



# UNIVERSITY OF PRETORIA

## FACULTY OF VETERINARY SCIENCE

# Faculty Day

September 16, 2004

## PROGRAMME AND SUMMARIES



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# FACULTY DAY

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**16 SEPTEMBER 2004**



## **SPONSORSHIPS**

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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 5<sup>th</sup> 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*

Once again, it is my privilege to welcome you all to Faculty Day. This day is one of the highlights on the calendar of the Faculty and is an event that we are very proud of. Thank you for making the time available to share this very special day with us

Research remains one of the core activities of the University. This day allows our staff to provide some insight into their activities and the efforts of the Faculty in the many fields in which we function. It is also an opportunity for the young researchers in the faculty, be they staff or students, to become accustomed to addressing an audience about their scientific endeavours

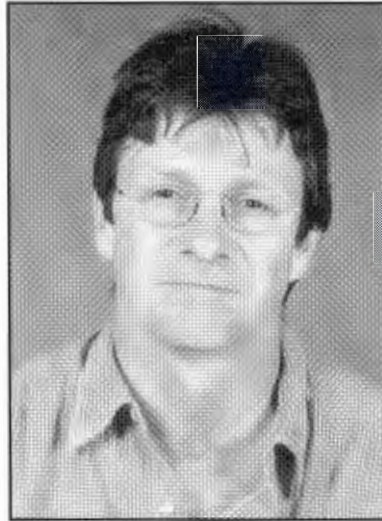
Today allows us too to award excellence. During the course of the day the Researcher of the Year, the Young Researcher of the Year, and the Lecturer of the Year awards will give recognition to outstanding performance in these fields. In addition, there will be awards for the best scientific presentation during the course of the day and the best poster.

Faculty Day has the purpose of bringing together colleagues, friends, and alumni from various walks of life. It is my privilege to welcome each and everyone here to the Faculty. I trust that you will enjoy the day and gain some insight into the research activities of our personnel

I also wish to extend my sincere appreciation to everyone that contributed to make this day special and a success. A particular word of thanks to Prof John Soley, the chairman of the organizing committee, and its members for the time and effort that they put into organizing this day. Congratulations on a job well done

NICK KRIEK  
DEAN





*Dr. Richard Kock*

## **DOCTOR RICHARD KOCK**

Richard Kock was born in Bulawayo in 1956. He qualified from the Cambridge University within the Medical Sciences Tripos (BA) in 1977 and from the CU School of Veterinary Medicine (VET MB) (MA Cantab) in 1980, when he was registered as a member of the Royal College of Veterinary Surgeons. Soon after qualifying he entered the world of the wildlife veterinarian in which he has been immersed for 22 years. He is accredited to the Royal College of Veterinary Surgeons in the UK, as a specialist in zoo and wildlife medicine and is currently registered with CU for their new VetMedDr. He has spent 22 years of his career affiliated to the Zoological Society of London (ZSL). Initially he worked as a veterinary officer and curator for ZSL's zoological parks and subsequently on conservation and animal health programmes around the world mainly in Africa, where he was born and has been resident for most of his life.

In 1991 he was seconded from ZSL to the Kenya Wildlife Service (a parastatal institution mandated to manage and conserve all wildlife in Kenya) to head a new Veterinary Unit (5 Vets, 2 Technicians, 30 officers and field staff) for a period of 7 years. The programme was successful with a now functional and sustained Unit at KWS with an excellent track record of wildlife disease diagnosis and support to the veterinary authorities and wildlife management in the country.

From November 1998-2004, he was seconded to a regional body, the African Union/Inter African Bureau African Resources (AU-IBAR Pan African Rinderpest Campaign and Pan African Programme for the Control of Epizootic Diseases). For the last 4 years, he has been a member of the Epidemiology Unit at PACE-IBAR headed by Dr Gavin Thomson recently of OVI. His work has focused on; the organisation and implementation of extensive sero-surveillance and disease investigation in susceptible wildlife species at the livestock interface throughout Eastern Africa and also in certain countries in Central and Western Africa; working mainly on rinderpest and PPR, as well as on training veterinary colleagues from these countries and establishing a surveillance network for wildlife disease.

He is well travelled having undertaken consultancy missions for ZSL, the International Union for the Conservation of Nature and Natural Resources, the World Wide Fund for Nature, The World Bank and The Food and Agriculture Organisation of the United Nations (GREP). He also contributes on a voluntary basis to the IUCN network as co-chair of the IUCN, Veterinary Specialist Group, and as Treasurer of the newly formed Wildlife Disease Association (Africa and Middle East section). He promotes wildlife veterinary matters through extensive publications in books and refereed journals (>65), and at professional meetings.

FACULTY OF VETERINARY SCIENCE, UNIVERSITY OF PRETORIA

# FACULTY DAY

THURSDAY 16<sup>TH</sup> SEPTEMBER 2004

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## PROGRAMME

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07:45-08:15    **Registration and Coffee**  
Master of Ceremonies: *Professor J O Nöthling*

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: *Professor C M Veary*

**Repeated use of the GNRH analogue deslorelin to down-regulate reproduction of male cheetahs (*Acinonyx jubatus*)**  
*H J Bertschinger, M Jago, J O Nöthling, A Human*

**Hypochromic, poikilocytic anaemia of neonatal roan antelope (*Hippotragus equinus*): a perinatal thalassaemia?**  
*S D C Parsons, B L Penzhorn, F Reyers, J C A Steyl, P J Becker*

**Risk factors contributing to an outbreak of copper and manganese deficiency in sheep utilising harvested maize fields or baled maize stover**  
*J G Harmse, G H Rautenbach*

**A histopathological study of mobile and immobile mice injected subcutaneously with necrotising snake venom: preliminary results**  
*N T Ndudane-Tyumre, R S M Blaylock, M Williams*

**South African field trials of a gametocyte antigen derived from *Eimeria maxima*: Immunization of breeder pullets for the protection of their progeny against coccidiosis**  
*SPR Bisschop, J M Greyling*

**A phylogenetic study of South African Newcastle disease virus strains isolated between 1990 to 2002 suggests epidemiological origins in the Far East**  
*C Abolnik, R F Horner, S P R Bisschop, M E Parker, M Romito, G J Viljoen*

- 09:30-10:20 **Sir Arnold Theiler Memorial Lecture: “Wildlife Domestic Animal Disease Interface – Hard or Soft Edge?”**  
*Doctor Richard A Kock*
- 10:20-11:00 **TEA and Viewing of Posters, Commercial Exhibits and Photographic Exhibition**
- 11:00-12:00 **RESEARCH PROGRAMME: ORAL PRESENTATIONS II**  
**SESSION CHAIRPERSON:** *Doctor J E Crafford*
- Response of the splenic dendritic cell population in a mouse model of malaria infection**  
*A L Leisewitz, K A Rockett, B Gumede, M Jones, B Urban, D P Kwiatkowski*
- New *Babesia* parasites in cheetahs and other wild felids**  
*A-M Bosman, E H Venter, N Collins, B L Penzhorn*
- Confirmation of occurrence of *Babesia canis vogeli* in domestic dogs in South Africa**  
*P T Matjila, B L Penzhorn, C P J Bekker, A M Nijhof, F Jongejan*
- Infection of roan antelope (*Hippotragus equinus*) with theileriosis using a cryopreserved stabilate derived from *Rhipicephalus evertsi***  
*J Steyl, L Prozesky, J Lawrence, B Penzhorn*
- The effect of haemolysis on AT III concentration as determined by a chromogenic method**  
*L L van der Merwe, L S Jacobson, J P Schoeman, R Delport*
- 12:00-12:30 **RESEARCH PROGRAMME: PRESENTATION OF POSTERS**  
**SESSION CHAIRPERSON:** *Professor E H Venter*
- P1. **Medicinal value of edible stink bug, *Encosternum delegorguei*, consumed in the Limpopo Province of South Africa**  
*L S Teffo, J N Eloff, R Toms*
- P2. **Screening of twenty-four South African *Combretum* species (Combretaceae) for antifungal activities.**  
*P Masoko, J Picard, J N Eloff*
- P3. **Antibacterial and antifungal activity of a by-product of the grape seed extraction process**  
*H Chikoto, J N Eloff, G E Swan*
- P4. ***In vitro* estimation of the number of corpora lutea in the ovaries of bitches by means of magnetic resonance imaging**  
*J O Nöthling, K de Cramer, D Gerber, P Irons*
- P5. **Effects of heterologous semen plasma and semen extenders on progressive motility of frozen-thawed ram sperm**  
*Gracinda Mataveia, S J Terblanche, J O Nöthling, D Gerber*

- P6. **Determination of pregnancy status of African buffalo by faecal steroid analysis**  
*P Irons, V King, L Otte, H Bertschinger, B Colenbrander, D Gerber*
- P7. **Basic macroscopic features of the venous drainage of the reproductive organs of the male ostrich (*Struthio camelus*)**  
*M Z Elias, T A Aire, J T Soley*
- P8. **Practical treatment of keratoconjunctivitis in Saanen goats**  
*JG Harmse, CAP Carrington*
- P9. **Ultrastructural assessment of primary ciliary dyskinesia in a Staffordshire bull terrier**  
*R G Lobetti, M de Scally, E van Wilpe*
- P10. **The importance of the vomeronasal organ in modulating the “buck effect” in does**  
*K K Booth, E C Webb, H J Jansen van Vuuren*
- P11. **Detection of bovine papillomavirus DNA in sarcoids in the Cape Mountain Zebra (*Equus zebra zebra*)**  
*E van Dyk, A-M Bosman, E H Venter, P J Nel, D Zimmerman*
- P12. **Withdrawal periods and tissue tolerance of intramammary antibiotics in dairy goats: preliminary results**  
*J Karzis, E F Donkin, I M Petzer*
- P13. **Assessing the level of knowledge of Animal Health Technicians involved in extension to control internal and external parasites of cattle**  
*M J Sekokotla, C M E McCrindle, M Mefane*
- P14. **The influence of rearing systems on gut development of kids**  
*T Songabe, E F Donkin, E D Green, E Gruys*
- 12:30-13:00 **VETERINARY EDUCATION SYMPOSIUM: An Online Distance Education Master’s degree programme in Veterinary Tropical Diseases**  
**CHAIRPERSON:** *Prof M van Vuuren*
- New primarily Web-based Master of Science degree programme in Veterinary Tropical Diseases**  
*J A W Coetzer*
- Paradigm shifts in Veterinary Tropical Medicine Education: Course development for online delivery – a case study**  
*E Mostert*
- Paradigm shifts in Veterinary Tropical Medicine Education: preparing Lecturers to teach in the online environment – a case study**  
*A Strehler*

- 13:00-13:45    **Light LUNCH in Cafeteria**
- 13:45-15:00    **RESEARCH PROGRAMME: ORAL PRESENTATIONS III**  
**SESSION CHAIRPERSON:** *Doctor M-C Madekurozwa*
- Canine cancer in South Africa: Prevalence and Outcomes (1997 – 2004)**  
*A B Zambelli, J P Schoeman*
- Feline cancer in South Africa: Prevalence and Outcomes (1997 – 2004)**  
*A B Zambelli, J P Schoeman*
- The use of a bacterin vaccine in broiler breeders for the control of *Ornithobacterium rhinotracheale* in commercial broilers**  
*S P R Bisschop, M van Vuuren, B Gummow*
- A study of the efficacy of a thermostable Newcastle disease vaccine in village chickens when administered by different routes**  
*S P R Bisschop, B L Mogoje, M M O Thekisoe*
- An immunohistochemical and histological characterization of atretic ovarian follicles in the immature ostrich (*Struthio camelus*)**  
*W H Kimaro, M-C Madekurozwa*
- Survey of small scale dairy farmers in Central North West Province using participatory rural appraisal**  
*P J Sebei, L Prozesky, C M E McCrindle*
- Duration of repellency of selected agents against *Culicoides* spp. when applied to polyester mesh**  
*P C Page, A J Guthrie, G J Venter, P Stadler, K Labuschagne, J Nurton*
- 15:00-16:00    **RESEARCH PROGRAMME: ORAL PRESENTATIONS IV**  
**SESSION CHAIRPERSON:** *Doctor J Schoeman*
- Ultrasonography of the solar aspect of the distal phalanx of the horse**  
*A Carstens*
- Wobbler syndrome in the Boerboel**  
*M J Gray, R M Kirberger, T C Spotswood*
- A biomechanical investigation of the static stabilisers of the glenohumeral joint in the dog**  
*M J Gray, N E Lambrechts, K E Joubert, N G J Maritz*
- Diagnostic imaging assisted diagnosis of discospondylitis in two horses**  
*L Sweers, A Carstens*
- Intralesional Carboplatin for the treatment of cutaneous mastocytoma in a Siberian hamster (*Phodopus sungorus*)**  
*A B Zambelli, C Elton, T Meiring, J P Schoeman*

## PROGRAMME

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

16:30-17:30 **RESEARCH PROGRAMME: ORAL PRESENTATION V**

**SESSION CHAIRPERSON:** *Professor C J Botha*

**Is searching for high activity antimicrobial compounds from plants an exercise in futility?**

*JN Eloff*

**Development of an antibacterial and antioxidant rich extract from the leaves of *Combretum woodii***

*V K Zishiri, J N Eloff, C J Botha*

**Biological activity and chemical comparisons of *Leonotis* (Wild Dagga) species**

*L J McGaw, J N Eloff*

**Preliminary antibacterial and antioxidant properties of *Dicerocaryum zanguebarium***

*D T Ntloedibe, J N Eloff*

**Anti-bacterial, anti-inflammatory and cyto-toxicity study of medicinal plants used in the treatment of wounds and retained placenta in livestock**

*D Luseba, J van Staden, E E Elgorashi, D T Ntloedibe*

**Novel biologically active triterpenoids from *Combretum imberbe* and *Combretum padoides* leaves**

*J E Angeh, J N Eloff, G E Swan, S E Huang, I E Sattler.*

17:30-

**COCKTAIL FUNCTION and PRIZE GIVING**

**During the cocktail function the following awards will be presented:**

**Lecturer of the Year; Researcher of the Year; Young Researcher of the Year; Best Scientific Paper; Best Scientific Poster; Photography prizes**

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

1. **PHOTOGRAPHIC EXHIBITION**

An exhibition of photographs taken by staff and students. The photographs will be judged by Derek and Norma Pearman (Hon. FPSSA, FPSSA) who have extensive national and international judging experience.

