopsies were collected on the last day of treatment.

Messenger RNA was extracted from the biopsies and reverse transcribed to complementary DNA. The MDR-gene was quantified using RT-PCR. The quantity was expressed as a ratio, relative to the conservative GAPDH gene. Differences between pre and post treatment levels of the MDR-1 gene within a given dog were analysed.

Individual dogs appeared to respond differently to the same treatments, as was evident from the fluorescence graphs, the means of the responses and from differences in medians. In the grape seed extract treatment; there was induction in dog 1, repression in dog 3 and a no effect observed in dog 2. The analysis of medians showed that the responses in dog 1 and dog 3 were highly significant with $P<0.01$. Analysis of the means showed that post treatment values of dog 1 were 65 times higher than pre-treatment values. In dog 3, the MDR–1 gene was lowered by a magnitude of 11. No statistical differences between pre and post treatment MDR-1 levels were evident when the animals were grouped. This overall effect was hardly surprising, given the opposing trends seen in the individual dogs.

From an individual dog point of view, grape seed has a dual effect on the MDR-1 gene. The population conclusion of a no-effect would be flawed because of the small number of animals used. Future studies would thus involve a larger group of animals.

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

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Bovine respiratory disease complex (BRD) consists of a largely single clinical entity of bronchopneumonia that is usually associated with the assembly of large numbers of especially weaner-cattle into a feedlot environment. It has a multifactorial aetiology and develops as a result of complex interactions between environmental factors, host or animal factors and pathogens, which include bacteria, viruses and mycoplasmas. The presence of *Mycoplasma* species has been shown to increase the severity of respiratory disease, but their role in BRD complex as a primary or secondary pathogen remains controversial. The aim of the study was to identify the isolation rates of *Mycoplasma* species in feedlot cattle in South Africa.

Transtracheal aspirate samples were collected from 442 clinically affected animals and 33 healthy animals from more than 25 feedlots throughout South Africa during the period 2000/1 to 2004. The samples were routinely cultured for aerobic bacteria and mycoplasmas

The percentage sample isolates of the most common respiratory pathogens from affected animals are reflected in the table presented below.

	Mycoplasma	M.haemolytica	P.multocida	P.trehalosi	H.somni	No microbial growth	Mycoplasma with no bacteria
2001	59.4	72.3	16.8	–	6.9	16.8	10.9
2002	44.1	29	40.3	4.8	4	18.5	9.9
2003	57.9	28	36.8	9.6	0	16	10.7
2004	46.6	32.6	22.8	5.4	1.1	15.2	12.5
Av.	52.3	40.5	29.2	6.6	3	16.6	11

According to the literature, mycoplasmas are isolated from 25% to 80% of pneumonic lungs and from the lungs of 5% to 10% of healthy feedlot cattle. In this survey the average isolation rate in affected animals was 52.3% (se above table) and 22.6% from healthy cattle (results not shown in abstract).

It is concluded from this study that there is a statistically significant (p=0,001 and OR=3,75) association between *Mycoplasma* isolation and respiratory disease in feedlot cattle in South Africa. The high incidence of mycoplasmas reported in this study makes it imperative that future research be aimed at possible vaccine development as well as the determination of the role of these organisms in mastitis.

Evaluation of a method to detect *Mycobacterium bovis* in formalin-fixed paraffin-embedded tissues of domestic and wild animals

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A presumptive diagnosis of tuberculosis can be made if a tissue sample has characteristic histopathological changes and acid-fast organisms are observed on a fresh smear of the lesion after Ziehl-Neelson staining. A definite diagnosis currently requires culture and species identification including bio-chemical testing of the causative mycobacterium, a process that takes several weeks. The aims of this study were to (1) evaluate the sensitivity of a polymerase chain reaction (PCR) for the rapid diagnosis of *Mycobacterium bovis* in formalin-fixed paraffin-embedded tissue samples and to compare the results to culture, and (2) to evaluate the effect of formalin fixation of the organism on the sensitivity of this PCR.

A total of 70 tissue samples were randomly selected from fresh samples received as tuberculosis suspects in the Bacteriology-TB laboratory of the ARC-OVI. These samples were cultured and submitted to the pathology laboratory for routine histopathology. Paraffin sections were also made for DNA extraction and PCR. The primers used for PCR amplified a 123-bp fragment of the IS6110 gene, an insertion sequence that is specific for organisms in the *Mycobacterium tuberculosis* complex (*M. tuberculosis*, *M. bovis*, *M. microti*, *M. africanum*). In order to evaluate the effect of formalin fixation on the PCR, a tissue sample with visible lesions and later confirmed by culture to be *M. bovis,* was fixed in buffered formalin at room temperature and processed for paraffin embedding at different time intervals i.e. 10 hrs, 60 hrs, 80 hrs, 108 hrs, 14 days and 22 days. Sections were then made from the different blocks and DNA extraction and PCR was performed.

Of the 70 samples collected 54 were PCR positive and 16 negative, and for culture, 49 were positive and 21 negative. There was also no evidence of DNA degradation for up to 22 days post formalin fixation.

The PCR provides a sensitive rapid diagnostic test for bovine tuberculosis, compared to culture, and its simplicity is ideal for diagnostic purposes. Buffered formalin can also be used to fix samples that may need molecular analysis without negatively affecting the results.

Demonstration of equine encephalosis virus in tissue culture using immunoperoxidase staining with ultrastructural controls

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Immunoperoxidase (IMP) is a staining technique frequently used for detection of antigen in tissues for diagnostic purposes and has also been used as a research tool for pathogenesis studies. The aim of this study was to ensure that anti-EEV antibody consistently recognized Equine Encephalosis Virus (EEV) under controlled conditions, and to determine at what stage of the virus cycle and with what concentration of antibody, optimal staining was obtained. A polyclonal antibody directed against the group-specific surface protein VP-7 of EEV was used to detect virus in inoculated BHK-21 tissue culture monolayers grown on microscope slides. Application of the technique to tissues from experimentally infected horses is intended to provide additional information on the tissue and cell affinity of the virus.

Tissue cultures were inoculated with decreasing concentrations of EEV serotype 1 in 8-well chamber slides. Each slide had an uninfected control well. Replicates of slides were fixed at 6, 12, 18, 24, 36 and 48 hours post-inoculation and were stained with 1:1000, 1:5000 or 1:10000 dilutions of antibody. A 75cm tissue culture flask was inoculated with EEV serotype 1 as an ultrastructural control corresponding to each time interval[2]. A single uninoculated flask was used as a negative control. An additional set of 8-well chamber slides were inoculated with all 7 serotypes of EEV, one serotype per well (8th well used as an uninfected control) and fixed at 24 or 48 hours post-inoculation.

The antibody dilution of 1:5000 was optimal for detection of virus in all time intervals and at all dilutions. At earlier time intervals and higher viral dilutions the positivity rate was less reliable. At 1:10000 dilution of antibody, viral detection was only reliable at higher magnification (400x). At higher concentrations of antibody (1:1000), background and non-specific staining occurred. Staining was confined to the cytoplasm in the majority of positive cells and could be either diffuse or granular, with some cells showing both patterns. Diffuse staining was pale or dense, usually involving the entire cytoplasm of a single cell. Granules ranged in size from finely stippled pinpoints, through small and medium sized granules, to large amorphous coalescent clumps suggestive of inclusion bodies. Granules tended to stain with a distinctive dense, well-demarcated pattern. Cellular response to infection was characterized by swelling with associated diffuse cytoplasmic staining. Some cells showed shrinkage and dense granular staining with nuclear pyknosis.

All seven known serotypes of EEV could be detected using this polyclonal antibody. The ultrastructural study confirmed the presence of orbivirus particles in tissue culture cells with associated cytopathology. Polyclonal antibody diluted 1:5000, is optimal for detection of infected cells at screening (100x) magnification using light microscopy.

Development of an ELISA for the detection of interferon-gamma as a diagnostic tool for tuberculosis in black rhinoceros (*Diceros bicornis*) and white rhinoceros (*Ceratotherium simum*)

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Bovine Tuberculosis (BTB) is believed to have entered the Kruger National Park (KNP) in the 1960's and was first diagnosed in July 1990 in an African buffalo (*Syncerus caffer*). Since then, in addition to buffalo, BTB has been found in at least 14 other mammalian species, including kudu (*Tragelaphus strepsiceros*), baboon (*Cynocephalus papio*) and lion (*Panthera leo*). This has raised concern about the spillover into other potentially susceptible species such as the rhinoceros, jeopardising breeding and relocation projects. Practical and reliable procedures for the diagnosis of BTB in black rhinoceros (*Diceros bicornis*) and white rhinoceros (*Ceratotherium simum*) need to be developed. Skin testing, as a diagnostic method for BTB in pachyderms, has important practical limitations. In addition, intrinsic values of the test, ie sensitivity and specificity, are unknown. In cattle, the bovine Interferon-gamma (IFNg) assay is used as a routine diagnostic test for BTB.

As a first step towards the development of an *in vitro* diagnostic test for BTB in the rhinoceros, a capture ELISA for the detection of rhinoceros IFNg (RhIFNg) was developed. The RhIFNg was cloned, sequenced, expressed and purified. Subsequently, two hybridoma cell-lines producing monoclonal antibodies (MoAbs) specific to recombinant RhIFNg (rRhIFN-gamma) were established. In parallel, polyclonal anti-rRhIFNg antibodies were produced in chicken eggs. Specific binding of the two MoAbs to rRhIFN-gamma was demonstrated in an indirect ELISA. In the development of a capture ELISA, the two MoAbs were independently used for the capture of rRhIFNg and the chicken antibodies anti-rRhIFNg in the detection step. Both assays were shown to detect rRhIFNg. Subsequently, both systems were shown to detect native RhIFNg in tissue culture supernatant, obtained after stimulation of purified rhinoceros lymphocytes with Concavalin A (Con A).

The RhIFNg ELISA that has been established will now enable further development of a whole blood assay that will be an effective diagnostic tool in the detection of BTB in the rhinoceros.

Isolation of lumpy skin disease virus (LSDV) from experimentally infected semen samples using different diagnostic techniques

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Lumpy skin disease virus (LSDV), the causative agent of lumpy skin disease (LSD) of cattle is an important pathogen that can be shed in the semen of infected bulls. The isolation of the virus in cell cultures is a sensitive diagnostic test which can be used to screen semen for infectious virus prior to artificial insemination. A major problem is, however, the toxic effect that semen has on the cells. The polymerase chain reaction (PCR), on the other hand, detects nucleic acid and can be used to compare the results obtained by cell cultures. The goal of this study was to find a method that decreases the toxic effect of semen in cell cultures without losing the sensitivity of the technique.