*Organisor: Dr E van Dyk*

2. **EXHIBITS BY SPONSORS**

3. **SCIENTIFIC POSTERS**

## Wildlife Domestic Animal Disease Interface – Hard or Soft Edge?

*R A Kock* (Richard.kock@oau-ibar.org)

Pan African Programme for the Control of Epizootic Diseases, African Union Inter-African Bureau for Animal Resources, Nairobi, Kenya

Written records of the transmission of diseases between species are almost as old as civilization itself. There are classical examples such as anthrax and tuberculosis, with relatively more recent reporting of the role of multiple wild and domestic hosts in the epidemiology of diseases such as influenza (birds-mammals), rabies (mammal – mammal) and Foot and Mouth Disease (buffalo – impala – kudu - cattle), as well as the improved understanding of the complex vector-host relationships of many diseases (African Horse Sickness, African and Classical Swine Fever, trypanosomosis, theileriosis, Rift Valley Fever). Land use (in support of the development of human society) has in part, been influenced by disease risk at this interface and the current landscape in South Africa is a good example. Here land parcels are usually fenced from each other according to government, commercial, private or community interests, and often according to the regulatory framework for disease control – creating the so-called *hard edge* between animal populations. This is similar to landscapes seen in most so-called developed nations. The situation in Eastern Africa is significantly different with large tracts of land much as they have always been since pre-ice age, without a clear land use policy and landscaping, the main landscape influences being climatic. Here there are much larger numbers of small-scale farmers, traditional pastoralists, and wilderness areas with wildlife and livestock often mixing on a continuous or seasonal basis – *the soft edge*. These wildlife ecosystems are attractive for tourism and have been the basis of strong industries in Kenya and Tanzania, but this has not compensated for their weak agricultural sectors. There continues to be a sporadic need for food aid to sustain the growing population in times of drought. The belief that this form of land management is ecologically sound has even influenced some of the recent thinking in Southern Africa, with the development of Peace Parks and larger transboundary wildlife management areas, whereas ironically in East Africa there is a move to develop a more commercial approach to wildlife and livestock management, similar to what is now, a highly developed industry in the south. Looking at the advantages and disadvantages of the different land management scenarios, from a disease perspective continues to challenge our thinking but both regions can learn from each other.

In terms of disease control, the policies in the south have worked effectively within the framework of international trading (sanitary) regulations to prevent the spread of certain infections and this has enabled a strong livestock economic sector to grow, whilst in the east, livestock (and wildlife) disease is widespread and uncontrolled, constraining commercial development and international trade. Although, in the more open systems, the actual impact of these diseases at a smallholder or pastoral level is minimal and it is unlikely the majority of farmers would in reality benefit from a strong exporting industry. There are exceptions to this, for example in Somalia where the majority of the population is dependant on livestock and there is a large live animal (international) trade. Removal of the rinderpest and RVF threat would be very beneficial to this community. What is best for the respective regions depends entirely on the perspective of the proposed beneficiary but it is likely to be more complex than we have hitherto thought, and more so as the objectives of development philosophy change .

Perhaps the over-riding concern is the increasing intensity at which the human species, as a result of its population explosion (along with its domesticated animals), is interacting with what is left of the natural world. This is resulting in an apparent increase in so-called emerging or novel diseases.

## SIR ARNOLD THEILER MEMORIAL LECTURE

Recent examples are Hendra Virus (Human - Equid) a novel morbillivirus; “lion” distemper, and emergence of a well-known morbillivirus of dogs, in a wild felid possibly through a hyena link; and SARS, involving most likely a human – civet interface. Although to prove these associations after the fact is difficult. In addition, in the era of so-called globalization, pathogen dispersal is more dependent on communication pathways than distance, through an increase in trans-continental movements of people and animals, particularly by air. This is most probably resulting in more frequent introductions of pathogens to new host populations with predictable results. The scenario with FMD type O viral movements between Asia, South Africa and the UK is an example and the current West Nile Virus epizootic in the USA, which might be a case of the introduction of an infected mosquito, person or horse through air transport (possibly from Israel according to one source) or through infected migrant bird species. This is also influencing our thinking when dealing with wildlife and livestock (trade) introductions to countries for exotic pet, commercial or conservation purposes.

Much of the hype surrounding disease in this “communication” age, leads us to believe that the plague of plagues is about to emerge and wipe us all off the planet, but what is the reality on the ground? When it comes to the veterinary field and the wildlife-livestock interface we have some interesting and contemporary problems under investigation. Examples are the Bovine tuberculosis epidemic in the Kruger National Park, the affect of badger population control methods on TB incidence in cattle in the UK, rinderpest virus persistence amongst cattle and wildlife in the Somali ecosystem of East Africa, West Nile Virus in the New World and research on Foot and Mouth Disease, with topo-typing of viral strains, improving traceability of disease outbreaks (and therefore significance of wild hosts). The epidemiological study of these diseases has been greatly enhanced with modern molecular diagnostic technology, of which some of the most exciting work has been undertaken here in Onderstepoort.

Globally, the importance of these interface diseases, relates to our (mis)perceptions on food safety and the effect of disease on global trade in animals and their products. This is in fact consistent with the general emphasis in global health on human and zoonotic diseases, which attract the most media attention, finance, research and control effort. Whether we opt for a hard or soft edge these concerns are likely be driving forces in the future direction we take in animal and land management.



## Repeated use of the GnRH analogue deslorelin to down-regulate reproduction of male cheetahs (*Acinonyx jubatus*)

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The GnRH analogue deslorelin, in a long-acting biocompatible implant, was originally designed for the control of reproduction in the domestic dog and cat. Since then it has been used as a contraceptive agent in a variety of wild carnivores, both male and female<sup>1,2</sup>. Contrary to the side effects that have been experienced in some carnivore females with progestogen implants<sup>3</sup>, no adverse side effects were observed with deslorelin. This paper describes the use of deslorelin implants repeatedly on an annual basis to contracept cheetah males in a semi-captive environment.

The cheetahs concerned were housed in camps, ranging from 10 to 1500 ha, in mixed sexes and the primary reason for treatment was to suppress fertility. The first four animals were treated in 1999 and by 2003 there were observations on 23 males. On an annual basis, two had been treated once, 7 twice, 9 thrice, 4 four times and one five times. Observations carried out on the first day of contraception and on an annual basis were: blood testosterone concentrations; testicular measurements; appearance of penile spikes; semen evaluation and mating of females in the same camp. During the first two years, adult females were also treated with deslorelin.

Two of the first four males treated (12 mg/male) showed undetectable concentrations of testosterone after 6 weeks whereas sperm were still present. The two others were evaluated after 3 months when they had zero testosterone and no sperm in the ejaculates. Two years later, they were still down-regulated. During the second year the dose was reduced to 6 mg, a year later to 5 mg and since then 4.7 mg has been used. A dose of 6 mg was found to be sufficient to suppress reproduction for at least a year whereas with 5 and 4.7 mg 3 of 17 males ejaculated a few sperm, most of which were dead. All testosterone concentrations, however, were still basal. A good subjective indicator of blood testosterone concentrations was the size of the penile spikes that are very well developed in cheetahs with normal concentrations. Aberrant sexual behaviour shown by one cheetah towards humans could be suppressed for a period of two years by annual treatments with double the dose (9,4 mg). No mating or attempted mating was observed and no conceptions occurred over the whole period. In addition, no side effects were seen.

It is concluded that deslorelin is a safe and reliable method to down-regulate sexual function in male cheetahs. The optimal annual dose to use is 6 mg.

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## Hypochromic, poikilocytic anaemia of neonatal roan antelope (*Hippotragus equinus*): a perinatal thalassaemia?

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The occurrence of a neonatal poikilocytic anaemia in certain members of the Hippotragini tribe has previously been documented but not fully investigated. High mortality rates amongst juvenile roan (*Hippotragus equinus*) and sable antelope (*H. niger*) from theileriosis have fueled speculation as to the underlying physiology of the anaemia and its potential role in haemoparasitic disease in these species. This study was undertaken to describe the erythrocyte morphology of new-born roan antelope over time, and to identify aspects of haemoglobin (Hb) production that might be implicated in this neonatal anaemia syndrome.

Blood samples from 29 roan antelope calves were taken in potassium ethylenediamine-tetraacetate (EDTA) on, or close to, one, seven, 14 and 28 days after birth. Three blood smears, made immediately after sampling, were stained with a Romanowsky, New Methylene Blue and Prussian Blue stain, respectively, and erythrocytes from these smears were categorised according to morphology and inclusions present. High performance liquid chromatography (HPLC) and electrophoresis were performed on haemoglobin in haemolysates prepared from the EDTA samples, and microhaematocrit and total Hb concentrations were determined for each sample.

Findings indicate a predictable change in the composition of roan calf blood with regard to erythrocyte morphology during the first four weeks after birth. There is a reduction in keratocytosis, dacryocytosis, schizocytosis, elliptocytosis and siderocytosis and an increase in acanthocytosis and spherocytosis. There was a peak in reticulocytosis at day seven, and in numbers of cells containing Howell-Jolly bodies at day 14.

Two haemoglobin types, foetal Hb (HbF) and adult Hb (HbA), were identified. The perinatal onset of HbA production appears to be delayed, resulting in the neonatal anaemia observed. Also, a significant correlation was shown to exist between total Hb concentration and spherocytosis and between HbA concentration and acanthocytosis and elliptocytosis. Goats, closely related to roan antelope, display a similar HbF/A switching pattern but produce a third Hb, HbC, during this period. We propose that these findings might suggest the defective production of the  $\beta$ -globin moiety of HbC in the roan antelope. This, by definition, represents a thalassaemia and would account for the observed poikilocytic anaemia and the associated time-related changes. This would constitute the first description of a naturally occurring thalassaemia in a non-human animal.

## **Risk factors contributing to an outbreak of copper and manganese deficiency in sheep utilising harvested maize fields or baled maize stover**

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Sheep from the Lichtenburg area were referred to the Onderstepoort Veterinary Academic Hospital, Faculty of Veterinary Science, University of Pretoria. Clinical signs included ataxia, testicular atrophy, lameness and a history of foetal skeletal deformities.

After clinical examination of the animals and a visit to the farm a tentative diagnosis of a combined copper and manganese deficiency was made. This was confirmed by histology (demyelination of the white matter in the spinal cord with multifocal vacuolization of the nerve tracts, hypoplasia of the testis with poor spermatogenesis) as well as feed and liver analysis. A video-clip of the clinical signs will be shown.

The sheep were fed a total mixed ration consisting of maize stubble combined with maize grain and a home recipe high protein concentrate (HPC) mix. The HPC had no trace minerals added. This type of feeding system as well as direct grazing of stubble off the fields during winter is practiced on a large scale in the maize producing areas of South Africa.

In order to identify possible risk factors contributing to the problem, a literature search was done. Apart from the well-known interaction between sulphur, molybdenum and copper, it was also found that a high soil concentration of iron, as found in especially red soils, could influence the absorption of copper<sup>1,3</sup> and manganese<sup>2</sup> and that liming of soil reduces the uptake of manganese and copper<sup>1</sup>.

Apart from the fact that maize grain is also low in both copper and manganese, these risk factors are present in large areas in the maize producing areas of South Africa and warrants special consideration when rations or licks are formulated.

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## A histopathological study of mobile and immobile mice injected subcutaneously with necrotising snake venom: preliminary results

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Tissue necrosis following snakebite in southern Africa is most common following bites by the Mozambique spitting cobra, *Naja mossambica*<sup>1</sup>, stiletto snake *Atractaspis bibronii*<sup>2</sup> and puff adder, *Bitis arietans*<sup>3</sup> among others. The present study was undertaken to determine histologically whether mobility affected the outcome of local necrosis in mice injected subcutaneously with puff adder venom (PAV) or Mozambique spitting cobra venom (MSVC).

Forty mice received 2.5µg of PAV subcutaneously in the right hind foot and another 40 mice were injected similarly with MSCV. Twenty mice from each group were anaesthetized with 1mg ketamine and 0.2mg xylazine hydrochloride, by intramuscular injection in the contralateral thigh, prior to venom injection. The other 20 mice from each group, immediately after injection, were permitted to swim in water (32°C), for 10 min, after which they were dried and allowed to ambulate or rest. The PAV and MSCV mice were euthanized at 95 and 144 hours, respectively. Tissues were obtained from the injection sites and prepared for light microscopy, conventionally. The microscopic slides were evaluated in a blind manner. Histopathological changes were characterised as mild, moderate or severe.

Tissue necrosis occurred less often in mobile PAV-injected mice than in immobile PAV mice ( $P = < 0.001$ ) with a tendency towards the same in MSCV mice ( $P = < 0.062$ ). The histological findings here were in consonance with gross observations following injections of venoms in the same groups of mice. It is thus confirmed that the dispersal of venom from the bite site has an ameliorative effect on tissue necrosis. Mobility after subcutaneous injection of PAV and MSCV tends to reduce local necrosis without increasing mortality.