Semen samples from LSDV sero-negative bulls were collected and spiked with a field strain of LSDV, strain V248/93. Aliquots of each semen sample were treated to one of four different methods before they were inoculated into monolayers of bovine dermis cells. The treatments were: centrifugation, serial dilution, filtration and chemical treatment with kaolin. Gentamycin was added to the samples after the centrifugation, serial dilution, and filtration methods.

In all the methods used, except that of centrifugation, the semen retained its toxicity for the cell cultures. Supernatants of semen samples centrifuged at 2000 rpm / 1min and then serially diluted in a solution containing antibiotics showed limited toxicity to confluent monolayers of bovine dermis cells. This method was also more sensitive compared to the other methods used and has been applied to field samples that tested positive using PCR, with similar results.

The efficacy of a thermostable Newcastle disease vaccine in village chickens when administered by community volunteers – a South African experience

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Initial work done on the vaccination of village chickens in the communal areas of South Africa demonstrated that the Nobilis Inkukhu® commercial thermostable vaccine was able to protect chickens against virulent Newcastle disease challenge when applied by eye-drop, water or in-feed (cooked maize meal) application. In the initial trial all vaccines were prepared and administered by University staff or graduate students, rather than poultry owners themselves. In order for vaccination of the village chickens to be carried out on a more extensive scale it is obviously necessary for more people to be enabled to vaccinate chickens. It was believed that, once community members had to make an effort to get their chickens vaccinated, it would be possible to gain insight into community perceptions of poultry vaccination. Serosurveillance allowed researchers to determine vaccine efficacy.

The trial was carried out in the village of Disaneng which lies in the Northwest Province of South Africa. Visual and practical training material was prepared and presented to community-elected and volunteer "vaccinators". Vaccinators were then required to register all the poultry owners in their ward who wished to have their chickens vaccinated. Once an indication of the number of chickens to be vaccinated had been made, Inkukhu vaccine was supplied to vaccinators free of charge. Vaccinators were responsible for the organisation of the vaccination campaign, including the storage and preparation of the vaccine for application.

All nine wards in the village were initially involved in the vaccination campaign with a total of 482 households owning 6 141 chickens participating. Detailed survey work carried out in three of the participating wards indicated that this represented slightly in excess of 60% of the chickens in the area. Involvement in a second round of vaccinations, one month later, was far poorer with only 211 households owning a total of 1 636 chickens participating.

Approximately one month after each vaccination campaign, blood samples were collected from a random sample of about 150 chickens that had been vaccinated and tested for circulating antibodies to Newcastle disease, using the HI test. These results showed variable levels of protection, but were influenced more by the area (vaccinator) from which they came, than the vaccine application method used.

Work will be done to investigate the reason for the sudden drop in community participation between vaccination campaigns as well as to obtain further information about vaccine handling and preparation by the community vaccinators. Another unexpected finding was the rate at which chicken flock numbers appeared to alter between vaccination campaigns. The reason for this is yet to be established but may indicate that chickens are moved between homesteads belonging to a single family, depending on what forage is available, or other unidentified disease problems.

The oral application of the Onderstepoort Biological Products fowl typhoid vaccine, its safety, efficacy and duration of protection in commercial laying hens

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Outbreaks of fowl typhoid caused by *Salmonella* Gallinarum (SG) still occur in laying hens in South Africa despite extensive vaccination using live and inactivated vaccines. This project was initiated to compare the efficacy, safety and duration of protection of a live SG vaccine when applied orally to the results obtained when the vaccine was applied by injection, in accordance with its current registration. Oral application of the vacine would of course be simpler than injection in large commercial poultry concerns.

Commercial brown layer hens were vaccinated with the Onderstepoort Biological Products Fowl Typhoid Vaccine (OBP FT vaccine). The oral administration of the OBP FT vaccine was compared to the intra-muscular injection of the same vaccine, and the use of a single vaccination was compared to the standard double vaccination. Birds were euthanased at time intervals after vaccination, autopsies were performed and organ samples were taken and cultured for SG. Safety testing was performed to detect the potential damaging effects of the OBP FT vaccine against layers, and whether shedding of the vaccine strain would occur. Serological responses to the different vaccination strategies were also compared.

In the safety trial: No clinical signs or mortalities could be attributed to the OBP FT vaccine. No active shedding of vaccine could be detected. Slight pathological changes occurred in both groups of pullets after vaccination, however this returned to normal within the observation period. The injected groups showed a better serological response than the orally vaccinated groups, and the serological response observed after one vaccination was weaker than that observed after two vaccinations.

In the duration of protection trial: The oral administration of the OBP FT vaccine was compared to the intra-muscular injection of the same vaccine, and both were compared to an unvaccinated control group. Birds were challenged at time intervals after vaccination, and samples were taken and tested. The efficacy and duration of protection testing was performed to test how well the oral application of the OBP FT vaccine would perform in commercial layer hens. There was 100% mortality in the control birds, attributed to fowl typhoid (FT) infection. The oral vaccination gave 70% protection against FT challenge up to 16 weeks post vaccination, on a par with the registered intra-muscular injection of the same vaccine. However by 24 weeks post vaccination both application routes showed a dramatic drop in vaccine protection against challenge.

A survey of *Campylobacter jejuni/coli* in a high throughput poultry abattoir using PCR

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The reported incidence of human campylobacteriosis has markedly increased in developed countries over the last 20 years. The prevalence and importance of *Campylobacter* spp as a cause of human gastroenteritis in developing countries is not known due to a lack of national surveillance programmes in these countries. The aim of this study was to determine the extent of contamination and cross-contamination of poultry products with *Campylobacter* in one high throughput poultry processing plant in South Africa. Both strains, *C. jejuni* and *C. coli,* were the subject of this study and will be collectively referred to as *Campylobacter* unless stated otherwise.

During the winter of 2004, 300 samples were randomly selected during routine slaughter. The samples originated from 50 chicken carcasses, taken directly after evisceration, as well as 25 samples from ready-to-sell packages of fresh intestines (mala) and livers. The samples were taken in batches over a time period of 4 weeks to allow randomised sampling spread over different supplying farms.

The polymerase chain reaction (PCR), a convenient and practical method for the screening of food samples for *Campylobacter* was used. While conventional culture-based detection methods of *Campylobacter spp* usually need 4-6 days to produce a result, the PCR used for this research project took less than 32 hours.

PCR screening of the samples revealed that average contamination rates with *Campylobacter* in both the skin and liver samples were 24%, and 28% for intestines.

Chicken and chicken products, especially livers and intestines form an integral part of the traditional diet, as they are cheap and readily available in bulk and un-chilled for direct distribution, mainly through street vending and other informal retail outlets. This research project proves that *Campylobacter* spp is prevalent in poultry in South Africa. Therefore the handling of contaminated poultry meat and products with this organism in households and the potential for cross-contamination of other foods presents a high risk of infection to consumers in South Africa. It also emphasises the need for further research in this field.

Athrixia phylicoides (bush tea): possibilities for a new health-promoting beverage

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Athrixia phylicoides DC. (Asteraceae), commonly known as Zulu or bush tea, is widely used as a beverage in South Africa. The plant is reputed to have medicinal properties, being used as a cough remedy, blood purifier and anthelmintic. The commercialization of this tea in a similar vein to popular herbal teas such as rooibos (*Aspalathus linearis*) and honeybush tea (*Cyclopia intermedia*) is being pursued. The aim of this study was thus to screen extracts of *Athrixia* for cytotoxicity, presence of caffeine, antioxidant activity and phenolic content.

Decoctions and infusions of *A. phylicoides* and a related species, *A. elata* Sond. (daisy tea) were prepared following the traditional approach. In this method, aliquots of approximately 50 g of dried plant material was boiled with 1.5 L of water (decoction) or steeped for 15 min in 1.5 L of boiling water (infusion), allowed to cool and filtered. Aqueous and ethanol extracts were also prepared in the laboratory by sonicating 1 g of plant material with 10 ml of distilled water or ethanol. The cytotoxicity of the extracts was investigated using the brine shrimp lethality test and the MTT cytotoxicity assay against the Vero cell line. Antioxidant activity was detected using the Trolox equivalent antioxidant capacity (TEAC) assay. The total phenolic content in each sample was determined spectrophotometrically according to the Folin-Ciocalteu method and calculated as gallic acid equivalents. The detection of caffeine was carried out following extraction of the infusions, decoctions and water extracts with chloroform, and analysis using thin layer chromatography and I/HCl spray reagent.

The infusions, decoctions and water extracts all showed negligible cytotoxic activity in both the brine shrimp and cell line assays. Ethanol extracts prepared in the laboratory, however, showed noteworthy cytotoxic activity in both assays. Excellent antioxidant activity was detected in the *Athrixia* preparations. The TEAC values of the extracts were found to be comparable to that of rooibos (0.257). The highest TEAC value was calculated to be 0.269 for the *A. phylicoides* decoction, whereas the other extracts displayed slightly lower values. The *A. phylicoides* decoction exhibited a total phenolic content of 45.18 (expressed as gallic acid equivalent) compared with a value of 35.64 for rooibos. There was no detectable caffeine in the *Athrixia* infusions and decoctions.

The lack of caffeine and the presence of antioxidant activity (which may contribute to a decreased incidence of certain cancers and other ailments), as well as the pleasant taste, support the use of bush tea as a healthy alternative to caffeine-containing beverages.

Antifungal activity of *Markhamia* species

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During a systematic screening of antimicrobial activity of leaves of Southern African tree species it was found that two *Markhamia* spp. (Bignoniaceae) displayed interesting activity against *Candida albicans*. Little work has been reported on the phytochemistry and medicinal use of the genus *Markhamia*. The study of the efficacy of plant extracts, or compounds isolated from those plants, against opportunistic pathogens such as *C. albicans* is an important focus of medicinal plant research. The aim of this study was to examine in more depth the antifungal activity of *Markhamia obtusifolia* and *M. lutea*.

Acetone extracts of the leaves of *M. obtusifolia* and *M. lutea* were assayed qualitatively using bioautography to determine the number of antifungal components in the extracts. The acetone leaf extracts were also screened quantitatively using a serial microdilution assay to determine the minimum inhibitory concentration (MIC) values of each extract against *C. albicans*. Methanol extracts of *M. obtusifolia* and *M. lutea* leaves were also tested for antioxidant activity by determining the degree of 1,1-diphenyl-2-picrylhydrazyl-2-radical (DPPH) scavenging activity.

In the bioautography experiments, acetone extracts of both species inhibited growth of isolates of *C. albicans*, as indicated by clear zones of fungal growth inhibition on bioautograms. *M. obtusifolia* extracts had more antifungal compounds than *M. lutea* (3 and 1 respectively). Chromatograms obtained with a mobile phase system composed of chloroform, ethyl acetate and formic acid (CEF) in the proportion 10:8:2 provided the best resolution of active compounds. The MIC value for *M. lutea* after 24 h was 0.16 mg/mL; after 48 h it was 0.08 mg/mL and after 72 h it was 0.08 mg/mL. The MIC for *M. obtusifolia* after 24 h was 0.32 mg/mL, after 48 h it was 0.32 mg/mL and after 72 h it was 0.64 mg/mL. Contrary to the results obtained in the antifungal bioautography assay, methanol extracts of *M. lutea* showed stronger antioxidant activity when compared with methanol extracts of *M. obtusifolia*.

This study revealed that *Markhamia* species are important sources of antifungal agents, and further studies aimed at isolating the compounds active against *Candida albicans* are in progress.

The diversity of antifungal compounds of six South African *Terminalia* species (Combretaceae) determined by bioautography

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Opportunistic fungal infections represent a significant cause of morbidity and mortality in immunocompromised patients, including those with AIDS, cancer and organ transplants. Despite the increase in fungal infections, therapeutic options are very limited and are often unsatisfactory because of elevated toxicity and an inability to eradicate infections. In the last few years the incidence of fungal infections in the immunocompromised host has increased greatly. The emergence of these pathogens has been followed by both primary drug resistance and the secondary development of azole-resistant isolates of *Candida albicans* and *Cryptococcus neoformans*. The search for new antifungal agents led us to the screening of antifungal compounds in *Terminalia* species (Combretaceae). The selection of this genus is based on uses in traditional medicine, since *Terminalia* species are widely used medicinal plants both in Africa and in Asia. *Terminalia sericea* has recently been selected as one of the 50 most important medicinal plants in Africa by the Association for African Medicinal Plant Standards. The use of decoctions of several *Terminalia* species is widespread in Africa, and many species are reputed to contain antimicrobial constituents.

As a first step in the isolation of antifungal compounds from *Terminalia* species, six *Terminalia* species (*T. prunioides*, *T. brachystemma*, *T. sericea*, *T. gazensis*, *T. mollis* and *T. sambesiaca*) were screened for antifungal activity. Dried powdered leaves were extracted with acetone, hexane, dichloromethane and methanol. Chemical constituents of the extracts were analysed by TLC, using three eluent systems. For detection of chemical compounds chromatograms were sprayed with a vanillin sulphuric acid spray reagent. Bioautography procedures using 2 mg/ml p-iodonitrotetrazolium (INT) were performed to determine activity of extracts against *Candida albicans*, *Aspergillus fumigatus*, *Cryptococcus neoformans*, *Sporothrix schenkii* and *Microsporum canis*. The R_f values and relative activities of separated compounds were determined.

Hexane and dichloromethane extracts had at least three times more antifungal compounds than the other extracts indicating the nonpolar character of the antifungal compounds. From the R_f values, the non-polar character of the antifungal compounds was confirmed indicating that the antifungal activity is not due to tannins. *M. canis* had the highest number, up to ten, of antifungal compounds with all *Terminalia* extracts. All *Terminalia* species contained a compound of $R_f = 0.46$ in benzene/ethanol/ammonium hydroxide (90/10/1) active against all tested pathogens. *T. sericea* and *T. brachystemma* were the most promising candidates for isolating antifungal compounds.

The results demonstrate the value of bioautography in examining plant extracts with antifungal activity and selecting species for further study.

Anthelmintic activity of *Peltophorum africanum* extracts against parasitic gastrointestinal nematodes of livestock

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Gastrointestinal parasitoses are the most prevalent and economically important diseases of man and livestock. Parasitic nematodes are a major cause of production losses in small ruminants. Synthetic and semi-synthetically produced anthelmintic drugs have long been used to control helminthosis. However, widespread use has created multiple anthelmintic resistance, necessitating a search for alternative compounds and ways of parasitic control. There is a renaissance in phytotherapy, with 80% of people in developing countries depending on herbs for primary health care. As traditional use of medicinal plants doesn't follow the western paradigm for testing of efficacy, most medical and veterinary professionals distrust the use of herbs. The objective of the present study was to establish efficacy of extracts of *Peltophorum africanum*, against the important livestock parasitic nematodes, *Haemonchus contortus* and *Trichostrongylus colubriformis*.

Leaves, bark and roots collected from mature, naturally growing *Peltophorum africanum* trees were dried in the dark. The dried materials were ground and stored in dark glass bottles, prior to extraction with acetone. Various concentrations of acetone extracts of the leaf, bark and root of *P. africanum* were incubated (at 23°C) with the eggs and larval stage (L_1) of these parasites, for two and five days respectively, *in vitro*. Thiabendazole and water acted as positive and negative controls respectively. Concentrations of 0.2-1.0 mg/mL of the leaf and bark, and root extracts were found to be completely ovicidal and larvicidal. At concentrations of 5-25 mg/mL the extracts lysed the eggs and L_1 of both *H. contortus* and *T. colubriformis*.

The *in vitro* model results support the traditional use of *P. africanum* against helminths. We will attempt to isolate and characterize the active compounds within the extracts and to establish their mode of action. The work may lead, not only to possible isolation of novel anthelmintics from the plant, but also to better methods of plant extraction readily adaptable for use by rural communities against helminthosis.

Isolation and identification of antioxidants from *Bauhinia tomentosa* Linn (Fabaceae)

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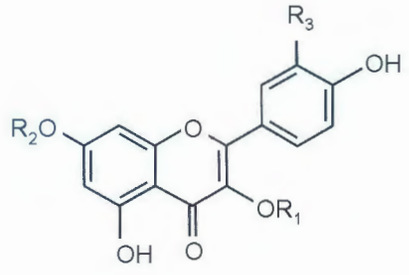
Recent advances in medical sciences have linked excess free radicals generated in the body to various chronic diseases such as cancer, hypertension and cardiovascular disease. Antioxidants protect against free radicals and are important in obtaining and maintaining good health. In this study, leaf extracts of *Bauhinia tomentosa* claimed to be effective as an anthelmintic, diuretic and aphrodisiac were investigated in our quest for novel antioxidants from natural sources.

An activity-guided fractionation procedure was used to identify the antioxidant constituents of the 50% ethanol dried leaf extract of *B. tomentosa*. The active compounds were isolated using chromatographic techniques including silica gel and Sephadex LH-20 column chromatography. The structures of the compounds were established by nuclear magnetic resonance (NMR) spectroscopy and mass spectrometry (MS). The DPPH (1,1-diphenyl-2-picrylhydrazyl) spectrophotometric technique was employed to follow the antioxidant activity in the separated fractions, and to evaluate the activity of the isolated compounds.

Four flavonol glycosides: kaempferol-7-O-rhamnoside (1), kaempferol-3-O-glucoside (2), quercetin-3-O-glucoside (3) and quercetin-3-O-rutinoside (4) were isolated from *B. tomentosa*. The structures of compounds 1 – 4 is as follows:



Compound	R_1	R_2	R_3
1.	H	Rhamnose	H
2.	Glucose	H	H
3.	Glucose	H	OH
4.	Rutinose	H	OH

All the compounds exhibited considerable antioxidant activity when compared to the control antioxidant substances used in the assay. Compounds 1-3 were reported for the first time from this species.

Bauhinia tomentosa is a rich source of antioxidant compounds that may protect against various diseases. The ethnopharmacological approach where medicinal plants are evaluated for biological activity has been shown to be useful in yielding active compounds.

Characterization of South African *Theileria parva* isolates

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Differentiation between *Theileria parva* subtypes has traditionally been based on differences in numbers of schizonts and piroplasms present in infected animals and the epidemiology of the disease. In this study, restriction fragment length polymorphism (RFLP) analysis of the variable regions of the parasite antigen genes p104, PIM and p67 was used to differentiate *T. parva* isolates.

Parasite DNA was extracted from blood collected from buffaloes from Hluhluwe (48 samples), Mabalingwe (7), Free State (1) and Kruger National Park (46) and cattle (4) from KwaZulu Natal (Mr Green's farm) and Welgevonden (2). Primers for amplification of the variable regions of the antigenic genes p67, p104 and PIM were designed, and the polymerase chain reaction (PCR) was used to amplify these regions. The sizes of the p67 amplicons were determined by agarose gel electrophoresis. Restriction enzymes *Bcl*I and *Alu*I were used to digest p104 and PIM amplicons respectively and RFLP profiles were analysed by polyacrylamide gel electrophoresis.

In East Africa a 130 bp insert in the p67 gene has been used to distinguish buffalo-derived from cattle-derived *T. parva* isolates. Most of the buffalo-derived isolates in this study lacked this insert, indicating that this criterion cannot be used to distinguish between buffalo- and cattle-derived isolates in South Africa. This finding, however, does not rule out the possible existence of other markers in the p67 gene.

The majority of the p104 RFLP profiles were typical of buffalo-derived isolates. A new profile was identified from the Welgevonden isolate, whilst the profiles of isolates from cattle on Mr Green's farm were similar to that of Muguga, a cattle-derived isolate from Kenya. PIM profiles from these isolates were homogenous and also resembled Muguga. Most of the buffalo-derived isolates had highly polymorphic PIM profiles, except for those from the Hluhluwe buffalo which were homogenous. The PIM profile of the Free State isolate was similar to the Hluhluwe profile.

The p67, p104 and PIM profiles can be used to characterize and distinguish between *T. parva* isolates, but it has not been established if the profiles correlate with the pathology of the isolates.

Molecular characterization and treatment of a chronic *Babesia gibsoni* infection in South Africa in an imported pit-bull puppy

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Canine babesiosis is a disease caused by two species of *Babesia* parasites, the large piroplasms or the *B. canis* group and the small piroplasms or the *B. gibsoni* group. The *B. canis* group consists of the three subspecies: *B. canis canis*, endemic to Southern Europe, *B. canis rossi*, endemic to Southern Africa and *B. canis vogeli*, endemic to tropical and subtropical areas.