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## South African field trials of a gametocyte antigen derived from *Eimeria maxima*: Immunization of breeder pullets for the protection of their progeny against coccidiosis

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For the past 40 years, coccidiosis control in intensive broiler production systems has mostly been achieved through the use of in-feed anticoccidials. Concerns about the continued efficacy of these drugs as well as consumer demands for “drug-free” poultry products have stimulated the development of alternative coccidia control strategies.

Recently, three proteins associated with the gametocyte generation of *Eimeria maxima* were identified as effective in stimulating a strong protective immune response in chickens. It was established that immunised breeder hens were able to transfer immunity to their progeny which was able to protect broilers from the harmful effects of coccidial infestation for the first three weeks of life. During this time broilers were exposed to natural coccidial infestation in the poultry house and developed active protective immunity. Trials confirmed that broilers were protected from challenge by *E. tenella* and *E. acervulina* as well as *E. maxima*.

The gametocyte antigen was applied to two flocks of broiler breeders in two South African provinces and the production results of approximately 80 000 broilers hatched from these flocks and raised without in-feed coccidiostats were compared with results of unvaccinated broilers raised under identical conditions with standard prophylactic coccidiostats in their feed.

Comparison of production results between broiler progeny of vaccinated breeders (treatment group) and broilers raised with in-feed coccidiostats (control group)

Parameter	KwaZulu-Natal (2 group average) 31 200 broilers/placement		Gauteng (2 group average) 9 000 broilers /placement		Combined (weighted average)	
	Treatment	Control	Treatment	Control	Treatment	Control
Average age at final weighing	35 days	35 days	41 days	41 days	36.3 days	36.3 days
% mortality to slaughter	2.70 %	3.68 %	6.02 %	6.37 %	3.44 %	4.28 %
Average final live weight	1.61 kg	1.68 kg	1.76 kg	1.79 kg	1.64 kg	1.70 kg

Production results in the trial broilers were similar to the control group, with lower mortality but poorer weight gain. Litter oocyst counts indicated exposure to coccidia in all flocks and although oocyst counts in vaccinated flocks were higher than in unvaccinated flocks, no difference in intestinal lesion scores were observed.

## A phylogenetic study of South African Newcastle disease virus strains isolated between 1990 to 2002 suggests epidemiological origins in the Far East

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Newcastle disease (ND), classified as a list A disease by the *Office Internationale des Epizooties* (OIE), remains one of the most serious poultry diseases in developing countries. The velogenic form of ND is a severe and acute disease of chickens and other domestic poultry, frequently causing mortality in excess of 80% during outbreaks in unvaccinated flocks. Sporadic outbreaks have occurred in South Africa since the 1940's, and consequently ND was thought to be endemic here. It was speculated that village chickens could serve as reservoirs of NDV. The objectives of this study were to investigate the phylogenetic relationships of local ND strains and, if possible, to identify a local wildtype lentogenic strain with the potential to mutate into a velogenic virus.

155 Newcastle disease virus (NDV) isolates collected in South Africa between 1990 and 2002 were analysed. Viruses were propagated in embryonated eggs, and viral ribonucleic acid (RNA) was extracted from infective allantoic fluids. The RNA was reverse-transcribed, and polymerase chain reaction (PCR) was used to amplify a region of the viral fusion (F) protein which contains the F<sub>0</sub> cleavage site, an important molecular determinant of viral virulence. DNA was sequenced, aligned and phylogenetic trees were constructed.

All of the lentogenic field isolates were shown to be derived from commercial vaccines. No true South African lentogenic wildtype was identified. Three outbreaks of velogenic NDV caused by three distinct NDV genotypes occurred during this period. The proposed link to the Far East is supported by the following evidence: (a) all causative genotypes were present and active in the Far East in months prior to outbreaks, but absent from South Africa, (b) within each of the three outbreak genotypes there was minimal genetic drift which suggests recent introduction events and viruses that did not circulate for long periods in the country and (c) viruses from the most recent KZN outbreaks in 2000 were extremely similar to isolates obtained from geese in western China during 1998. In a parallel serological study conducted during 2002, no evidence of the disease was found among village chickens from three provinces, which suggests that they do not act as reservoirs of NDV's.

The results of this study imply that ND is not endemic to South Africa, but is possibly introduced periodically from an external source. The epidemiological pattern does not support importation via harbors or airports but migratory aquatic birds are prime candidates: aquatic birds are known to be the natural asymptomatic hosts of NDV and avian influenza viruses, and can shed viruses in high quantities through the faeces into the environment. Several species of Charadriidae and Scolopacidae are known to migrate from Asia. Surveillance of wild aquatic birds has begun.

## Response of the splenic dendritic cell population in a mouse model of malaria infection

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Dendritic cells, and particularly those resident in the spleen, are thought to orchestrate acquired immunity to malaria, but it is not known how the splenic dendritic cell population responds to malaria infection and how this compares with other antigen-presenting cells. The present study investigated this question in a murine model.

C57BL/6 mice were infected with *Plasmodium chabaudi* AS, and parasitaemia was followed on thin blood smears made daily. All experiments were repeated at least 3 times. Spleens were prepared for immunohistochemistry by snap freezing and for routine histology by formalin fixation. Single cell suspensions of three pooled fresh spleens from days 3, 5, 7 and 9 were prepared for cell phenotyping and intracellular cytokine detection by flow cytometry. All antibodies used were monoclonal and fluorescently conjugated (BD Biosciences or Serotec, Oxford, UK)

It was found that dendritic cells, defined here by the CD11c marker, migrated from the marginal zone of the spleen into the CD4<sup>+</sup> T-cell area within 5 days of parasites entering the bloodstream. This contrasted with the macrophage and B-cell populations which expanded greatly but did not show any comparable migration. Over the same time period dendritic cells showed upregulation of CD40, CD54 and CD86 co-stimulatory molecules that are required for successful T cell activation. In dendritic cells, intracellular IFN $\gamma$  expression (as shown by FACS) rose to a peak on day 5, two days earlier than in B-cells or macrophages.

These findings show that splenic dendritic cells are actively engaged in the earliest phase of malarial infection *in vivo* and are likely to be critical in shaping the subsequent immune response<sup>1</sup>.

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## New *Babesia* parasites in cheetahs and other wild felids

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*Babesia*, an intracellular erythrocytic haemoprotozoan parasite of mammals, has also been reported in reptiles and birds. A diagnostic assay, the Reverse Line Blot (RLB) hybridization technique is currently used to detect *Babesia* and other blood parasites in a variety of animal species. Results obtained from this test showed that there might be undescribed *Babesia* parasites other than *B. felis* and *B. leo* present in wild felids.

Specimens from the field are submitted directly to our laboratory by various collaborators. In addition, specimens submitted to the Clinical Pathology Laboratory and found to harbour piroplasms are forwarded to our laboratory for further processing.

DNA was extracted from domestic cats (*Felis domesticus*); lions (*Panthera leo*); cheetahs (*Acinonyx jubatus*); black-footed cats (*Felis nigripes*) and serval cats (*Felis serval*) using the QIAamp DNA extraction kit. Polymerase chain reaction (PCR) was performed using primers that amplified a variable region of the 18S ribosomal rRNA gene. The PCR products were analysed using the RLB. The parasites that tested positive only with the genus-specific *Babesia/Theileria* probe were cycle sequenced, using an ABI PRISM BigDye™ Terminator v3.0 Ready Reaction Cycle Sequencing Kit. Electrophoresis of the sequencing reactions was done on the ABI 310 automated sequencer, according to instructions of the manufacturer (Applied Biosystems). The sequencing data were analysed using the Staden package.

Results showed that *B. felis* occurred in both lions and domestic cats and can occur concurrently with other haemoparasites. *Babesia* was also detected in cheetahs, a black-footed cat and in a serval, but the species could not be determined. Sequencing data from the parasite in cheetahs differed from the known *B. felis* and *B. leo* sequences (Genbank), but closely resembled that of the *B. gibsoni* group of organisms.

These results indicated the presence of a possible new *Babesia* parasite in cheetahs, but these results must be still confirmed by phylogenetic analysis and serology.



## Confirmation of occurrence of *Babesia canis vogeli* in domestic dogs in South Africa

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*Babesia* species are intraerythrocytic protozoan parasites affecting a wide range of vertebrate hosts and are among the most common tick-borne pathogens of dogs in South Africa. There are currently three recognized subspecies of *B. canis*, which differ on the basis of geographical distribution, vector specificity and antigenic properties. The subspecies also differ in pathogenicity. *B. c. rossi* causes a frequently fatal infection in domestic dogs, even after treatment; *B. c. vogeli* causes a moderate often clinically inapparent infection, and *B. c. canis* infections result in a more variable pathogenicity intermediate between *B. c. rossi* and *B. c. vogeli*. Molecular diagnosis has previously been used as a tool for the detection and characterization of *B. canis* infections in dogs and in tick vectors. The aim of this study was to conduct a survey of *Babesia* parasites in dogs using molecular methods.

A total of 226 blood samples were collected from dogs in shelters of the Society for the Prevention of Cruelty to Animals (SPCA). An additional 55 samples were obtained from dogs at the Onderstepoort Veterinary Academic Hospital (OVAH) and 15 samples were from Keringa Kennels in Johannesburg. Approximately 2–4 ml of blood was collected from the cephalic vein into citrate buffered vacutainer tubes. About 200 µl of blood was aliquoted into 1.5 ml eppendorf tubes and stored at “20 °C until DNA was extracted. After DNA extraction, the polymerase chain reaction (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. The reverse line blot (RLB) was performed using PCR products. In order to be able to differentiate between the three large piroplasms of dogs, species-specific oligonucleotides were deduced in the amplified V4 region. Partial sequences (530–540 bp) from the products were sequenced .

Amplified products, with the aid of the RLB, showed that 18 samples from OVAH were positive for *B. c. rossi* and one was positive for *B. c. vogeli*. Twelve samples from Bloemfontein were positive for *B. c. vogeli* and one sample for *B. c. rossi*, whereas 12 samples from East London contained *B. c. rossi* DNA. The remaining samples from Durban, Johannesburg and Keringa Kennels were negative for *Babesia* infections. Partial sequencing of approximately 530 bp derived from samples positive for *B. c. vogeli* Bcv 6 (accession no. [AF548006](#)) and Bcv 28 (accession no. [AF547387](#)) were 100% identical.

The occurrence of *B.c. vogeli* was confirmed for the first time in SA. However, we conclude that the presence of both *B. c. rossi* and *B. c. vogeli* may contribute to the varied clinical manifestations that are typical to South Africa. Attempts are underway to isolate *B. c. vogeli* in order to study its pathogenicity and also to confirm that *R. sanguineus* is the tick vector.

## Infection of roan antelope (*Hippotragus equinus*) with theileriosis using a cryopreserved stabilate derived from *Rhipicephalus evertsi*

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*Theileria* spp. have been known to occur in roan antelope, *Hippotragus equinus*, in West Africa since 1912<sup>1</sup> and in South Africa since 1974<sup>2</sup>. They are responsible for roan mortality in several areas of RSA<sup>3</sup>. Gene sequencing and comparison of isolates from recent cases in western roan in Malelane, RSA revealed that the *Theileria* sp. involved is unique, and naming it is currently under discussion. A PCR and DNA-probe for this *Theileria* sp. has been developed in collaboration with Utrecht University to aid in specific diagnosis of clinically infected and carrier animals.

The tick vector(s) of the Malelane *Theileria* sp. were identified by microscopic examination and DNA-probe testing of salivary glands dissected from adult ticks that had fed on infected roan. Immature stages of proven vector species were fed on known carrier roan using ear bags. Adult ticks were ground up after moulting to produce a tick derived stabilate that was cryopreserved. Eight hand reared, four-week-old roan antelope calves were divided in four pairs for titration of each stabilate. Three pairs were inoculated subcutaneously in the left suprascapular region with a dose equivalent to 60, 10 and 5 ticks respectively, with a control pair receiving 2 mL of physiological saline. The animals were monitored for four weeks using the following parameters: rectal temperature, blood smears, left suprascapular lymph node fine needle aspirate smears, lymph node palpation and mucous membrane colour.

*Rhipicephalus appendiculatus* and *Rhipicephalus evertsi* adults were found to be infected with the *Theileria* sp. microscopically and with the DNA-probe. Only the stabilate produced from *R.evertsi* resulted in infection of the roan calves with theileriosis. The animals which received the higher doses of stabilate, ie 60 and 10 tick equivalent doses, died from clinical theileriosis. In the 5 tick dose group, one animal develop mild signs of theileriosis and survived with supportive therapy, with the other not reacting clinically but testing PCR positive. The control animals did not react and stayed negative with the DNA-probe.

From the results it is clear that *R.evertsi* is the main vector of roan theileriosis, and that *R.appendiculatus* possibly only becomes infected if it feeds on parasitaemic animals during outbreaks. Work is in progress to develop a practical vaccination strategy for roan antelope calves using an *R.evertsi* stabilate.

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## The effect of haemolysis on AT III concentration as determined by a chromogenic method

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The presence of free haemoglobin in serum or plasma can markedly affect the outcome of laboratory tests by increasing the spectrophotometric absorbance of tests run at wavelengths within the absorbance range of 510 nm. Nothing is published about the effect of haemolysis on the determination of ATIII levels in canine plasma samples and the assay insert warns against use in hemolysed serum. This study aimed to determine what effect haemoglobin would have on AT III determination.