B. gibsoni has been reported to be endemic to Asia, North America, North and Eastern Africa and Europe. Amongst the small piroplasms or the *B. gibsoni* group, there are at least three genetically distinct small piroplasms of dogs. These isolates are differentiated into the Asian, North America (California) and Spanish isolates. In the United States the most reported *B. gibsoni* infections are those of the Asian genotype. During early summer of 2004, a case of a *B. gibsoni* infected dog was encountered at Crowthorne Veterinary Clinic in Bryanston, South Africa. The dog was a three-month-old pit-bull puppy imported from Philadelphia, USA. The dog had come to the clinic for routine microchip implantation and de-worming. Canine babesiosis caused by *B. gibsoni* is a controlled disease in SA and this is the first time that it has been encountered in a dog residing in SA. Until recently there has been no effective treatment against this parasite.

On physical examination, the dog had no typical signs of clinical babesiosis, except for a temperature of 39°C. *Babesia gibsoni* infection was confirmed by way of smear examination, PCR, Reverse Line Blot (RLB) and sequence analysis. Approximately 2–4 ml of blood was collected from the cephalic vein into EDTA vacutainer tubes. About 200µl of blood was aliquoted into 1.5 ml eppendorf tubes and stored at +20 C until DNA was extracted. After DNA extraction, PCR was conducted with a set of primers that amplified a fragment of 460–540 bp of the 18S SSU rRNA gene spanning the V4 region. Reverse line blot (RLB) was performed using PCR products. Partial segments (400–540 bp) of the PCR products were sequenced. Sequence analysis revealed that the sequence had a 100% homology with the Asian genotype (accession no. AF205636).

Treatment was initiated with diminazene aceturate (Berenil RTU) followed by 2 doses of imidocarb diproprionate (Forray) three days and 14 days later. *B. gibsoni* DNA was still detectable two weeks post-treatment on the PCR/RLB test. A recently published 10-day combination drug therapy of atovaquone and azithromycin was initiated. Blood samples taken on day one and day nine after completion of treatment were negative for *B. gibsoni* DNA on PCR/RLB test.

Increased travel and movement of animals into South Africa has increased the possibility of new tick-borne pathogens being introduced in non-endemic areas. Without vigilant surveillance and correct treatment, the presence of potential tick vectors increases the chances of this pathogen becoming established in South Africa.

Central and peripheral *Babesia canis rossi* parasitaemias and their association with outcome of infection

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Data is presented from 100 dogs enrolled in a prospective, cross sectional, descriptive study of clinical cases of canine babesiosis. The objectives were to compare the use of central and peripheral smears for the detection of parasites and to determine whether the level of the parasitaemia was associated with outcome. In addition, we quantified the repeatability of our sampling and scoring methods.

Previous investigators disagreed on whether central or peripheral smears are most sensitive at detecting *Babesia canis* parasites. In a similar human haemoprotozoal disease (malaria due to *Plasmodium falciparum*), outcome is related to parasite density and sequestration of parasitised red blood cells (pRBC) in microvascular beds. These aspects of canine babesiosis have not been investigated.

Dogs were enrolled if large babesias were found on capillary smears. Infection with *B. canis rossi* was confirmed by reverse line blot (RLB). Dogs in which RLB detected *Ehrlichia canis* (n = 5) or *B. canis vogeli* (n = 2) were excluded. Peripheral smears were made from an ear prick. Central smears were made from jugular or cephalic blood collected within 10 minutes of making the capillary smears and prior to treatment. Parasitaemias were manually counted and expressed as the percent pRBC. Repeated scoring of the same smear and of paired peripheral and central smears was performed. Scoring was blinded. Dogs were grouped according to the following outcomes: death (D), admission for treatment (A), treatment as outpatient (H).

Peripheral parasitaemia (median 0.59%, range 0.04-73.9%, interquartile range (IQR) 0.19-3.70%) was significantly greater than central parasitaemia (median 0.14%, range 0-30.58%, IQR 0.046 – 0.55%) with $P<0.0001$. Five dogs had higher central parasitaemias. Within the individual outcome groups, the difference between central and peripheral parasitaemias was also significant. Pairs of peripheral smears showed a significantly lower intraclass correlation coefficient (ICC) than pairs of central smears with ICCs of 0.958 and 0.998 respectively ($P = 0.015$). There was insufficient evidence to show that the difference between central and peripheral parasitaemias increased with worsening outcome ($P = 0.35$).

It is concluded that peripheral smears are usually more sensitive at detecting *B. canis rossi* parasites, whereas results from central smears are more repeatable. Peripheral parasitaemias are variable between sites or over time and this variability increases when the animal shows signs of circulatory compromise. Both central and peripheral parasitaemias are significantly associated with outcome, although the ranges of the outcome groups overlap widely.

The elevated serum urea: creatinine ratio in canine babesiosis is not of renal origin

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The early diagnosis of acute renal failure in canine babesiosis is crucial but complicated by various biochemical issues. It has been observed that serum urea is often raised despite serum creatinine being normal in this disease. Free serum haemoglobin due to haemolysis may interfere with the determination of serum creatinine. Assay for cystatin-C (an excellent measure of glomerular filtration rate) in serum is unaffected by free serum haemoglobin. The origin of the high concentrations of urea in babesiosis is undetermined. We hypothesised that the raised urea and urea:creatinine ratio seen in babesiosis is not of renal origin. We also compared the concentrations of creatinine and cystatin-C in dogs with and without babesiosis- induced renal failure.

Twenty eight dogs with canine babesiosis and 13 normal control dogs were examined. Twenty five of the babesia infected dogs had a PCV <20%, representing a severely anaemic group. Three of the 28 had a PCV >50%, representing a haemoconcentrated group. The mean serum urea:creatinine ratios were significantly elevated in the anaemic group, when compared to the control group at 46.3 and 15.9 respectively, P <0.001. Serum urea was significantly correlated with the serum urea:creatinine ratio across all 3 groups, $r = 0.74$, P <0.001 Neither serum creatinine nor serum cystatin-C were significantly correlated with the elevated serum urea:creatinine ratio across all 3 groups, $r = 0.30$, $P = 0.06$ and $r = 0.19$, $P = 0.24$, respectively. The mean serum urea was also significantly elevated in the anaemic group when compared with the control group, P <0.001. The means for serum creatinine and serum cystatin-C did not differ significantly between the anaemic babesia group and the control group, $P = 0.27$ and $P = 0.45$ respectively. Serum urea therefore, as expected, was not significantly correlated with serum cystatin-C, $r = 0.17$, $P = 0.3$. Serum creatinine, however, was significantly correlated to serum cystatin-C, $r = 0.39$, $P = 0.01$. Serum urea was above control concentrations in 23/25 anaemic dogs; none of these dogs had elevated concentrations of serum creatinine or serum cystatin-C. Serum urea was elevated in three out of three haemoconcentrated dogs; two of these dogs also had elevated concentrations of serum creatinine and serum cystatin-C. Both of these dogs died and the one available for post mortem was diagnosed with acute renal failure.

The elevated urea:creatinine ratio in the anaemic group is therefore considered to be of non-renal origin. Serum creatinine appeared to be a more specific measure of renal disease than serum urea in canine babesiosis in this study. Serum creatinine appeared to be as specific and sensitive as serum cystatin-C in detecting azotaemia of renal origin in canine babesiosis in this study.

The impact of two dipping systems on endemic stability to bovine babesiosis and anaplasmosis in cattle at four communally grazed areas in Limpopo Province, South Africa

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A 12-month study was conducted at four communal grazing areas in the Bushbuckridge region, Limpopo Province, South Africa. The main objective was to investigate the impact of reduced acaricide application on endemic stability to bovine babesiosis (*Babesia bigemina* and *Babesia bovis*) and anaplasmosis (*Anaplasma marginale*) in the local cattle population. To this end 60 cattle at each communal grazing area were bled at the beginning and the end of the experimental period and their sera were assayed for *B. bovis*, *B. bigemina* and *Anaplasma* antibodies. Cattle in the intensively dipped group were dipped 26 times and maintained on a 14-day dipping interval throughout the study, whereas cattle in the strategically dipped group were dipped only 13 times. Three cattle, from which adult ticks were collected, were selected from each village, while immature ticks were collected by drag-sampling the surrounding vegetation. During the dipping process, a questionnaire aimed at assessing the prevalence of clinical cases of tick-borne disease, abscesses and mortalities was completed by an Animal Health Technician at each diptank.

An increase in seroprevalence to *B. bovis* and *B. bigemina* and a decrease in seroprevalence to *Anaplasma* was detected in the strategically dipped group whilst in the intensively dipped group the converse was true. *Amblyomma hebraeum* was the most numerous tick species on the cattle, and *Rhipicephalus (Boophilus) microplus* was more plentiful than *Rhipicephalus (Boophilus) decoloratus*. Drag samples yielded more immature stages of *A. hebraeum* than of *Rhipicephalus (Boophilus)* spp. The incidence of clinical cases of tick-borne disease and of abscesses increased in the strategically dipped group during the survey. These results were extrapolated from the questionnaire.

It was concluded that it was not necessary to dip intensively at fortnightly intervals in this region especially when one considers the relatively low tick burdens on the cattle and on the local vegetation. The increase in seroprevalence to *B. bovis* and *B. bigemina* in the strategically treated group indicates that if the decrease in dipping frequency could be maintained for long enough an endemically stable disease situation should result. Outbreaks of clinical cases of disease could be treated and vaccination could be used to supplement the natural tick challenge if it is not sufficient to maintain endemic stability. The increased seroprevalence to both *B. bovis* and *B. bigemina* in calves suggests that calf vaccination is unnecessary and that tick control should therefore be aimed mainly at preventing excessive tick worry.

Villous architecture and feto-maternal interdigitation in the African buffalo (*Syncerus caffer*) during different gestation stages

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Anchorage of the ruminant placenta is achieved by complementary indentation of chorionic villi into endometrial crypts. Geometrical patterns of interdigitation vary greatly among ruminants. Fetal villousity has been studied in related bovid species, cattle (*Bos taurus)* and water buffalo *(Bubalus bubalis*). The present study provides first information on villous architecture and feto-maternal anchorage in the African buffalo.

Tissue samples from 6 pregnant cows (crown/rump length in cm: 2,3,14,17,full term) were collected during disease eradication programs in the Hluhluwe Game Reserve and from a buffalo breeding centre at Phalaborwa (afterbirths, n=3). Tissue was fixed with 2.5% glutaraldehyde in phosphate buffer by immersion or, if possible, via vascular perfusion through uterine and umbilical arteries prior to separation of feto-maternal tissue for scanning electron microscopy (SEM) preparation. Samples were further prepared for light microscopy and SEM using standard techniques. SEM samples were examined in a Philips XL 20 Microscope operated at 7 kV.