Two plasma pools were prepared with [ATIII] 100% and [ATIII] 70%. The ATIII 70% pool was prepared by diluting plasma with 0.9% saline in a ratio of 7:3. A unit of whole blood was collected from a donor dog. The erythrocytes were lysed by freezing (-80°C) and thawing. The solution was centrifuged, the supernatant collected and sequentially filtered using 1.2 µm and 0.22 µm filters to remove residual red cell stroma. The haemoglobin concentration ([Hb]) of the solution was determined using a modification of the automated Drabkin method. Intermediate hemoglobin solutions of increasing concentrations were prepared. Equal volumes of the intermediate hemoglobin solutions were added in a 1+9 manner to create the test plasma. A series of samples were prepared with a final calculated and measured [Hb] of 0; 0.5; 1.5; 2.5; 3.5; 4.5 and 5.5 g/L. The ATIII determinations were performed using a functional chromogenic assay and the spectrophotometric absorbances were read using a 405 nm filter, as specified.

The within batch co-efficient of variation (CV) and between batch analytical CV were 1.41% and 5.57%, respectively. Increasing [Hb] resulted in decreases in the [ATIII] measured. A linear regression analysis was performed on both 70% and 100% samples using a two step regression analysis. The results were  $R^2 = 0.979$  for the ATIII 100% plasma and  $R^2 = 0.986$  for the AT III 70% plasma. Linear regression analyses for AT III 100% gave the equation:  $AT\ III = -5.74x + 115.24$ , and for the ATIII 70% the result  $AT\ III = -4.20x + 66$ . A conversion table (see below) was created by interpolating between these two lines. These results show that it is possible, using a conversion table, to perform the ATIII assay in haemolysed serum thus opening the way to further evaluation of the coagulation status in patients with haemolytic disease.

[Hb] g/l	AT III (%)						
[Hb] ≤1.5g/l (true value)	<b>60.74</b>	<b>68.45</b>	<b>76.15</b>	<b>83.86</b>	<b>91.57</b>	<b>99.27</b>	<b>106.98</b>
1.5	60.74	68.45	76.15	83.86	91.57	99.27	106.98
1.75	59.66	67.31	74.96	82.62	90.27	97.92	105.57
2	58.58	66.18	73.78	81.37	88.97	96.57	104.17
2.25	57.50	65.04	72.59	80.13	87.67	95.21	102.76
5	45.62	52.56	59.51	66.45	73.39	80.33	87.28
5.25	44.54	51.43	58.32	65.20	72.09	78.98	85.87
5.5	43.46	50.29	57.13	63.96	70.79	77.63	84.46

## Canine cancer in South Africa: Prevalence and Outcomes (1997 – 2004)

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No information exists on the relative proportions, incidences or outcomes of diagnosis and treatment of canine cancer, in South Africa, barring one survey of histopathology reports<sup>1</sup>. Standard texts of veterinary oncology quote data in various US and European studies, which are subject to wide geographic variations.

In this preliminary report of a retrospective analysis, the medical database of the Onderstepoort Veterinary Academic Hospital (OVAH) was analysed for details of canine cancer patients admitted for period 1998 – 2004 ( $N = 1022$  including a small number of duplicate entries for multiple neoplasms of  $N = 47,245$  admissions, or 2.163% of total canine admissions). Patients were categorised according to diagnosis (histopathology, cytology or presumed); tumour location and metastasis; survival (where known); whether or not any medical or surgical treatments were performed; and for *Spirocerca lupi*, type of neoplasm or granuloma diagnosed.

Mesenchymal tumours formed the majority of neoplasms (47%), even if mast cell tumours (MCT) were considered as round cells (thereby reducing prevalence to 33%). Adenomas and carcinomas were the next most common tumours (32%), followed by round cell tumours (28% including MCT, 12% without MCT). Eight percent of tumours were undiagnosed or unclassifiable on the basis of incomplete records. The two most common cancers diagnosed were mammary (15.17%) and mast cell tumours (14.09%). Other notable tumours diagnosed were: lymphosarcoma (9.2%), non-*Spirocerca* osteosarcoma (8.51%), haemangiosarcoma (5.97%) and various sarcomas (5.28%). The proportion of cutaneous squamous cell carcinoma was lower than expected (1.17%) given its frequency in a similar study performed in felines (55%)<sup>2</sup>, but this result may be artificially lowered by the high number of “unclassified” tumours (7.8%) and “other carcinomas” (6.46%). Further investigation is expected to clarify these data into more discrete and representative figures.

Mesenchymal tumours are the most common cancer for which patients receive attention at the OVAH. Mammary and mast cell neoplasia are the two most numerous types diagnosed. Mast cell tumours should reasonably form the major South African canine oncology research focus. Early (pre-pubertal) ovariohysterectomy, which has a powerful protective effect against mammary cancer<sup>3</sup>, should be more strongly advocated by all veterinarians in the Republic.

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## Feline cancer in South Africa: Prevalence and Outcomes (1997 – 2004)

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No information exists on the relative proportions, incidences or outcomes of diagnosis and treatment of feline cancer, in South Africa, barring one survey of histopathology reports<sup>1</sup>. Standard texts of veterinary oncology quote data from the Northern hemisphere, and geographic differences are apparent even within these figures. In this retrospective analysis, the medical database of the Onderstepoort Veterinary Academic Hospital (OVAH) was analysed for details of feline cancer patients admitted for the period 1998 – 2004 (N = 73 including 1 duplicate record for 2 different neoplasms on 1 patient out of N = 4274 feline admissions, or 1.71% of total feline admissions).

Patients were categorised according to diagnosis (histopathology, cytology or presumed); tumour location and metastasis; survival (where known); whether or not any medical or surgical treatments were performed; and, for squamous cell carcinoma, colouration (white/not white).

In contrast to published reports of US<sup>2</sup>, Australian<sup>3</sup> and European<sup>4</sup> data where lymphosarcoma is the most common cancer of cats, squamous cell carcinoma (SCC) forms the predominant neoplasm diagnosed at the OVAH (55% of feline cancer patients, 81% in white or part-white cats). Lymphoma was the second most common diagnosis (19%) followed by various carcinomas and adenocarcinomas (10% combined but excluding 3% various mammary tumours). Only one apparent case of vaccine-associated sarcoma was recorded, and this was based on a cytological diagnosis. A large proportion (55%) of patients received some form of treatment, but only 68% of neoplasms were confirmed by cytology or histopathology.

Squamous cell carcinoma, followed by lymphoma, form the majority of feline cancers diagnosed in South Africa (74% combined). The average age of patients was 9.5 years at presentation, and feline cancer represented 1.71% of the case load at the OVAH. SCC should reasonably form the major South African feline oncology research focus.

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## The use of a bacterin vaccine in broiler breeders for the control of *Ornithobacterium rhinotracheale* in commercial broilers

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In 1991 a previously unidentified bacterium associated with signs of respiratory disease was isolated from the airsacs of broilers in South Africa. The organism was named *Ornithobacterium rhinotracheale* (ORT) in 1994. The organism has subsequently been isolated from chickens and turkeys worldwide. The control of ORT by prophylactic treatment, often by means of in-feed tetracyclines, is widespread in South Africa. In view of growing consumer concern about antibiotic residues in food products as well as evidence that ORT rapidly acquires resistance to antibiotics, this approach is clearly not sustainable.

A newly developed ORT bacterin vaccine (Nobilis® ORT Inac) was tested under South African field conditions. As injection of the vaccine into broilers is impractical on a large scale, the vaccine was applied twice to each of three (n= 43 000 birds) flocks of broiler breeder pullets during rearing, with the aim of stimulating a strong immune response in the hens that could be transferred to their progeny transovarially. Vaccine safety was evaluated. Commercial production results including total and average live weight, mortality rate, feed consumption and feed conversion ratio from approximately 208 000 broilers hatched from the vaccinated breeder flocks were compared with those of broilers hatched from similar but unvaccinated breeder flocks. Samples of broilers were also bled at slaughter and tested for antibody titres to ORT.

Clinical evidence indicated that the birds experienced no adverse systemic reactions to the test vaccine or its application. Prior to vaccination all flocks tested were serologically negative for ORT. In response to the vaccinations given, the titres of the vaccinated flocks rose and remained significantly higher than the titres in unvaccinated breeder flocks for the duration of the production cycle.

No conclusions could be drawn as to whether the vaccination of broiler breeders resulted in sufficient immunity to be beneficial to their progeny. Confounding factors that made it difficult to draw conclusions were the poor quality of the data supplied by the commercial partner and the small number of broiler cycles that could be compared. The results suggest that breeder vaccination with the ORT test vaccine may be beneficial to their progeny, but is probably less effective than the application of oxytetracycline in the birds' feed. This difference may be due to the fact that oxytetracycline controls a wide range of pathogens besides ORT.

## A study of the efficacy of a thermostable Newcastle disease vaccine in village chickens when administered by different routes

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Newcastle disease is a viral disease of poultry with a potentially devastating impact on both commercial and free-ranging poultry flocks. The disease is considered endemic in South Africa and vaccination is routinely practiced in commercial poultry flocks. In village chickens vaccination is not widely practised, for a variety of reasons, including difficulties maintaining the cold-chain as well as the application of the vaccine to free-ranging birds.

The former problem has been partially addressed by the development of thermostable Newcastle disease vaccines, most famously the I2 vaccine developed in Australia. The vaccine is unavailable in South Africa as a result of stringent registration requirements. This study aimed to investigate the efficacy of a commercially available thermostable vaccine (Nobilis Inkhuku<sup>®</sup>) for use in rural communities in South Africa. In view of the difficulty of catching free-ranging chickens, three different vaccine application techniques were used – eye-drop, drinking water and in-feed (maize).

Antibody titres measured using the haemagglutination inhibition (HI) test showed a small decline over three months after vaccination, indicating that an interval of more than three months between vaccinations would be appropriate for these flocks. Challenge studies showed an excellent correlation between HI titres obtained and survival after experimental challenge.

HI titre group	Mean Survival
>3	95%
1-3	47%
0	23%

Challenge survival rates varied between vaccine application groups and between the two study areas used. In both study areas, the overall survival rate for birds vaccinated by eye-drop was slightly better than for birds receiving their vaccine via the drinking water. In the case of in-feed vaccination, the challenge survival rate was only slightly poorer than the other two routes in birds from the Northwest Province (NW), while birds vaccinated in-feed in Qwaqwa showed a very poor survival rate. No unvaccinated birds survived challenge.

Treatment	% survival NW	% survival Qwaqwa	Mean Survival
Water	86%	60%	73%
Feed	75%	10%	43%
Eye-drop	80%	70%	75%
Controls	0%	0%	0%

These preliminary results were considered most promising, especially the use of in-feed vaccination which is simple to apply. Further work is planned to explore vaccine application techniques in rural communities.

## **An immunohistochemical and histological characterization of atretic ovarian follicles in the immature ostrich (*Struthio camelus*)**

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Relatively little is known about the occurrence of atresia in the ovary of the immature ostrich. Hence, the present study was undertaken to investigate the different forms of atresia in this species. In addition, the distribution of the intermediate filament proteins, vimentin, desmin and smooth muscle actin, in atretic follicles, was studied using immunohistochemical procedures.

A total of 14 immature female ostriches, aged between 12 and 14 months were used in the present study. The birds were sampled during the non-breeding season. Immunostaining was performed on 5µm sections of ovarian tissue using a LSAB plus kit (Vector Lab. Inc., USA). Antibodies against vimentin, desmin and smooth muscle actin were used at dilutions of 1:100, 1:300 and 1:50, respectively.

Glandular and invasion atresia were observed in small ( $\leq 324 \mu\text{m}$  in diameter), medium sized ( $\leq 449 \mu\text{m}$  in diameter) and large ( $\geq 450 \mu\text{m}$  in diameter) follicles. Glandular atresia was characterized by the presence of a multilayered granulosa cell layer, which contained several pycnotic nuclei. Immunostaining for desmin was either weak or absent in the granulosa cell layer of all the glandular atretic follicles studied. However, the thecal layers of these follicles were immunopositive for desmin. Granulosa cells in small and medium-sized glandular atretic follicles were immunopositive for vimentin, whilst those of the large follicles were immunonegative. The thecal layer was immunonegative for vimentin in all follicles studied. Smooth muscle actin immunoreactivity was not observed in any of the follicles displaying the glandular form of atresia.

Invasion atresia was marked by the infiltration of the oocyte by vacuolated granulosa cells, adipose cells and connective tissue fibres. The connective tissue fibres were immunoreactive for smooth muscle actin. Weak immunostaining for vimentin was observed in the theca interna, as well as in the vacuolated granulosa cells of large atretic follicles. Immunostaining for desmin was weak in the granulosa cells of medium-sized follicles, as well as in the connective tissue fibres accompanying the vacuolated granulosa cells in the large follicles.

These findings indicate that ovarian follicles in the immature ostrich undergo a period of regression which is similar to that reported in other avian species. Furthermore, from the results of the immunohistochemical study it is apparent that the occurrence and distribution of intermediate filament proteins is influenced by atresia.



## **Survey of small-scale dairy farmers in Central North West Province using participatory rural appraisal**

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The Department of Land Affairs has revised its Land Reform Programme to support sustainable rural development policies and interventions. Its focus will shift over the medium term to the implementation of an integrated programme of land redistribution and agricultural development. The programme is designed to provide grants to previously disadvantaged people to access land, specifically for agricultural purposes. Dairy farming has been identified as a priority by the North West Province (NWP) of South Africa, particularly in the Central Region, as it has the potential, not only for job creation, but also as a sustainable source of high quality protein for rural communities. This paper reports on the results of a survey of small-scale dairy farmers in Central North West Province using participatory rural appraisal.