Fetal villi consisted of a vascularized mesenchymal core covered by trophoblast epithelium. At a crown/rump length of 2 and 3 cm, the cotyledons comprised numerous short finger-like villi (length=0.15-0.46 mm) originating from the chorionic plate. Some of these primary (stem) villi divided longitudinally a short distance from their origin. These relative simple villi were accommodated within corresponding caruncular crypts. At later gestation (crown/rump length: 14 and 17 cm), fetal villi had lengthened (length=2-7.5 mm) and lateral (secondary) branching had started. The latter appeared in the form of bulbous or elongated structures projecting at approximately right angles from the stem villus. During the last month of gestation and in post partum samples, primary (8-12 mm) and secondary villi had developed considerably in length. Secondary villi displayed leave-like tertiary (terminal) villi of variable shape and size. A rich capillary network was observed within the connective tissue core of the tertiary villi as well as in the corresponding endometrial septae forming the walls of the caruncular crypts. Fully developed, each primary villus with its attendant secondary and tertiary villi formed a slender, slightly conical-shaped unit, also referred to as a cotyledonary villous tree.

The specific branching pattern of cotyledonary villi and complementary caruncular crypts in the African buffalo placenta, as described in this study, appears less complex when compared to the placenta in cattle. Villous-crypt architecture rather resembles structures occurring in the water buffalo placenta. This morphological similarity supports the assumption of a closer phylogenetic relationship between the two buffalo genera, than between buffalo and cattle.

Using the organic acid buffer MES to produce Foot and Mouth disease virus-free *in vitro* embryos

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The risk of transmitting Foot and Mouth disease (FMDV) virus by *in vivo* produced embryos is extremely small. *In vitro* produced (IVP) embryos carry a greater risk of transmitting FMDV. *In vitro*-produced day 7 embryos, exposed to FMDV can not be freed of the virus by washing. Similarly, IVP embryos, exposed to FMDV during *in vitro*-maturation (IVM) and *in vitro* fertilization (IVF) can not be freed of FMDV by washing. The aim of this study was to test if bovine IVP embryos, exposed to FMDV during IVM and IVF can be rendered free of infective FMDV by treating them with an organic buffer, MES (2-[N-Morpholino]ethanesulfonic acid) at a pH of 5.5.

Four groups of 300 and two groups of 320 oocytes were obtained from ovaries supplied by a local abattoir. Oocytes were matured, fertilized and cultured. Virus was added at a concentration of $2x10^6$ $TCID_{50}$ in all treatments. Treatment with MES buffer varied between 30s and 60s in all cases. After exposure to MES oocytes were washed 5 times in IVM, IVF or IVC medium. In all groups virus detection was attempted by PCR and culture using pig kidney cell monolayers.

Group 1 was not exposed to MES and not spiked with FMDV (control). In group 2 FMDV was added during IVM and IVF. Foot and Mouth virus detection was attempted after the denuded presumptive zygotes were exposed to MES. Group 3 was treated as for group 2, except that presumptive zygotes in this group were cultured up to day 7 before virus detection was attempted. In group 4, cumulus-oocyte complexes (COC) were treated with MES after IVM and viral detection attempted. Group 5 was treated as for group 4, but COC's were submitted to IVF. FMDV was added again during IVF. Denuded presumptive zygotes were again treated with MES and viral detection attempted. Group 6 was treated as for group 5, except that virus detection was not attempted after denuding, but presumptive zygotes were cultured up to day 7 before virus detection was attempted. No FMDV could be demonstrated in the control group. FMDV could be detected in all COC's. All denuded oocytes/embryos that were treated with MES were negative for the presence of infective FMDV. Treatment with MES is ineffective in rendering COC's free from infective FMDV. Denuded oocytes and presumptive zygotes treated with MES for 30-60s were free from infective virus. Treatment with MES did not negatively affect the blastocyst rate (Chi square test, $p<0.05$) in this trial.

It appears that FMDV is protected by the cumulus cells. It is not clear if the virus infects the cumulus cells *per se* or if it is protected in the intercellular spaces. It also appears that FMDV does not penetrate the zona pellucida and that if the surface of the zona pellucida is exposed to an acidic environment FMDV can be inactivated.

Effect of the acidic organic buffer MES on bovine *in vitro* embryo production

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Transferred embryos carry the risk of being vehicles of organisms causing diseases. Currently, the risk of *in vitro* produced embryos is more difficult to assess than the risk of *in vivo* produced embryos, since much less research has been published on the former. Foot and mouth disease virus (FMDV) is extremely sensitive to a low pH and could likely be destroyed if embryos were exposed to a low pH for a short period of time. MES (2-[N-Morphalino]-ethanesulfonic acid, an organic buffer with pKa 6.1; MES, Sigma, M2933) has been shown to destroy FMDV at a rate of 90% per minute at pH 6 and at a rate of 90% per second at a pH of 5. The aim of this study was to test whether exposing bovine oocytes and *in vitro* produced (IVP) zygotes to the organic buffer MES, buffered at pH 5.5, would be detrimental to the development of bovine IVP embryos.

In vitro maturation (IVM), fertilisation (IVF) and culture (IVC) was carried out using 1367 oocytes. Oocytes were divided into 3 groups; 484 were used as controls (no MES exposure), 437 in a maximal exposure group (MAX), ie MES treatment after washing of oocytes, after IVM and after IVF, and 446 in a minimal exposure group (MIN), ie MES treatment after IVF only. To treat the oocytes with MES, 100 oocytes (from ten droplets) were drawn up into a pipette in a maximal volume of 100µL solution and placed in 3mL of MES, swirled around for 10 seconds, picked up, again in a maximal volume of 100µL solution, and placed in 3mL of culture medium. Oocytes or zygotes were washed five times in culture medium before being further processed in IVM, IVF or IVC depending on their stage in the IVP program. Exposure time to MES varied between 30 and 60s (10 seconds swirling and a variable time period to pick up oocytes thereafter). A Chi-Square test was used to test for differences in cleavage and day 7 blastocyst yield between control and treatment groups ($p<0.05$).

Cleavage (70%; 340/484) and blastocyst rate (32%; 156/484) in the control group were not different from MIN (68%; 304/446 and 29%; 131/446 respectively), but were significantly higher than in MAX (57%; 249/437 and 18%; 79/437 respectively). In MAX the MES had a real harsh effect on the cumulus cells, making them granular and clumpy in appearance. Treated oocytes in MAX and in MIN adhered to the bottom of the dish in the MES solution making their handling difficult. Exposure time in MES was therefore variable and longer than initially planned.

It can be concluded that bovine IVP embryos can be exposed to MES without detrimental effect. Treatment with MAX still resulted in blastocysts, although it did not yield good embryo numbers. In future trials, treated dishes should be used to prevent oocyte and zygote adherence. Further research is necessary to test whether FMDV can be removed from bovine IVP embryos with the described method.

Effects of heterologous seminal plasma and semen extenders on progressive motility of frozen-thawed ram sperm

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Frozen-thawed ram semen crosses the cervix poorly, necessitating laparoscopic insemination. Acceptable fertility can be achieved with frozen-thawed ram semen deposited at the external cervical opening if ram seminal plasma (SP) is added. Homologous SP also improves the fertility of frozen-thawed sperm of boars and dogs. Heterologous SP may have effects as well; the addition of bovine SP increased the ability of buffalo sperm (*Syncerus caffer*) to fertilize bovine oocytes *in vitro*. The aim of the current study was to compare the effects of SP of rams (SPR), bulls (SPB) and dogs (SPD), protein-free TALP, Triladyl (Minitüb, Tiefenbach, Germany) and skim milk upon longevity and percentage progressively motile frozen-thawed ram sperm.

Three ejaculates from each of six rams (2 Dorpers, 2 Döhne merinos and 2 merinos), aged 2 4 years, were extended in Triladyl, pooled and frozen as a single batch per ram at 200 x 10^6/mL in 0.25 mL straws. SPR was obtained from the same rams and SPB from 5 bulls by centrifugation, while the post-sperm fractions were collected from 5 dogs (SPD). Within a species, the SP from different donors was pooled and frozen in aliquots at -18 °C. The 108 straws (6 rams, 6 diluents, 3 replicates) were thawed in random order. Once thawed, a straw was emptied into a tube with 0.85 ml of the appropriate fluid at 37°C and kept for 6 h. Percentage progressively motile sperm was estimated immediately, 2, 4 and 6 h after thawing at X200 magnification. One person thawed the semen and prepared motility specimens, while another performed all motility evaluations.

Data were evaluated by means of repeated-measures ANOVA, with rams as subjects and time and fluid as fixed effects. Non-significant interactions were removed from the model. Means were compared by means of Bonferroni's test ($P<0.05$).

The model included ram, time, fluid and ram x fluid, and time x fluid interactions, which were all significant ($P<0.01$). Mean motility decreased from each time to the next and were 39.0% (0 h), 26.0% (2 h), 19.6% (4 h) and 12.6% (6 h), SEM 1.38%, n=108. Mean motility was higher for skim milk (39.9%) than all fluids except Triladyl (27.7%), which was better than SPB (13.0%), whereas TALP (20.5%) and SPR (21.9%) were similar to Triladyl and SPB (n=72, SEM 2.85%). The interactions (ram x fluid or time x fluid) were mainly due to SPD, SPR, Triladyl and TALP, while milk resulted in the best and SPB in the lowest motility.

This study shows that heat-treated skim milk maintains progressive motility of frozen-thawed ram sperm better than SP of various species and protein-free TALP. In contrast to SPR, skim milk is known to result in poor fertility of frozen-thawed ram semen after cervical insemination. It would thus appear that maintenance of progressive motility *in vitro* may be a poor indicator of fertility after cervical insemination.

Validation of two different chromatographic methods for the analysis of urinary endogenous steroids in the Nile crocodile (*Crocodylus niloticus*)

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The Nile crocodile (*Crocodylus niloticus*) is considered to be a very valuable bio-indicator of aquatic pollution. Assessment of urinary steroid profiles in the Nile crocodile could provide important information about the functioning of the crocodile's endocrine system and, diagnostically, be used to determine exposure to aquatic pollutants. This study sought to determine whether an analytical method could be established to effectively measure urinary steroid metabolite levels in the Nile crocodile.

Analytical methodologies for the quantitative determination of urinary endogenous steroids include high performance liquid chromatography (HPLC) and gas chromatography-mass spectrometry (GC-MS). These two methods were validated and detection limits, quantitation limits, accuracy, precision, ranges of linearity, and stabilities of steroids and their metabolites were determined.

Before and after enzymatic deconjugation of steroid-sulphates and –glucuronides, sample preparation was achieved with solid phase extraction (SPE). After elution with ethyl acetate, the samples were dried at 37°C under a stream of nitrogen. Samples for gas chromatography/mass spectrometry were derivatized (methoxylation followed by tri-methyl silylation), and samples for high performance liquid chromatography were reconstituted in the mobile phase before analyses were performed. HPLC separation was performed on a C18 reverse phase column, employing 70% acetonitrile as mobile phase at 1 mL/min. Detection was done at 254 nm on a diode array detector (DAD) at 355 nm. A SE-30 capillary column was used as stationary phase for GC/MS, using helium (ultra high purity) as carrier. Electron impact ionisation was combined with a quadrupole detector.

R^2's obtained were >0.99. Sensitivity of analytes varied from the ppb range in HPLC to the ppt range in GC/MS analysis. Within- and between batch CV's were <10 and <15 respectively for GC/MS analysis. Stability of dried and derivatized samples was found to be prolonged when stored at -25°C.

It was found that both methods are sensitive and allow reliable detection of low levels of urinary endogenous steroids, and could be applied in future investigations for diagnostic and research purposes.

The microvasculature of the testis, epididymis and proximal ductus deferens of the ostrich (*Struthio camelus*) as revealed by India ink injection

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Although the basic pattern of arterial supply and venous drainage of the male reproductive tract of the ostrich has been established, only limited information is available on the vascularization of the individual reproductive organs. This paper reports on the vascularization of the testis, epididymis and proximal ductus deferens using India ink injection to identify the vessels.

The torsos of five male ostriches were obtained from various abattoirs. The arterial system of the testis, epididymis and ductus deferens in three torsos, was rinsed with physiological saline through the renal artery after which India ink was injected into the arteries via the same route. A similar technique was used for injecting the venous system in the remaining two torsos except that the testicular veins were used. Small tissue blocks were trimmed from the various organs, immersion-fixed in buffered 10% formalin for 2 days and then conventionally processed for light microscopy.

Arteries and veins could be identified in histological sections of the testis, epididymis and proximal ductus deferens due to the presence of India ink in the lumen of the vessel. In the testis, arteries were sparsely distributed in the tunica albuginea of the testicular capsule but were common in the interstitial tissue, especially in the wages between three or more seminiferous tubules. In some instances individual tubules were almost entirely surrounded by arteries, which varied greatly in size. In contrast, the venous network appeared to be concentrated in the testicular capsule with numerous variably sized vessels being observed in the tunica albuginea. Only few randomly distributed veins were observed in the interstitial tissue.

The parenchyma of the epididymal region contained few arteries and veins, none of which appeared specifically oriented in respect of any of the tubular elements (rete testis, proximal and distal efferent ducts, connecting ducts and epididymal ducts) present in the region. However, small arteries/arterioles were occasionally associated with few of the efferent ducts. Large arteries and veins were encountered in the connective tissue capsule surrounding the epididymal region. As in the testis, the veins were larger and more numerous than the arteries. Only a few arteries and veins were present in the proximal ductus deferens and large veins were seen superficially in the wall of the duct.

This study indicates that the large network of superficial vessels previously noted in the ostrich testis is venous in nature. The relative paucity of blood vessels, particularly of the arteries, observed in the parenchyma of the reproductive organs of the ostrich, when compared to those of the chicken, may be due to the route chosen for injecting the India ink. It is possible that most of the ink injected into the cranial renal artery goes to the kidney and that too little reaches the reproductive organs for ideal filling of all the vessels. The use of alternate injection strategies, possibly in conjunction with other techniques such as the preparation of corrosion casts and electron microscopy, may have to be considered to allow for more meaningful assessment of the microvasculature of the reproductive organs of the ostrich.

Immunoreactivity of protein gene product 9.5, neurofilament protein and neuron specific enolase in the ovary of the sexually immature ostrich (*Struthio camelus*)

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The innervation of the ovary has been studied in various species of birds and mammals. Research carried out in the domestic fowl has shown a high density of nerve fibres in the walls of mature follicles, thus suggesting that the innervation of the ovary plays a role in follicular growth and maturation[1]. Despite the fact that the innervation of any organ is an essential factor in controlling its growth and function, no information is available on the distribution of nerve fibres in the ovary of the sexually immature ostrich. Thus, the present study was undertaken to investigate the distribution of nerve fibres in the ovary of the sexually immature ostrich, using antibodies against neurofilament protein (NP), protein gene product 9.5 (PGP 9.5) and neuron specific enolase (NSE).

A total of 26 sexually immature female ostriches, aged between 12 and 14 months were used in the present study. Immunostaining was performed on 5mm thick sections of ovarian tissue using a LSAB plus kit (Dakocytomation). Antibodies against NP and PGP 9.5 were used at dilutions of 1:25, 1:50, respectively. A ready-to-use solution of antibodies against NSE was also used.

Strong immunostaining to NP, PGP 9.5 and NSE was observed in nerve bundles, which coursed through the ovarian stalk and extended into the medulla and cortex. In addition, NSE immunoreactive nerve cell bodies were observed in the cortex and medulla. Neurofilament protein, PGP 9.5 and NSE immunoreactive nerve fibres were present in the thecal layer of the follicular wall.

The current study has highlighted the distribution of NP, PGP 9.5 and NSE-immunoreactive nerve fibres in the ovary of the sexually immature ostrich. This appears to be the first study on the intrinsic innervation of the ovary in the ostrich using immunohistochemical techniques. The findings of the present study suggest that the distribution of nerve fibres in the sexually immature ostrich is similar to that of the domestic fowl[2].

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The ultrastructure of thecal gland cells in the ovary of the sexually immature ostrich (*Struthio camelus*)

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In birds, thecal gland cells are a common feature of the thecal layer in healthy follicles[1]. In addition, the theca interna cells of atretic vitellogenic follicles often differentiate into thecal glands[2]. Thecal gland cells are known to produce steroid hormones. The present study was undertaken to investigate the ultrastructure of thecal gland cells in the ovary of the sexually immature ostrich.

A total of 26 sexually immature female ostriches, aged between 12 and 14 months were used in the present study. Ovarian tissue was fixed by immersion in 2.5% glutaraldehyde in 0.1M cacodylate buffer. The samples were then processed and prepared for transmission electron microscopy, using standard methods.

Thecal gland cells in the thecal layer of previtellogenic and vitellogenic follicles occurred either singly or in groups of 3 to 6 cells. Two types of gland cells (type 1 and type II) were identified. The type 1 thecal gland cell contained an oval or elongated nucleus, which exhibited clumps of heterochromatin. The cytoplasm of these cells contained relatively few organelles, which included mitochondria and rough endoplasmic reticulum. In addition, bundles of fibrils were observed in these cells. The nuclei of type II thecal gland cells were round to oblong in shape. The cytoplasm of type II thecal gland cells contained several lipid droplets, as well as electron dense bodies.

Thecal gland cells, which originated from atretic vitellogenic follicles, contained eccentrically placed heterochromatic nuclei. The cytoplasm of these cells was filled with lipid droplets.

This study has shown marked differences in the ultrastructure of thecal glands located in healthy and atretic follicles. Further immunohistochemical studies need to be carried out to ascertain whether these thecal glands secrete similar steroid hormones.

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Gross morphological features of the oro-pharyngeal cavity of the ostrich (*Struthio camelus*)

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Although numerous studies have concentrated on the formulation of commercial diets for the raising of ostriches, very few descriptions of the morphology of the digestive tract have been published. This paper details the gross morphological features of the oral cavity and pharynx of the ostrich as part of a wider anatomical study of the upper digestive tract of this economically important bird

The heads of five one-day-old and five three-month-old ostrich chicks were obtained from birds sacrificed during a nutritional trial. The specimens were preserved in buffered formalin, rinsed in running water and incised at one commissure of the mouth to expose the oral cavity, pharynx and oesophagus. The macroscopic features were described and photographically recorded using a digital camera.

No morphological distinction could be made between the oral cavity and the pharynx and both cavi-ties formed a common chamber. The roof of the oral cavity was formed by the palate which contained a caudally positioned, bell-shaped depression, the choana through which the paired nasal cavities (separated by a sloping median ridge) communicated with the oral cavity. A longitudinal, median palatine ridge extended rostrally from the choana to the tip of the beak. The roof of the pharynx contained a median longitudinal fissure, the pharyngeal cleft which incompletely divided the roof into two medially overlapping, rounded mucosal flaps, each of which formed a retro-pharyngeal recess which was continuous with the longitudinal mucosal folds of the oesophagus. The common opening of the left and right pharyngotympanic tubes opened at the apex of the pharyngeal cleft. The roof of the oropharyngeal cavity was divided by a semi-circular white line into two distinct regions based on the colour of the mucosa: a pale rostral component and a pink caudal component.

The floor of the oropharyngeal cavity was formed by a rostral mucosal plate, the tongue and the laryngeal mound. The rostral mucosal plate, which presented numerous longitudinal folds, formed the floor of the oral cavity rostral to the tongue and extended bilaterally around both the tongue and the laryngeal mound, eventually merging with the oesophageal mucosa. The short, stubby tongue was folded back on itself dorsally, forming a distinct "pocket". The raised laryngeal mound was situated caudal to the base of the tongue and occupied the caudal third of the floor of the oro-pharyngeal cavity. The rostral half of the mound displayed three pairs of bulbous papillae which gave it a star-shaped appearance. The rostral pair were laterally directed whereas the caudal and medial pairs were caudally directed. This part of the mound contained the glottis which in the fixed specimens was opened wide in a V-shaped fashion. The lips of the glottis were prominent and elevated above the surface of the mound. The only difference observed between the two groups of birds was that the laryngeal papillae were more pronounced in the older birds.

This study confirmed the basic morphology of the oro-pharyngeal cavity of the ostrich, but in addition revealed previously non-described structural features such as the laryngeal papillae, the retro-pharyngeal recess and the presence of distinct mucosal regions in the roof of the cavity.

Ultrastructural features of the *ductuli efferentes* of the ostrich (*Struthio camelus*)

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Ductuli efferentes or efferent ducts (ED) are involved in fluid reabsorption in the epididymis, a phenomenon that is indispensable for post-testicular spermatozoal development and viability. In birds the ED are housed, together with various other ducts, in a specialized structure, the epididymal region or epididymis which is attached to the dorso-medial surface of the testis. Although a few reports are available on the structure of the epididymis of birds, very little is known about this region in the ostrich. The purpose of this study was to characterize the ultrastructure of the ED of the testis of the ostrich.