There are 791 dairy farmers in the province (excluding those who produce for home consumption and local sale). National statistics show that 46% of dairy farmers produce between 0 and 500l/day but contribute only 9% of total production. Therefore, a large number of the milk producers that are classified as commercial are in reality small-scale producers, as many of their herds number less than 20 cows in milk. With the current land redistribution program many smaller units are being created and semi-intensive or intensive enterprises have to be considered. The way in which the production system is managed plays an important role in profitability in terms of input costs and milk production at lower volumes.

After purposive selection of 15 farmers that met the criteria of the North West Province and project leader, a workshop was held with the farmers and their extension and animal health officers to discuss and prioritise short- and long-term objectives using facilitated focus group discussions. A structured interview was developed from the findings of the workshop. This was followed by farm visits. In addition to the structured interview, informal interviews were conducted with farmers, their families and beneficiaries. Observations were made and photographs taken to record the available infrastructure, management, farming system and cattle breeds used.

The interviews and on-farm observation revealed serious deficiencies in the skills, knowledge and attitudes of small-scale dairy farmers. Very few farmers had received training at any level in dairy management, and it is obvious that there is a need for training if small-scale farmers are to make a success in this field. On most farms the system of milking and calf rearing was closer to the traditional management of communal cattle than specific management for dairy production. Most farmers did not feed suitable concentrates and roughage to their cows, and this deficiency was reflected in the low milk yields. Milk hygiene was found to be sub-optimal and was probably partly responsible for the poor keeping quality of milk. Few of the farmers had milk-cooling facilities or bulk tanks and most sold to the informal market. Only one of the farmers used a milking machine.

A serious deficiency on most of the farms was the paucity or absence of records. Poor records make it impossible to assess the efficiency of an enterprise and to identify the constraints affecting profitability. However, the average price of R4.00 per litre received for milk by small-scale farmers was high and was approximately double that received by commercial producers. At these prices there is a good potential for these farmers to make a profit and improve food security in outlying areas if their production capacity and milk hygiene are improved through training.

## Duration of repellency of selected agents against *Culicoides* spp. when applied to polyester mesh

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*Culicoides* spp. (Diptera: Ceratopogonidae) are small biting midges, one to three millimetres in size. Their importance to equids is that, of the approximately 120 species of *Culicoides* known to occur in South Africa, *C. imicola* and *C. bolitinos* have been implicated as vectors of the virus that causes African horsesickness. Of additional importance is their association with equine encephalosis virus and with equine insect hypersensitivity dermatitis. Whilst recommended control measures to prevent equine disease associated with *Culicoides* spp. include the application of insect repellents to the horse and/or its direct environment, little information regarding efficacy and duration of repellency of the various agents, with specific reference to equines, is available for South African conditions. The objective of this study was to compare citronella oil 0.6%,  $\alpha$ -cyano-cypermethrin 0.3%, and N,N-diethyl-3-methylbenzamide (DEET) 15% as repellents against *Culicoides* spp. when applied to polyester mesh.

Five 220V down-draught suction blacklight traps fitted with polyester mesh were used to collect *Culicoides* spp. in a randomised, blind, field experiment according to a previously reported technique.<sup>1</sup> The meshes used were treated with citronella oil 0.6%,  $\alpha$ -cyano-cypermethrin 0.3%, DEET 15%, or ethanol solvent, along with an untreated control mesh. The locations of the light traps in five outside camps were randomised for 15 nights in a Latin square design. A horse in each camp served as an attractor for *Culicoides* midges. Light traps were operated from 1800 h to 0600 h. Catches were collected hourly and the *Culicoides* spp. in each catch counted. Climatic variables (outside temperature, relative humidity, rainfall and wind speed) were recorded hourly. Treatment variables were evaluated by one way ANOVA. Where any statistically significant effect of treatment ( $P < 0.05$ ) was found, means were compared with the Student-Neuman-Keuls test for multiple comparisons.

The results of the study showed that significantly fewer *Culicoides* spp. were caught in the DEET 15%-treated light trap compared to the untreated control light trap during the 2<sup>nd</sup>, 4<sup>th</sup> to 7<sup>th</sup>, and 9<sup>th</sup> hours of collection ( $P < 0.05$ ). No statistically significant difference was demonstrated between the numbers of *Culicoides* spp. caught between the control, citronella oil 0.6%,  $\alpha$ -cyano-cypermethrin 0.3%, or ethanol solvent-treated light traps.

The study concluded that DEET 15% has a significant repellent effect against *Culicoides* spp. for up to nine hours when applied to polyester mesh, as tested with a down-draught suction light trap.

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## Ultrasonography of the solar aspect of the distal phalanx of the horse

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The solar aspect of the distal phalanx of the horse can be affected by laminitis, pedal osteitis, sagittal and marginal fractures, solar abscesses, keratoma and remodeling in flexural deformity. The impar distal sesamoidean ligament and the insertion of the deep digital flexor tendon (DDFT) on the distal phalanx may be affected in navicular disease. Abnormalities of blood flow to the distal phalanx and these associated structures may be present in laminitis, pedal osteitis, fractures, insertional deep digital flexor tendinitis, abscessation and navicular disease and have clinical relevance as to prognosis and response to therapy. This study was performed to evaluate the sagittal solar aspect of the normal distal phalanx ultrasonographically and to visualize the blood flow in the same structures.

Ultrasonographic examinations were performed on the solar aspect of the distal phalanx of 10 feet of five normal live horses (Group one), 22 feet of seven normal cadavers (Group two) and nine feet of five horses with pathology of the dorsal solar aspect of the distal phalanx (Group three). Lateromedial radiographs of the distal phalanx were made in all groups, and in Group two, digits were sagittally sectioned after imaging. The ultrasonographic appearance of the sagittal solar aspect of the distal phalanx was described. Measurements of the distance between the sole and the distal tip of the distal phalanx (A), the solar aspect of the apex of the frog and the distal phalanx (B), and the body of the frog's surface and flexor surface of the distal sesamoid bone (C) were made ultrasonographically, radiographically and on the sectioned cadaver specimens.

There was no statistical difference between the radiographic, ultrasonographic and direct cadaver measurements in A and C. In B there was a statistical difference among the measurements – most likely as result of the difference in trimming of the frog apex.

Color flow and power Doppler ultrasonography were performed on the normal sagittal solar distal phalanx, the impar distal sesamoidean ligament and at the insertion of the DDFT on the *facies flexoria* of the distal phalanx. Power Doppler in these horses showed blood flow at 0.16-0.48 kHz at the tip of the distal phalanx and at 0.16 kHz at the DDFT insertion and in the impar distal sesamoidean ligament. Using colour flow Doppler in normal horses, mean blood flows ranged from 1.8-5.4 cm/s at the tip of the distal phalanx and 1.8-2.0 cm/s at the deep digital flexor tendon insertion and in the impar distal sesamoidean ligament.

Trans-solar ultrasonography can be used to evaluate and locate the distal phalanx relative to the sole and visualize the blood flow to the phalanx and associated structures. This information can be applied in the evaluation of clinical cases such as laminitis with distal phalanx displacement and pedal osteitis. The technique can be used in future research to ultrasonographically evaluate blood flow to the distal phalanx in syndromes such as developmental and acute laminitis.

## Wobbler syndrome in the Boerboel

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The term “wobbler syndrome” has been applied to dogs with symptoms resulting from compression of the cervical spinal cord caused by a malformation/malarticulation syndrome and/or degenerative disease. It is most prevalent in Dobermans and Great Danes, but has been reported in other breeds. This paper describes the radiographic findings in wobbler syndrome in the Boerboel.

The records of the Department of Companion Animal Clinical Studies at the Onderstepoort Veterinary Academic Hospital (OVAH) were searched for Boerboel dogs that presented with symptoms of wobbler syndrome between January 1998 and December 2001. Only cases where the diagnosis was confirmed with diagnostic imaging (radiographs or magnetic resonance imaging (MRI)) were included.

Seven cases fitted the inclusion criteria. Two other cases that presented with symptoms mimicking wobbler syndrome are described separately as “atypical wobblers”.

*Typical cases:* These dogs were all 2 years or younger when presented. All showed similar bony deformities on survey radiographs. Articular facets appeared to be flared and/or bulbous, and some vertebral pedicles deviated medially. Myelographic studies generally showed no or mild changes on lateral views and then usually only when C2/3 was involved where slight attenuation of the dorsal contrast column was noted. The VD/DV views showed medial deviation of the contrast columns adjacent to the medially deviating pedicles and bulbous articular facets. Single or multiple sites could be involved. Four cases were treated conservatively – 2 responded and made full recoveries, 2 were euthanized due to progression of symptoms. Two dogs underwent surgery – 1 made a full recovery and 1 died of post operative complications. One dog was euthanized immediately at the owner’s request.

*Atypical cases:* The one case was diagnosed with cervical synovial cysts on MRI after normal survey radiographs and myelogram. The dog made a full recovery after surgery. The second atypical case was diagnosed with lateral cord compression by medially deviating pedicles in the region of T4-6. This dog was successfully managed by conservative means.

The Boerboel clearly fits into that category of wobbler that affects immature dogs, which have structural bony malformation leading to stenosis of the vertebral canal (malformation-associated wobblers). Lesions appear to affect the articular facets and pedicles with no vertebral body deformity. These malformations can often only be seen on DV/VD views. Myelography is essential for surgical planning and to confirm the diagnosis. The small number of cases and variability of treatment does not allow for prognostication in this study.

## A biomechanical investigation of the static stabilisers of the glenohumeral joint in the dog

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There is very little published data describing the biomechanical function of the canine glenohumeral joint (GHJ). The increased availability of advanced diagnostic techniques such as arthroscopy, magnetic resonance imaging and computed tomography has led to the description of a number of previously unrecognised conditions. The lack of information regarding normal function has hampered our ability to determine the pathogenesis or best treatment for many of these conditions. The purpose of this study was to examine the contribution of the glenohumeral ligaments/joint capsule (GHL/JC) complex and the limited joint volume and intermolecular adhesion/cohesion (LJV/AC) mechanisms to the static stability of the canine GHJ.

The GHJ of 32 complete cadaver specimens were manipulated to estimate laxity (>2mm translation) at joint angles of 150°, 135° and 90°. Following the removal of the peri-articular muscles, laxity was measured after applying a 15N force to the scapula in a variety of horizontal directions whilst the humerus was fixed to a jig. This was repeated for the abovementioned angles in intact joints and after venting and flushing to eliminate the LJV/ACM. Results of clinical palpation and biomechanical studies were compared.

Manipulation revealed a marked variation between joints although there was a tendency for progressive laxity as the joint was flexed. This finding was supported by the biomechanical study that also demonstrated no significant difference between intact and vented joints except in a cranio-caudal direction at 135° and 90°, and in a medial/lateral direction at 135°. Static stabilisers permitted a wide range of motion. Clinical palpation did not correlate well with biomechanical findings.

Translation and rotation variables were significantly less at a joint angle of 150° than at other angles. This was believed to be due to a “locking mechanism” created by tension on the caudal joint capsule and compression of the supraglenoid tubercle and tendon of origin of the *m. biceps brachii* within the intertubercular groove of the humerus (resulting in a concavity compression effect). At joint angles of 135° and 90°, the locking mechanism was released and progressively greater translation and rotation was permitted. Venting joints distinguished stability provided by the GHL/JC complex from that provided by the mechanisms of LJV/AC by neutralising the latter. The lack of a significant difference between intact and vented joints showed that the LJV/AC mechanisms are unlikely to play a significant role in providing constraint to the canine GHJ during loading, except in the cranio-caudal direction at 135° and 90° and the medial/lateral direction at 135°. Translation in these directions requires separation of joint surfaces and an increase in joint volume. With the exception of extension, the static stabilisers do not appear to play a significant role in providing joint constraint through the normal range of motion. Manipulation is unlikely to be a reliable method for determining subtle joint laxity and a wide variation in normal should be expected. Diagnoses of pathological laxity attributable to the dysfunction of the static stabilisers should be made with caution and surgical treatments should take cognisance of the above-mentioned biomechanical features.

## Diagnostic imaging assisted diagnosis of discospondylitis in two horses

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Two cases of discospondylitis in two horses are discussed with emphasis on the diagnostic imaging modalities used and their contribution to the final diagnosis and outcome. The radiographic appearance of discospondylitis in horses and small animals is well documented. However, the use of ultrasound as a diagnostic imaging modality in this condition has not received much attention in the literature. Our cases illustrate the practical use of ultrasound and the ultrasonographic findings likely to be expected in cases of discospondylitis.

In the first case, a 2-year-old, Warmblood-Friesian cross gelding was examined for progressing ataxia, hindquarter weakness and muscle atrophy. Radiographs of the lumbar spine revealed vertebral endplate lysis with sclerosis and proliferative new bone formation at the L3-4 disc space. This was associated with dorsal subluxation of L4 in relation to L3 and bridging ventral spondylosis, which was also seen on transrectal ultrasound. Due to an extremely poor prognosis, the patient was euthanased at the owner's request and the above findings were confirmed at *post mortem*. The second case, a 10 year old, Belgian Warmblood mare, showing lameness and muscle twitching over the ventrolateral neck region, was examined. Radiographs of the cervical spine revealed a 3cm radiolucent zone of lysis and mild sclerosis at the C3-4 disc space with loss of the ventral aspect of the sclerotic caudal vertebral endplate of C3. A narrowed C3-4 disc space was also found on follow-up radiographs. Ultrasonography confirmed discospondylitis and abscess formation at the site and identified the correct site for a fine needle aspirate to be performed. Scintigraphy was performed to evaluate the possibility of additional affected sites along the vertebral column before surgical curettage and removal of the abscess was performed. The patient subsequently recovered successfully.