Epididymal tissue, obtained with minimum delay from five adult sexually active male ostriches slaughtered at local abattoirs, was fixed by immersion in 4% glutaraldehyde in Millonig's phosphate buffer. The tissue blocks were subsequently processed, using conventional standard procedures for light and electron microscopy.

The epididymis of the ostrich consists of a maze of ducts, including the rete testis, the efferent, connecting, and epididymal ducts. Each ED consists of two successive segments: proximal efferent duct (PED) and distal efferent duct (DED). The diameter and epithelial height are greater in the PED than in the DED. Three distinct cell types were identified in the columnar epithelium of the EDs: Ciliated (C) cells (found in both segments), as well as the non-ciliated (NC) type I (in the PED) and type II (in the DED) cells. The C cells were predominant in the DED, and their nuclei were large, often irregularly shaped and displaced apically, hence the pseudostratified appearance of the epithelium. The apex of the NC I cell extended into well-developed long microvilli and tubular coated pits opening into an elaborate sub-apical endocytic apparatus, which included a large vacuole containing flocculent material. Dense globular bodies, an inconspicuous Golgi apparatus and rough endoplasmic reticulum were also present in the supra-nuclear region. Mitochondria were numerous in the supra-nuclear region, but were also frequently aggregated in the infra-nuclear area. Dilated intercellular spaces occurred from about the apical one third of the cell, and were particularly conspicuous basally. The NC II cell also displayed long microvilli, as well as a large, conspicuous, usually heterogeneous and supra-nuclear, but occasionally infra-nuclear, electron-dense lipid droplet, a small Golgi apparatus, and a few profiles of smooth and rough endoplasmic reticulum. Their nuclei were basally located and were either oval or irregular in shape. Mitochondria were not uncommonly seen to aggregate at the base of the cells. However, the NC II cell conspicuously lacked dense globular bodies in the supra-nuclear region and demonstrated a poorly developed sub-apical endocytic apparatus.

The NC I cells of the PED displayed features that are typical of an absorptive cell, including well developed microvilli, an elaborate sub-apical endocytic apparatus, numerous basal mitochondria, and dilated basal intercellular spaces. This segment therefore appears, as in other birds, to be the main fluid-absorbing segment of the excurrent duct system of the testis of the ostrich.

The genome of the heartwater agent, *Ehrlichia ruminantium*, contains multiple tandem repeats of actively variable copy number

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Heartwater is a tick-borne disease of domestic and wild ruminants caused by the intracellular rickettsia *Ehrlichia* (previously *Cowdria*) *ruminantium*. It is of major economic importance throughout sub-Saharan Africa and in parts of the Caribbean, but there is currently no effective vaccine. Immunity to the organism is T-cell mediated, which makes vaccine development very difficult, so a genome sequencing project was undertaken, in order to facilitate the search for vaccine candidate genes. Over 90% of the work was done in South Africa, with contributions from other international centres. The results were published in January 2005 (*Proceedings of the National Academy of Sciences, USA*. 102:838.)

The genome is a 1,516,355 bp circular chromosome, predicted to encode 888 proteins and 41 stable RNA species. The most unusual feature is the presence of a large number of tandemly repeated and duplicated sequences, some of which were shown to undergo variation of copy number during replication of the organism. These repeats have mediated numerous translocation and inversion events, resulting in gene duplication and truncation, and also giving rise to new genes. The products of several of the new genes have been demonstrated to be readily visible to the immune system of the host, suggesting that they play a role in immune recognition. Free-living bacteria frequently evolve and adapt by acquiring genes from other species, but for intracellular bacteria this mechanism is not available. *E. ruminantium* therefore appears to have evolved a different mechanism for increasing its antigenic diversity.

Reconstruction of the central metabolic pathways of *E. ruminantium* depicts an aerobic organism which does not ferment glucose. The primary carbon sources are likely to be proline and/or glutamate, and the latter is transferred into a complete TCA cycle. The organism also synthesises its own ATP rather than acting as an "energy parasite", like Chlamydiales and some other Rickettsiales which make use of host cell ATP. Almost 30% of the genome is devoted to genes for proteins which are membrane-associated in some way. We also discovered several new families of membrane proteins, none of which have a known function, nor even any known homologues in other bacteria. These may well turn out to be valuable vaccine candidate genes.

E. ruminantium has been studied at Onderstepoort for nearly 80 years, and yet in the two years since the completion of the genome sequence we have learned more about how it functions than was ever known before. This demonstrates just how much new knowledge can become available from the new genomic-based techniques which are the frontiers of biology in the 21st century. It is essential for South African scientists to be active in these new technologies in order to remain relevant to the study of medical, veterinary, and agricultural problems which continue to hinder development in Africa.

Repetitive DNA in the complete genome sequence of *Ehrlichia ruminantium*

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The genome sequence of *Ehrlichia ruminantium* (Welgevonden), an intracellular rickettsia which causes heartwater, was completed recently. The genome sequence was obtained by whole-genome shotgun sequencing of clones from two small insert *E. ruminantium* genomic libraries. Sequences were assembled using PHRAP and GAP4. One of the most striking features of the *E. ruminantium* genome sequence is the large number of repeated sequences which constitute 8.3% of the chromosome. Repeats contribute to the high proportion of non-coding sequence (38%) which results in a larger size for the *E. ruminantium* genome than for other Rickettsiales. An analysis of the repeats was performed in an attempt to elucidate their role. Tandem repeats in the genome sequence were identified using the programs Mreps and Tandem Repeats Finder, while dispersed repeats were detected using Dotter and the repeat finder function in GAP4.

Many large tandem repeats (six bp up to a several hundred bp) were identified in the *E. ruminantium* genome sequence. Large tandem repeats which overlap at the beginning or end of a gene account for 25% of the pseudogenes identified in *E. ruminantium*. Four sites were identified where slipped-strand mispairing appeared to occur more frequently than at other tandemly repeated sequences in the genome. Interestingly three of these are tandem repeats of different 7 bp motifs, with vastly variable numbers of the repeated sequence motif. The fourth is a 122 bp repeat which occurs with continuously variable frequency from 1.5 - 7.5 times. Of the 30 coding sequences containing longer tandem repeats, 86.7% were genes whose products are predicted to be membrane-associated or genes unique to *E. ruminantium*. There were numerous duplicated sequences in the genome, including both direct and inverted repeats. 75 such repeat units were identified, most of which were present twice in the genome. Translocation and inversion events have resulted in duplication and truncation of a number of genes; in fact, 65.6% of the putative pseudogenes that were identified appear to have been produced in this way. Four large duplications (> 1 kb) in the genome were identified and duplication events appear to have resulted in the formation of several genes.

Reductive evolution is thought to occur through intrachromosomal recombination events at repeated sequences, which lead to deletions. Contrary to this hypothesis, in *E. ruminantium* duplicated and tandemly repeated sequences appear to contribute to an increase in the size of the genome in comparison to related intracellular pathogens and may be involved in increasing its genetic repertoire. In the isolated intracellular environment, intrachromosomal recombination and duplication events may be mechanisms used by *E. ruminantium* to increase its antigenic diversity by modifying gene functions and creating new genes. Whatever the role of the repeats, they are maintained and generated in the *E. ruminantium* genome in the face of reductive evolution, suggesting that they provide some selective advantage to the organism.

18S sequence data reveals genetic diversity in *Theileria equi* and *Babesia caballi*

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Equine babesiosis (piroplasmosis) is an infectious tick-borne disease of the equidae caused by two different intraerythrocytic protozoa, *Babesia caballi* and *Theileria equi*. Tests for equine prioplasmosis prescribed by the OIE for international trade are serological assays (ELISA and IFA) which detect antibodies. An attempt was made to develop a real-time polymerase chain reaction (PCR) test to detect the organisms themselves.

Species specific Simple Probes were designed based upon sequence differences in the V4 hypervariable region of the published *B. caballi* and *T. equi* 18S rRNA gene sequences. In addition, amplification primers for the specific amplification of a 180bp (*B. caballi*) and a 160 bp (*T. equi*) portion of this region were designed for the real-time PCR test. The real-time test was used to detect three known positive *B. caballi* samples and seven *T. equi* known positives. Positive signals were obtained from all of the *T. equi* samples, but one of the *B. caballi* samples, EQ158, was not detected. Primers designed in conserved domains of the 18S rRNA gene of *Theileria* and *Babesia* species were therefore used to amplify and sequence the hypervariable V4 region from all ten samples. The sequences of two of the *B. caballi* samples and four of the *T. equi* samples differed from the published sequences at several nucleotides. Three nucleotide differences at the 3' end of the *B. caballi* reverse primer in the sequence of sample EQ158 explained the failure of the real-time PCR test to detect the parasite in this sample.

It will therefore be necessary to redesign the primers and probes for the real-time PCR test. However, before this can be done, it will be necessary to further assess the sequence diversity in the 18S gene amongst protozoal parasites that cause equine piroplasmosis. One hundred and eighty-three samples from different geographical locations in South Africa were obtained. Samples containing parasites with novel genotypes were selected for sequencing using the reverse line blot (RLB) assay. The RLB allows the simultaneous identification of distinct piroplasm species present in the same sample and gives an indication of the possibility of a new species or genotype. *T. equi* was identified in 86 samples and 13 samples were *B. caballi* positive. Twenty-one samples hybridized only to the *Theileria/Babesia* "catch-all probe" (which detects any *Theileria* or *Babesia* species), but not to any of the species-specific probes included in the assay. These samples are currently being sequenced and based on the new sequence data, a new set of real-time probes will be designed and tested on the lightcycler.

New *B. caballi* and *T. equi* 18S genotypes were identified during the development of a real-time PCR assay to detect these parasites. Sequence diversity in the 18S gene must be further assessed before a new real-time PCR test can be designed.

Phylogenetic analysis of *Babesia* parasites in cheetahs in South Africa

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Conventional diagnosis of piroplasmosis relies on the identification of organisms on blood smears, which is unreliable. Specimens from a variety of species are submitted routinely to our laboratory for molecular screening for the presence of parasites. The Reverse Line Blot hybridisation assay (RLB) was used to screen 140 cheetah (*Acinonyx jubatus*) speciments which had been submitted by various collaborators.