Radiography is the mainstay of diagnosis of discospondylitis. However, radiation exposure to patients and personnel remains a limitation. In addition, the radiographic diagnosis of thoraco-lumbar spinal conditions in the equine patient is challenging due to the size of the patient and the difficulty in obtaining diagnostic quality radiographs. It requires the use of a more expensive high-output x-ray machine, which limits most private practitioners who have low-output machines. Scintigraphy is used to localise possible additional lesion sites to provide a more realistic prognosis for the owner, but again falls out of the scope of regular practitioners. Nowadays, most practitioners have access to reasonably good diagnostic quality ultrasound. It is useful to localise abnormal sites, to assess additional soft tissue or bony pathology and to perform guided fine needle aspirates for cytology and culture of the causative organism. It is helpful in setting a realistic prognosis, as in our case one, where vertebral subluxation was indicative of a degree of spinal cord compression and thus a worse prognosis. Ultrasound of the spine may be the only imaging modality a clinician may be able to perform in a field setting where radiography of the vertebral column is either impractical or impossible. A systematic approach should be followed, carefully comparing adjacent disc spaces and vertebrae for identification of abnormal sites based on irregular endplates or bone surfaces, widened or narrowed disc spaces, the ability to see into the depth of the disc space, spondylosis, vertebral step formation, abscessation or other soft tissue pathology as described in our cases.

## Intralesional Carboplatin for the treatment of cutaneous mastocytoma in a Siberian hamster (*Phodopus sungorus*)

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The Siberian, or Djungarian hamster *Phodopus sungorus*, is a common laboratory animal and children's pet with an average adult mass of 35 – 45 g and a lifespan of 18 – 24 months<sup>1,2</sup>. Its size, the perceived costs and benefits of treatment, and a dearth of clinically relevant information on its diseases usually result in no treatment or euthanasia should one fall ill.

A two-year-old Siberian Hamster presented to the Outpatients clinic of the Onderstepoort Veterinary Academic Hospital with a large facial mass (4 x 4 x 3 mm). A working diagnosis of mastocytoma was obtained through fine needle aspirate cytology and later confirmed on histopathology.

Complete regression of the tumour was obtained after intralesional carboplatin (P&U Carboplatin CSV, Pharmacia) and subcutaneous methylprednisolone treatment. A disease free interval and survival time of 81 days was obtained, at which point the patient died from other causes. A post-mortem and histopathology of the organs was performed and the findings reported. Details of dosage calculations based on body surface area<sup>3</sup> and histopathology of the tumour and other affected organs are also discussed.

This is only the second report of cutaneous mastocytoma in this species<sup>4</sup>, and the first of its treatment, although other authors have used surgery to remove a different and more accessible tumour<sup>5</sup>. This tumour may be considered controllable with intralesional chemotherapy, subject to certain practical limitations.

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## Is searching for high activity antimicrobial compounds from plants an exercise in futility?

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A survey in 1984 found that at least 25% of prescriptions in the USA and Canada contain bioactive compounds derived from plants or modeled after plant natural products. There have been hundreds of publications on screening plant extracts for potent new antimicrobial compounds. Yet there has been very little success in developing a product to enter the pharmaceutical market.

Based on our work in isolating anti-infective compounds from the Combretaceae we have identified the following possible explanations:

1. The agar diffusion bioassay method used by most scientists usually does not work well with plant extracts and there is little correlation between minimum inhibitory concentrations (MIC) values and inhibition zone diameter.
2. The extractants used frequently do not extract the antimicrobial compounds.
3. Many scientists have followed ethnobotanical leads to investigate purported antimicrobial activities. In our experience most antimicrobial compounds are non-polar. As traditional societies do not have non-polar extractants such as hexane available, scientists have therefore probably followed the wrong leads.
4. The evidence is growing that in many plant extracts synergistic effects occur. Isolating a single active compound frequently does not increase activity as much as can be expected.
5. It is difficult to compare results of different scientists because different strains are used for evaluation and there is no agreement on what constitutes an active extract. Many small laboratories start screening plants for especially antibacterial activity and consider minimum inhibitory concentration (MIC) values of 5 mg/ml as proof of antibacterial activity.
6. In many cases promising data is not followed up because it requires specialized expertise.

All of these aspects will be illustrated from our results in screening and isolating antibacterial and antifungal compounds from *Combretum* and *Terminalia* species in South Africa. By applying methods that we have developed for extraction and bioassay and by screening plants on a random base we have found that crude leaf extracts of several trees had MIC values as low as 20-40 µg/ml towards e.g. *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli* and *Enterococcus faecalis*, *Candida albicans*, *Cryptococcus neoformans* and *Mycobacterium smegmatis*.

By applying the methods we have used and screening plants widely the chance of identifying new magic bullets from plants may be increased.



## Development of an antibacterial and antioxidant rich extract from the leaves of *Combretum woodii*

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There is an urgent need to replace antibiotic feed additives (AFAs) in poultry feed with other prophylactics due to the possible transfer of antibacterial resistance to human pathogens. In earlier work we have found that *C. woodii* leaves contain high concentrations of combretastatin B5 with high antibacterial activities. The aim of this study was to develop a leaf extract that could be used to replace antibiotic feed additives in poultry feed.

Dried, ground leaves of *Combretum woodii* were extracted with 5 different solvents (Hexane, methylene dichloride, ethyl acetate, acetone, and ethanol) to determine the best extractant for subsequent development of an extract of high antibacterial and antioxidant activity. Initially the antibacterial activity of all the extracts was tested against American Type Culture Collection (ATCC) strains of *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli* and *Enterococcus faecalis* using TLC bioautography as antibacterial activity indicator, and quantified by micro-dilution technique developed by our group using 96-well microplates and tetrazolium salts to determine minimum inhibitory concentration (MIC) values. The ethanol and acetone extracts had the highest antibacterial activity against the test organisms with MIC values as low as 0.08 mg/mL against *E. faecalis* in the crude extracts.

Based on these results, various extracts were developed by employing different pre-treatment procedures to the crude extracts of acetone and ethanol, antibacterial and antioxidant activity of these extracts was quantified by MIC and Trolox equivalent antioxidant capacity (TEAC) values respectively.

The acetone extract which had been pre-treated by a single direct extraction with hexane was shown to be the procedure of choice; MIC values had dropped to 0.04 mg/mL against ATCC strains of *S. aureus* and *E. faecalis* and antioxidant activity increased to a TEAC value of 2.2 i.e 2.2 times the anti-oxidant activity of vitamin E.

These results point to the potential use of *C. woodii* extracts as a replacement for antibiotic feed additives in poultry feed. However, although the *in vitro* efficacy of extracts was enhanced by up to 400%, *in vitro* activity of the extracts remains to be tested.

## Biological activity and chemical comparison of *Leonotis* (Wild Dagga) species

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Within the genus *Leonotis* (family Lamiaceae), *L. dysophylla* and *L. microphylla* are often classified as *L. ocymifolia* var. *raineriana* and *L. ocymifolia* var. *schinzii* respectively. In addition to the morphological differences between these two plants, chemical comparisons and biological activity studies may support the existence of two separate species.

Leaf material from *Leonotis dysophylla* and *L. microphylla* (and *L. leonurus* as a comparison) was collected from the Pretoria area. Acetone extracts were prepared and subjected to TLC analysis. The TLC chromatograms were sprayed with chromogenic reagents, including vanillin-sulphuric acid and anisaldehyde-sulphuric acid. *Leonotis* extracts were also screened for various biological activities. Antioxidant compounds with free radical scavenging ability were visualized upon spraying the TLC chromatograms with 1,1-diphenyl-2-picrylhydrazyl (DPPH). Bioautography was performed on TLC plates with various bacterial species. The extracts were tested against the free-living nematode *Caenorhabditis elegans* and against *Artemia salina* larvae in the brine shrimp assay.

The results of the TLC analysis revealed substantial chemical differences between all three plants. In the antioxidant investigation, zones of antioxidant activity with similar  $R_f$  values were distinguished in each of the three *Leonotis* extracts. Bioautography demonstrated that chemical constituents with similar  $R_f$  values in all three species displayed antibacterial activity against the Gram-positive *Staphylococcus aureus*. Additional active compounds visualized on the TLC plate were present in *L. microphylla*. All the *Leonotis* extracts lacked anthelmintic activity, but showed a limited degree of activity in the brine shrimp assay.

In conclusion, it appears that although a fairly similar biological activity profile emerged after testing extracts of various *Leonotis* species, substantial chemical differences between extracts of *Leonotis dysophylla*, *L. leonurus* and *L. microphylla* gives credibility to the division of the plants into separate species.

## Preliminary antibacterial and antioxidant properties of *Dicerocaryum zanguebarium*

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Devil's claw (*Dicerocaryum zanguebarium*) is a herbal plant used for food and medicine in humans. It is used in traditional medicine to aid the expulsion of retained placenta in women, for treating gonorrhoea and hydrocoele, as well as serving as a hair shampoo<sup>1</sup>. In ethnoveterinary medicine it is claimed to be useful for treating retained placenta, especially in cattle<sup>2</sup>. The present study was designed to evaluate the antibacterial and antioxidant activity of leaves of *Dicerocaryum zanguebarium*.

The leaf material was extracted with hexane, water, ethanol, methanol and acetone. The antibacterial activity of the various extracts was determined using the serial dilution assay<sup>3</sup> and bioautography<sup>4</sup>. For the assay 4 different organisms were tested, 2 Gram-positive (*Staphylococcus aureus* and *Enterococcus faecalis*) and 2 Gram-negative organisms (*Escherichia coli* and *Pseudomonas aeruginosa*). Preliminary antioxidant activity was determined by the TLC-DPPH assay<sup>5</sup>.

The acetone extract showed highest antibacterial activity for all bacterial strains tested, with the Gram-positive organisms being more sensitive (minimum inhibitory concentration (MIC) value for *S. aureus* of 0.31 mg/ml). All tested organisms were most sensitive towards the acetone and ethanol extracts, but were resistant towards the hexane extracts. All the extracts, except for the water extract, showed up to four bands of inhibition on the bioautogram after spraying with *S. aureus*. Preliminary antioxidant activity indicated all extracts had at least one band of activity.

This is the first report indicating that this specific plant has both antibacterial and antioxidant activity. The antibacterial compounds had intermediate polarity based on  $R_f$  values and antibacterial activity of different extracts.

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## Anti-bacterial, anti-inflammatory and cyto-toxicity study of medicinal plants used in the treatment of wounds and retained placenta in livestock

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Infection is a major problem in wound and afterbirth care in animals. Ticks and sharp materials such as thorns are the major causes of puncture wounds that result in infections. Retained placenta and the resultant infection, has been also reported as one the major problems in small-scale cattle farming. Traditional treatment of wounds and retained placenta involves the use of diverse medicinal plants such as *Aloe marlothii*, *Cissus quadrangularis*, *Cussonia spicata*, *Dicerocaryum eriocarpum*, *Jatropha zeyheri*, *Pouzolzia mixta*, *Pterocarpus angolensis*, *Ricinus communis*, *Sarcostemma viminalis*, *Schkuhria pinnata*, *Sclerocarya birrea* and *Ziziphus mucronata*. This study aimed at evaluating the use of these plants in the treatment of wounds and retention of placenta for their efficacy and safety.

Appropriate plant materials were collected as indicated by farmers during field investigations. Further processing included drying under shade, grinding and extracting with dichloromethane (DCM) and methanol as solvents. The anti-bacterial activity of plants was evaluated using the 96-well micro-dilution assay against bacteria *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Enterococcus faecalis*. The anti-inflammatory activity was assessed using the cyclooxygenase assay 1 and 2 (COX-1 and COX-2) against bacteria *Bacillus subtilis*, *S. aureus*, *E. coli* and *Klebsiella pneumoniae*. For safety study, the Ames assay was performed with *Staphylococcus typhimurium* strain TA98 using the plate incorporation procedure.

DCM extracts of *D. eriocarpum*, *P. angolensis*, *R. communis* and *S. pinnata* exhibited the greatest antibacterial activity compared to methanol extracts, with *D. eriocarpum* and *S. pinnata* showing some activity against gram-negative and positive bacteria. As an indication of anti-inflammatory activity, DCM extracts of *C. quadrangularis*, *J. zeyheri*, *P. angolensis*, *S. birrea*, *S. pinnata* and methanol extract of *Z. mucronata* inhibited a higher percentage of prostaglandin, mainly in COX-1 assay. None of the plant extracts was mutagenic in the bacterial Ames test.

It seems that most plants used traditionally for treating wounds and retained placenta in animals might be effective in combating infection and reduction of pain. Lack of mutagenicity suggests that these plants are most probably safe although other tests need to be conducted, for example, by using other cell lines. However, small-scale farmers use water extracts or simple decoctions and infusions that have been shown to be ineffective in most laboratory investigations. The ultimate goal of this research would be the development of effective simple technologies that would enable small-scale farmers to obtain better formulations.

## Novel biologically active triterpenoids from *Combretum imberbe* and *Combretum padoides* leaves

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*Combretum imberbe* (leadwood, hardekool) has been used for medicinal purposes and several studies have been carried out to investigate the chemical compounds present in the bark of this plant. Preliminary experiments indicated that leaves of this plant contain antibacterial compounds that do not occur in other *Combretum* species. This study was designed to identify the chemical compounds present in the leaves of this plant and to determine their antibacterial activity.

Leaves of *Combretum imberbe* and the closely related *Combretum padoides* belonging to the *Combretum* section, Hypocrateroptis of the African Combretaceae were extracted and fractionated by bioassay-guided fractionation.

Two new antibacterial pentacyclic triterpenoids (1,23-dihydroxy-12-oleanen-29-oic acid-3 $\beta$ -O- $\alpha$ -L-2, 4-diaetylramnopyranoside and 1,14-dihydroxy-12-oleanene-29-oic acid-3 $\beta$ -O- $\alpha$ -L-2-acetylramnopyranoside) (**5** and **6**) along with six known triterpenoids (**1-4**, **7** and **8**) [1,3-dihydroxyl-12-oleanen-29-oic acid (**1**), 3-hydroxyl-12-oleanen-12-oic acid (**2**), 3,30-dihydroxyl-12-oleanen-22-one (**3**), 1,3,22-trihydroxyl-12-oleanen-29-oic acid (**4**), 1,22-dihydroxyl-12-oleanen-29-oic acid (**7**) and 24-ethylcholesta-7, 22,25-trien-3-ol-O- $\beta$ -D-Glucopyranoside (**8**)] were isolated from both *C. imberbe* and *C. padoides*. The structure of each compound was elucidated on the basis of 1 dimensional and 2 dimensional nuclear magnetic resonance (NMR) experiments, as well as electron impact (EI) and electron spray impact (ESI) mass spectrometric techniques.

All compounds showed moderate to strong antibacterial activity against *Staphylococcus aureus*, *Bacillus subtilis*, *Mycobacterium vaccae* with compounds **2**, **5** and **7** being most active. Compounds **2** and **3** also showed strong anti-inflammatory activity against 3 $\alpha$ -hydroxysteroid dehydrogenase with an IC<sub>50</sub> of 10  $\mu$ g/ml and 7.8  $\mu$ g/ml as well as moderate cytotoxicity (CC<sub>50</sub> = 17.6  $\mu$ g/mL and CC<sub>50</sub> = 10.5  $\mu$ g/ml) against Hela cell lines. Compounds **2** and **5** also indicated moderate anti-proliferative (GI<sub>50</sub> = 16.5  $\mu$ g/ml, 13.2  $\mu$ g/ml and 9.0  $\mu$ g/ml, 8.7  $\mu$ g/ml) activity against K-562, L-929 cell lines.

The results of this study expand our knowledge of the phytochemistry of these two plant species and confirms the ethnomedicinal use of *C. imberbe*. Initial indications are that extracts from these plants may have a clinical application.

## **New primarily Web-based Master of Science degree programme in Veterinary Tropical Diseases**

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This new primarily web-based MSc degree programme in Veterinary Tropical Diseases focuses on animal health management issues of infectious and parasitic diseases of domestic and wild animals, particularly in the Afrotropical (sub-Saharan) Region. It also includes the development of specific technical knowledge and skills that are required to make a diagnosis in microbiology, parasitology and molecular biology laboratories. The programme comprises various modules/sub-modules that can also be taken for non-degree purposes in support of the continuing professional development of a candidate.

This new degree programme will be of great value for candidates from developing countries where trans-boundary diseases in particular are often the biggest constraint to socio-economic development. The programme will also be very useful to candidates from the developed world from which many of these diseases have been eradicated, often at great cost, but which nevertheless still pose a distinct threat because of increased international trade in animals and their products.

The primary education mode is web-based supplemented by contact/practical sessions. Experts from within and outside South Africa will be invited to take part in online group discussions. The focus of the programme is on the following:

Core modules: Research Methodology; Community-based Animal Health; and Introductory Epidemiology.

Elective modules with the following study themes: Laboratory Diagnostics; Ticks and Tick-borne diseases; Tsetse and Trypanosomosis; Helminth Infections; Ectoparasitic Infestations and Protozoal Infections; and Selected Infectious Diseases.

The degree programme and modules are presented by the Department of Veterinary Tropical Diseases, Faculty of Veterinary Science, University of Pretoria, in collaboration with the Department of Animal Health of the Institute for Tropical Medicine, Antwerp, Belgium, and with the support of the Department of Production Animal Studies (UP) and the Department of Infectious Diseases and Immunology, Faculty of Veterinary Medicine, Utrecht University, The Netherlands.

This presentation provides information on the background, objectives, educational approach, curriculum, entry requirements, course credits, and career paths of the web-based MSc degree programme in Veterinary Tropical Diseases.

## **Paradigm shifts in Veterinary Tropical Medicine Education: Course development for online delivery – a case study**

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Traditionally, Veterinary Tropical Medicine has been taught in a classroom or laboratory in a face-to-face situation. The need for a distance education Master's degree in Veterinary Tropical Diseases was identified and it was envisaged that it be presented online. This decision to present a Master's degree in Veterinary Tropical Diseases as an online distance education course necessitated that all role players think innovatively with regard to teaching and learning. Online learning in higher education institutions has developed in the last few years as one of the key modes of instruction due to the flexible learning environment that can be created and the possibilities for lifelong learning.

Lecturers from the Department of Veterinary Tropical Diseases, University of Pretoria, the Prince Leopoldt Institute for Tropical Medicine, Antwerpen, Belgium and the Utrecht University, Netherlands are all involved in the development and presentation of this programme. They are responsible for the development of the curriculum and content, the identification of resources and will eventually be responsible for the facilitation of the online course delivery.

The Department of Telematic Learning and Education Innovation (TLEI) of the University of Pretoria is responsible for the development of quality learning materials for all programmes presented by the university, as well as assisting, guiding and training lecturers to ensure that sound teaching practices are adhered to. The development team from TLEI consists of a project manager, educational consultant, instructional designers and media specialists, including graphic designers, animators, video specialists and photographers. Other support services who are involved with the development and delivery of the online programme are the Academic Information Service (AIS) and the Department of Information Technology (IT).

Factors taken into consideration in making key decisions about the development and delivery of the learning material include:

- the target market
- computer skills of the target market
- access to technology by the target market (internet, computers)
- bandwidth in South Africa and Africa
- mode of delivery of learning materials (web vs CD ROM)
- assessment in an online distance learning model
- communication between students and lecturers
- communication between students
- technical support available to students
- the role of other support services e.g. the Academic Information Service

Some of the learning materials (web and CD ROM) will be demonstrated to indicate the outcomes of some of the decisions made regarding the development of this course for online delivery.

## **Paradigm shifts in Veterinary Tropical Medicine Education: preparing Lecturers to teach in the online environment – a case study**

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Online learning challenges many of the assumptions lecturers may have about how people learn – assumptions which are developed over years of experience with teaching in a traditional face-to-face learning environment. Many of the strategies are effective and it is a very human characteristic to want to keep what works. Lecturers do not traditionally receive training in educational practice. The majority of lecturers, unless trained in appropriate teaching and instructional strategies, will model the teaching styles to which they have been exposed, which in most situations is the traditional face-to-face model of teaching. Lecturer development and support should include opportunities to equip them to teach in this new learning environment. This paper describes the development and delivery of a course aimed to develop skills and expertise in facilitating online learning.

Requests directly from lecturers for training provides impetus for the development of various training courses. Foremost on the list of training needs is the need to master the technology itself. There are many tools that can be used to facilitate learning in this new environment, and keeping up to date with these is an ongoing necessity. Facilitators of online learning need to come to grips with the instructional, psychological and social aspects of online learning, all of which are very different to the traditional teaching environment. The role of the teacher / instructor is changing – change which is accompanied by new instructional expectations. It is also vital that all those who are responsible in some way for teaching realise that technological competency is not the same as teaching competency. Good classroom teachers do not necessarily make good online facilitators. Once lecturers have started to master the technology itself, the following questions soon appear on the agenda:

- What are the most appropriate activities for an online classroom?
- How does one structure and pace a programme of online activities?
- How does one keep learners motivated in this faceless, open environment?

This paper looks at the training of lecturers to facilitate learning in the electronic learning environment under the following headings:

- Course outcomes
- Course delivery
- Content
- Assessment.
- Course evaluation
- Course evolution - the way forward

Distance learning is nothing new and neither is technology. It is the combination of the two that makes it imperative for those who are responsible for creating meaningful learning environments to look at understanding the technologies and seek ways to get the best out of this combination. Lecturers need to confront the issues that arise due to the nature and context of the online learning environment. They need to be supported in acquiring these skills in a way that supports heavy workloads and constrained resources.



## Medicinal value of edible stink bug, *Encosternum delegorguei*, consumed in the Limpopo Province of South Africa

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In rural areas, reliance on traditional medicine and food is high and attributable to both economic and cultural factors. The insect known as thongolifha or stink bug (*Encosternum delegorguei*) is consumed by the Venda tribe and is eaten raw or cooked. Thongolifha is important to the Venda in terms of its nutritional and economic value. In addition, the insects feed nearly exclusively on the leaves of the Sand Olive (*Dodoneae viscosae*) which are known to contain antibacterial compounds. The aim of this research project was to determine if the insect exhibited antibacterial properties and whether this activity could be ascribed to the same compounds present in its food source.

Thin layer chromatography (TLC) was used to separate components extracted from thongolifha and the presence of various compounds was noted after spraying the chromatograms with either vanillin or p-anisaldehyde spray reagents. The leaves of the host plant, *Dodoneae viscosa* were tested for antibacterial activity using the bioautography and minimum inhibitory concentration (MIC) assays. The bacteria used in the study were the Gram-positive *Staphylococcus aureus* and *Enterococcus faecalis*, and the Gram-negative *Escherichia coli* and *Pseudomonas aeruginosa*. Thongolifha extracts were also tested for antibacterial activity.

The hexane, dichloromethane, ethanol, ethyl acetate and acetone extracts of *Dodoneae viscosa* showed antibacterial activity against the Gram-negative bacteria *E. coli* and *P. aeruginosa*. The highest activity was found in the acetone extracts, while hexane exhibited less activity. The MIC value for the various extracts ranged between 0.04 - 0.31 mg/ml. The acetone extracts of the thongolifha also exhibited antibacterial activity against *E. coli*, *Enterococcus faecalis*, *P. aeruginosa* and *Staphylococcus aureus*.

Although the antibacterial activity of the insect extracts was much lower than that of the *D. viscosa* extracts, the results indicate that thongolifha may be of medicinal importance. Based on bioautography results, it appears that the same compound may be responsible for the antibacterial activity in both the plant and insect.

## Screening of twenty-four South African *Combretum* species (Combretaceae) for antifungal activities.

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Medicinal plants have become the focus of intense study recently in terms of their traditional uses, which are often supported by their actual pharmacological effects. In this study the antifungal activities of 24 South African *Combretum* species of the Combretaceae family were screened against five fungal animal pathogens (*Candida albicans*, *Cryptococcus neoformans*, *Aspergillus fumigatus*, *Microsporium canis* and *Sporothrix schenckii*) using a serial microplate dilution method.

Acetone, hexane, dichloromethane and methanol extracts from these *Combretum* species were tested. Chemical constituents of the extracts were analysed by thin layer chromatography (TLC), using three eluent systems. For detection of chemical compounds chromatograms were sprayed with a vanillin sulphuric acid spray reagent, and for detecting antioxidant activity with 0.2% DPPH.

Methanol was the best extractant in terms of quantity extracted in all the *Combretum* species, and methanolic extracts also had the highest antifungal activity. The methanolic extracts of *C. moggii* and *C. petrophilum* were very active against all the tested pathogens. All extracts of *C. nelsonii* were also very effective against all the pathogens. Some of the extracts had antioxidant activity. Bioautography procedures using 2 mg/ml p-iodonitrotetrazolium (INT) were performed to determine the activity of extracts against all pathogens. Based on bioautography results and minimum inhibitory concentrations (MIC) values, most extracts contained antifungal compounds. Most of the antifungal extracts had MIC values of c. 0.08 mg/ml, some with MIC values as low as 0.02 – 0.04 mg/ml. *Microsporium canis* was the most susceptible microorganism. Fractionation and bioassay-guided isolation of the antifungal active compounds are currently being undertaken on the crude extracts of *C. nelsonii*, *C. albopunctatum* and *C. imberbe*.

These results appear to validate the widespread use of *Combretum* species for infection related diseases.

## Antibacterial and antifungal activity of a by-product of the grape seed extraction process

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The phytomedicine laboratory developed and previously reported on an inexpensive and effective method of concentrating grape seed antioxidant compounds. The reported procedure is in the process of being patented and scaled up with the collaboration of an industrial partner. To investigate further applications a by-product of the process was fractionated and tested for antibacterial and antifungal activity with the aim of identifying the most active fraction and eventually isolating active constituents.

The by-product, 85g, was taken up in acetone and introduced on a silica gel 60 Vacuum Liquid Chromatographic column. The column was eluted with varying gradients of hexane-ethyl acetate-methanol. Twelve fractions were obtained and their minimum inhibitory concentrations and total activities were calculated using a microplate dilution assay<sup>1</sup> against bacteria and fungi species. Total activity represents the volume in ml to which the fraction can be diluted and still retain activity<sup>2</sup>. Bacteria used were *Staphylococcus aureus*, *Enterococcus faecalis*, *Escherichia coli* and *Pseudomonas aeruginosa* whilst fungi used were *Aspergillus fumigatus*, *Sporothrix schenckii*, *Microsporum canis* and *Candida albicans*.

The 90% hexane in ethyl acetate fraction had the highest yield of 26.86 g and the highest total activity for all bacteria tested. The fraction was most active against *E. faecalis*, where it had a total activity of 5925 ml compared to the lowest value of 1.6 ml from the 100 % methanol fraction against the same bacteria, indicating the successful fractionation already attained. Activity against fungi showed that the 90% hexane in ethyl acetated fraction had the highest total activity against *S. schenckii* (5266 ml/g) and *M. canis* (1723 ml/g). The 30% ethyl acetate in methanol fraction however had the highest activity against *A. fumigatus* (201 ml/g) and *Candida albicans* (1564 ml/g) indicating that different compounds are responsible for the activities.