DNA was extracted from blood samples using the QIAamp DNA extraction kit (Southern Cross Biotechnologies). A PCR was performed using primers that amplified a 460 – 520 bp fragment in the V4 variable region of the 18S rRNA of *Theileria* and *Babesia* species. The PCR amplicons were analysed using the RLB hybridisation assay and cycle sequenced (BigDye® Terminaror V3.1 Cycle Sequencing Kit; Applied Biosystems). *Babesia* and/or *Theileria* genus-specific probes, as well as *B. felis*, *B. leo* and *Cytauxzoon felis* species-specific probes were included. Sequencing electrophoresis was done on a 3130 XL Automated Sequencer (Applied Biosystems). Sequence analysis (Staden package) and alignments with known sequences of related genera using the ClustalX program was preformed. A phylogenetic tree was constructed using the neighbor-joining in combination with the bootstrap method.

Although some of the cheetah blood samples tested positive for *B. felis* and *B. leo,* a large number (29.45 %) gave a positive signal for only the *Babesia* and/or the *Theileria* genus-specific probe. A phylogenetic tree was constructed by using sequencing data from 16 of these cheetah isolates. The sequence similarity analysis showed that some isolates are closely related to *B. gibsoni* California, previously described in dogs and others grouped together with *B. felis and B. leo* sequences.

High bootstrap values support the possibility of the presence of two unknown *Babesia* parasites in cheetahs in South Africa. A probe is currently developed for the use in the RLB that will target the parasites that are closely related to the *B. gibsoni* California isolate.

Piroplasmosis as a possible cause of mortality in giraffes

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Piroplasms have previously been reported from giraffes in Kenya and Namibia and in one instance theileriosis was suspected of causing mortality. The diagnosis was based on the presence of small intraerythrocytic piroplasms on blood smears. Recently, three cases involving adult giraffes from different geographic areas in South Africa, which showed a sudden onset of disease, were reported. The course of the disease in each case was peracute and appeared to affect many organ systems. Some of the clinical signs noted were: severe depression, recumbency and severe weakness, hypothermia, dyspnoea, rumen stasis and oedema of the sclera and conjunctiva. Some of the post-mortem findings included haemoglobinuria, haemopericardium and macroscopic necrotic foci in the liver. Microscopic examination of thin blood smears revealed the presence of small piroplasms in red blood cells.

In an attempt to confirm piroplasmosis as the causative agent of the disease, DNA was extracted from blood and spleen samples. The V4 variable region of the parasite 18S rRNA gene was amplified and analyzed using the Reverse Line Blot (RLB) assay. The full-length 18S rRNA genes of the parasites were amplified and cloned using the Promega pGEM–T Easy Vector System and recombinants were sequenced. Sequencing data were assembled and edited with the GAP4 package and aligned with published sequences of related genera using ClustalX. A phylogenetic tree was constructed using the neighbor-joining method in combination with the bootstrap method.

RLB results showed that the PCR products amplified from all three giraffe samples hybridized only with the *Babesia/Theileria* genus-specific probe, but not with any of the *Babesia* or *Theileria* species-specific probes present on the blot. This probably indicated the presence of a novel *Babesia* or *Theileria* species or variant of a species. The 18S rRNA gene sequences of the parasites isolated from both GIR0105 and GIR229 showed the highest similarity with *B. orientalis*, previously isolated from a water buffalo, and a bovine-isolated *Babesia* species. The 18S rRNA sequence of the parasite isolated from GIR224 showed similarity with a group of *Theileria* parasites, which included *T.* sp. Duiker, *T. separata*, *T.* sp. Sable and *T.* sp. Namibia.

This study indicated that piroplasmosis could be considered as a possible cause of mortality in the three giraffes examined. Further molecular characterization of these parasites is, however, required as well as a determination of the prevalence of these organisms in giraffes.

Verification of parentage in African wild dog (*Lycaon pictus*) packs shows multiple paternity

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The African wild dog, second most endangered carnivore in Africa, has a well-developed, highly cooperative pack system. The usual structure of a pack consists of a dominant breeding pair, the alpha male and the female, several subordinates, non-breeding adults and dependent offspring. Domestic dog microsatellites were used to study the parentage in three packs and confirm that more than one dog, including the subordinate males, can sire pups within a litter as previously suggested[1].

The study was performed on two isolated populations of wild dogs in the North West Province of South Africa. In Madikwe Game reserve skin samples from 47 dogs were obtained by means of biopsy darts and stored in absolute ethanol. In Pilanesberg National Park blood samples from 18 dogs were collected in EDTA tubes during capture. The wild dogs were photographed and individually identified according to coat patterns. Behavioural data to determine ranking were collected from all three packs.

DNA was extracted using proteinase-K digestion followed by isolation of DNA with phenol/chloroform/isoamyl alcohol. A total of 16 microsatellite loci that consistently amplified and appeared to be polymorphic in wild dogs, were used. Polymerase Chain Reaction was performed using two panels of microsatellite loci in multiplex reactions. An amount of 1 µl of PCR product was loaded on to the 3130 *XL* Genetic Analyser with Genescan 500 LIZ (Applied Biosystems) size standard and analysed using *STRand* (Board of Regents, University of California) software program. CERVUS2.0 [2] software was used to calculate allele frequencies, expected and observed heterozygosity, frequency of null alleles, polymorphic information content and exclusion probabilities for parentage assignment. Parentage verification was also performed manually.

The parentage analysis revealed that in each of the five litters at least one pup was not sired by the alpha male. Although previous studies suggested that the alpha male sires the majority of offspring in the pack our results confirm that subordinate males commonly sire pups with the alpha female if and when the opportunity arises. Possibly this is a mechanism to decrease the chances of inbreeding.

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Identification of anti-babesial activity for four ethnoveterinary plants *in vitro*

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Babesiosis is a tick-borne disease affecting a wide range of vertebrate hosts in both the tropical and sub-tropical parts of the world. Clinically the babesial protozoan parasite is related to malaria by having an almost identical clinical presentation and belonging to the phylum Apicomplexa. In South Africa there are several plant remedies in use by small-scale rural farmers to treat babesiosis. Their choice in herbal remedies is based solely on knowledge passed down from generation to generation. As such the efficacy of their chosen remedies is always questionable and needs proper evaluation.

A commonly available *Babesia caballi* culture system was utilized to determine if *in vitro* anti-protozoal activity was present in four ethnoveterinary plants, *Rhoiscissus tridentata*, *Elephantorrhiza elephantina*, *Aloe marlothii* and *Urginea sanguinea*. Well-established *B. caballi* cultures, with 250 ìl of infected cells, were initially inoculated with either imidocarb diproprionate and diminazene aceturate for the validation of the assay. The degree of inhibition was determined using the colour change within culture flask and by calculating the degree of erythrocyte parasitaemia and converting the value to the drugs' effective concentration (EC).

The Imidocarb and Diminazene had an EC_{50} value of 0.08 and 0.3 ug/mL respectively. The degree of inhibition as evaluated from the colour change correlated with the mean cell parasitaemia. The acetone extracts from *E. elephantina, A. marlothii and U. sanguinea* had no inhibitory activity while only the *E. elephantina* rhizomes extracts had a reproducible effect at a concentration of 100 ug/mL (EC_{50} not calculated). The colour change method of evaluation appeared to be initially insensitive in indicating the inhibitory activity of the herbal acetone extracts. However, when inoculant size of infective parasites was reduced a positive colour change was evident.

From the above assay it would appear that ethno-veterinary leads may help in the elucidation of a new antibabesial drugs and therefore warrants further study. With the numerous side effects associated with current commercial remedies a novel antibabesial may help make treatment safer. It may even lead to the discovery of agents effective against malaria.

Faculty of Veterinary Science
University of Pretoria

Faculty Day 2005 : Committees

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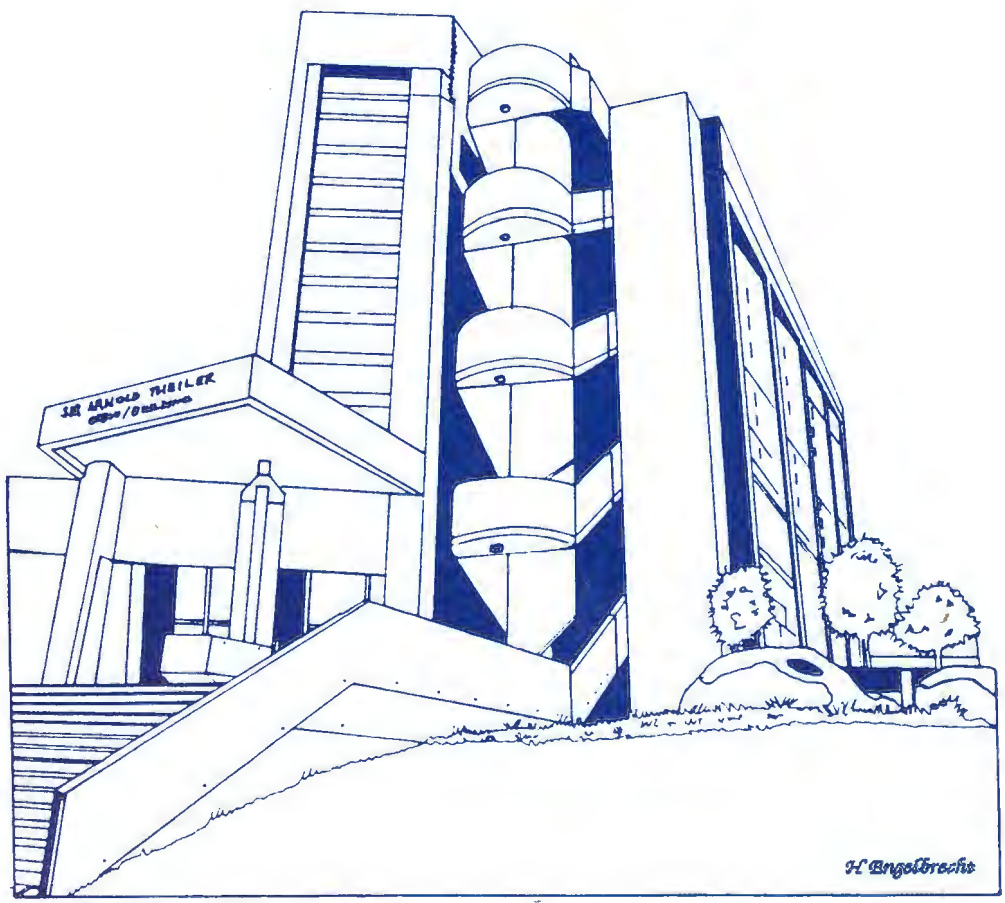
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