The 90% hexane in ethyl acetate fraction was used in initial isolation attempts. The fraction was eluted isocratically with 50 % hexane in dichloromethane. Chemically similar fractions were pooled and fractionated again on silica gel using 90 % hexane in chloroform followed by chloroform as eluents. This led to a compound that appeared pure by thin layer chromatographic analysis. The isolated compound was active against *S. aureus*, *E. coli* and *P. aeruginosa* based on bioautography results.

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## ***In vitro* estimation of the number of corpora lutea in the ovaries of bitches by means of magnetic resonance imaging**

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Fecundity in the bitch, as measured by litter size, depends on ovulation rate. The ratio between the number of corpora lutea and litter size provides a more sensitive measurement of fertility in artificial insemination trials than merely using litter size. If the number of corpora lutea could be counted accurately and non-invasively, it would be possible to use a bitch repeatedly, each time for a different experimental treatment, and analyze the data with bitches as subjects in a repeated measures design, thereby providing a more powerful experiment than when each bitch is exposed to only one treatment. The aim of this trial was to determine whether magnetic resonance imaging (MRI) could be used to accurately count the number of corpora lutea in the ovaries of bitches. The hypothesis was that each of three researchers would be able to estimate the number of corpora lutea correctly in at least 80% of ovaries from dioestrus bitches and, if the estimation was incorrect, the error would not differ by more than one corpus luteum per ovary.

Initially, 15 dioestrous bitches were spayed over two days and their ovaries, stripped of their bursae, placed in a 50% solution of glycerine in normal saline for one to 3 days before the ovaries were embedded in gelatin inside a plastic box of about 20 cm wide by 25 cm long by 5 cm deep to be exposed to magnetic resonance (MR). When the ovaries were removed from the glycerosaline some appeared autolysed and many were dehydrated. A further 5 dioestrous bitches were then spayed and their ovaries placed in isotonic saline for a few hours until they were similarly embedded in gelatine. In each instance the box with gelatin and ovaries was placed in the gantry of a 1.5 T Siemens Magnetom machine and a series of T1 images taken, with slices 2 mm apart, in two perpendicular planes. Three operators estimated the number of corpora lutea twice from the series of transverse sections and twice from the number of vertical sections through each ovary. The average of the 4 counts done by an operator, rounded up to the nearest integer, was taken as the estimated number of corpora lutea in the ovary for that operator. The three operators subsequently and simultaneously dissected the ovaries and counted the corpora lutea in each.

The three operators did not differ in the percentage of ovaries where they estimated the number of corpora lutea correctly (Chi-square,  $P > 0.2$ ). The three operators combined estimated the number of corpora lutea correctly in 60% of 10 ovaries stored in saline and 38% of 30 ovaries stored in glycerosaline (Chi-square,  $P = 0.03$ ). Further results refer to ovaries stored in saline. Each operator tended to underestimate the number of corpora lutea, and errors tended to become more common and larger when the number of corpora lutea in an ovary increased. Even for the 10 ovaries kept in saline, only one of the three operators estimated the number of corpora lutea correctly in 80% of ovaries while the remaining two operators each estimated the number correctly in only 50% of ovaries.

This study shows that, for the system used, the number of corpora lutea cannot be counted accurately *in vitro* by means of MRI. Further research is indicated to determine whether using a machine with higher field strength, reducing the distance between sections to one millimeter, using gadolinium as contrast-enhancing agent and taking the images *in vivo* will enable accurate determination of the number of corpora lutea in the ovaries.

## Effects of heterologous semen 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 intrauterine insemination. Acceptable fertility can be achieved with frozen-thawed ram semen deposited at the external cervical opening if ram semen plasma (SP) is added, but such plasma is difficult to obtain. Homologous SP also improves the fertility of frozen-thawed sperm of boars and dogs. Heterologous SP may have effects as well; the *in vitro* ability of buffalo (*Syncerus caffer*) sperm to fertilise bovine oocytes can be improved by the addition of SP of domestic bulls. The aim of the current study was to compare the effects of SP of rams (SPR), bulls (SPB) and dogs (SPD), protein-free Tyrodes albumin lactate pyruvate (TALP), Triladyl (Minit, b., Tiefenbach, Germany) and heat-treated skim milk upon longevity and percentage progressively motile sperm of frozen-thawed ram sperm.

Ejaculates of six rams (2 Dorpers, 2 Döhne merinos and 2 merinos), aged 2-4 years, were used. Three ejaculates from each ram, extended in Triladyl were pooled and frozen as a single batch at  $200 \times 10^6/\text{ml}$  in 0.25-ml straws and stored in liquid nitrogen. SPR from the same rams was obtained after centrifugation of additional ejaculates. SPB of 5 bulls was similarly harvested, while the post-sperm fractions of the ejaculates of 5 dogs (SPD) were collected. Within a species, the SP from different donors was pooled and frozen in aliquots at  $-18^\circ\text{C}$ . 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  $37^\circ\text{C}$  and kept for 6 h. Percentage progressively motile sperm was estimated immediately, 2, 4 and 6 h after thawing at X200 magnification. The same 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 progressively for each time interval - 39.0% (0 h), 26.0% (2 h), 19.6% (4 h) and 12.6% (6 h) ( $n = 108$ ,  $\text{SEM} = 1.38\%$ ). Mean motility for the respective fluids was 39.9% (skim milk), 27.7% (Triladyl), 21.9% (SPR), 20.5% (TALP) and 13.0% (SPB) ( $n = 72$ ,  $\text{SEM} = 2.85\%$ ). The interactions (ram x fluid or time x fluid) were mainly due to SPD, SPR, Triladyl and TALP, while skim milk produced the best and SPB 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 cause 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.

## Determination of pregnancy status of African buffalo by faecal steroid analysis

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Faecal sex steroid and metabolite concentrations have been applied in many species for the remote monitoring of reproduction. The aim of this work was to characterise the plasma progesterone concentration (PPC) and faecal steroid metabolites, oestrone and 20-oxo-pregnanes concentrations, in non-pregnant and pregnant African buffalo (*Syncerus caffer*) cows.

All subadult and adult female buffaloes in four herds were sampled during immobilization, four days after capture. Reproductive status was determined by palpation and ultrasound examination per rectum. Blood was taken from the jugular vein. Faeces was collected and frozen within 4h. Samples were collected from 67 animals. PPC was determined using a commercial radio-immunoassay (DPC 'Coat-A-Count'). Faecal samples were extracted by a wet method and enzyme immunoassays were done for oestrone and 5 $\alpha$ -pregnane 3,20-dione (University of Veterinary Medicine, Vienna), which has high cross-reactivity with all faecal 20-oxo-pregnanes.

Results from non-pregnant and pregnant animals are depicted in the table:

Assay	Non-pregnant (n=33)		Pregnant (n=34)	
	Mean ( $\pm$ S.D.)	Range	Mean ( $\pm$ S.D.)	Range
Faecal 20-oxo-pregnanes (ng/g)	169 ( $\pm$ 99)	40-359	1970 ( $\pm$ 1462)	311-7116
Faecal oestrone (ng/g)	63 ( $\pm$ 46.2)	1.7-196	631 <sup>#</sup> ( $\pm$ 275)	72->800 <sup>#</sup>
PPC (nmol/l)	7.4 ( $\pm$ 3.7)	2.3-16.9	35.5 ( $\pm$ 11.6)	10.8-63.2

<sup>#</sup> Faecal oestrone concentrations in 23 samples exceeded maximum limit of assay of 800 ng/g.

Differences between pregnant and non-pregnant animals were highly significant for all three assays, but least overlap was seen in faecal 20-oxo-pregnanes followed by PPC. Concentrations of 465 ng/g, 200 ng/g and 19 nmol/l were selected as cut-off points for faecal 20-oxo-pregnanes, oestrone and PPC, respectively, these approximating the mean plus three SD's for non-pregnant animals. Using these values, all assays had a specificity of 1. The sensitivity for pregnancy was 0.97, 0.85 and 0.93, respectively. Sensitivity was increased to 1 when either faecal 20-oxo-pregnanes or PPC was above the cut-off value. No animals in the third trimester of pregnancy were incorrectly classified on any assay. Based on faecal oestrone concentrations, both animals in the first trimester of pregnancy and 3 of 10 second trimester animals were wrongly classified as non-pregnant. One of the first trimester animals and one of the second trimester animals were wrongly classified using PPC. One of ten second trimester animals was wrongly classified as non-pregnant on 20-oxo-pregnanes concentration.

Faecal 20-oxo-pregnanes or PPC alone may be reliable indicators of pregnancy in the second and third trimester, and may be reliable throughout pregnancy when used in combination. Faecal oestrone concentrations appear to only rise in the second half of pregnancy. Further work is required with regard to the value of these methods in early pregnancy, the effect of capture, and sample collection and transport methods.

## Basic macroscopic features of the venous drainage of the reproductive organs of the male ostrich (*Struthio camelus*)

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In contrast to the situation in the poultry industry, ostrich production is hampered by a lack of information regarding the reproductive biology of this economically important bird. As part of a comprehensive study of the vascularisation of the male reproductive tract, this paper presents preliminary findings on the vascular drainage pattern of the ostrich and compares the findings to published information on the domestic fowl.

The torsos of five birds (one sexually mature male and four sub-adult males) with viscera intact were prepared as follows. The venous system was flushed free of blood by rinsing with physiological saline injected through the caudal vena cava after which blue latex was injected into the veins via the same route. The torsos were trimmed of excess tissue, immersion fixed in a 10% formalin bath for a minimum period of 5 days and then carefully dissected to expose the latex-filled veins.

The testicular veins were variable in number (ranging from 2 to 4 for each testis) and point of termination. The right testicular veins were observed to drain the right testis, epididymis and its appendix to the caudal vena cava just cranial to its origin, as well as to the right common iliac vein. In contrast, the left testicular veins emptied exclusively into the left common iliac vein just before the confluence of the left and right common iliac veins. In addition, the most caudal testicular vein was seen to drain the cranial aspect of the ureter and deferent duct by receiving the cranial ureterodeferential vein. The middle portion of each ureter and deferent duct was drained by the middle ureterodeferential veins. These vessels were variable in number, size and point of entry along the caudal renal vein into which they emptied. The caudal renal vein also drained the caudal part of the cranial division and all of the middle and caudal divisions of the kidney, as well as the ipsilateral ureter and deferent duct. The caudal renal veins fused with each other between the caudal and middle renal divisions. They drained into the common iliac veins. The internal iliac vein had both the internal pudendal and external pudendal veins as its tributaries. The pudendal veins drained the cloaca and the caudal part of the deferent duct through the caudal ureterodeferential veins. Several variably situated tributaries drained the epithelial covering and connective tissue, but not the substance, of the phallus.

The general pattern of the venous drainage of the reproductive organs of the male ostrich is similar to that described in the domestic fowl. However, an important difference was the fusion between the caudal renal veins that was observed in 80 % of the ostriches examined. This previously unreported venal fusion receives blood from the middle ureterodeferential veins.

## Practical treatment of keratoconjunctivitis in Saanen goats

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In early January 2002, an outbreak of keratoconjunctivitis started in a flock of Saanen goats at the Onderstepoort Campus of the University of Pretoria. More than eighty percent of the adult does (total 25) and some of the younger goats (lower incidence) were affected in one or both eyes during the outbreak period. Six does were totally blind. Pneumonia (with or without keratoconjunctivitis) was also diagnosed in some of the goats.

*Branhamella (Neisseria) ovis* had previously been isolated in this flock and initial treatment consisted of Cloxacillin topically (Orbenin<sup>®</sup> dry cow) and was followed up a week later with a subconjunctival injection of penicillin when no improvement was seen. *Mycoplasma conjunctivae* was isolated from additional conjunctival swabs and cytobrush samples. Because topical treatment was impractical, it was decided to use subconjunctival<sup>1</sup> oxytetracycline in a polyvinylpyrrolidone base (Engemycin<sup>®</sup> 10%, Intervet), to minimise the high incidence of tissue irritation reported in the literature<sup>1</sup>. A temporary tarsorrhaphy (closure of the eyelids) using a cyanoacrylate glue<sup>2</sup> (Superglue) was used on the worst affected eyes. The glue kept the eyelids closed for seven to ten days.

The oxytetracycline used did not appear to cause any obvious irritation or reaction and there were no corneal lesions when the glued eyelids opened.

*Branhamella ovis* would appear to be an opportunistic pathogen in goats and antibiotics more specific for *Mycoplasma*, *Chlamydophila* or *Rickettsia* should be considered for an outbreak of this nature. Cyanoacrylate glue for tarsorrhaphy proved to be a practical alternative to the more normal surgical techniques used for third eyelid flaps.

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## Ultrastructural assessment of primary ciliary dyskinesia in a Staffordshire bull terrier

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Clearance of mucus and other debris from the airways is achieved by the mechanisms of mucociliary activity, coughing and alveolar clearance. The cilia associated with respiratory epithelial cells have an important function in transporting mucus towards the pharynx. Cilia are motile, finger-like extensions of the cell surface which owe their motility to an inner core or axoneme composed of two central microtubules surrounded by 9 pairs of outer microtubules (doublets). The outer doublets are linked to the central pair by radial spokes and to each other by nexin links. Attached to each outer doublet is an inner and an outer dynein arm. When morphological abnormalities exist in these cilia, impaired mucociliary clearance occurs which predisposes to recurrent respiratory tract infections. Ciliary abnormalities may be primary, i.e. inherited (probably as an autosomal recessive trait), or secondary (caused by environmental factors). This paper gives an account of the first reported case of primary ciliary dyskinesia in a Staffordshire bull terrier in South Africa. The dog presented with early onset recurrent upper and lower respiratory tract disease that was poorly responsive to antibiotic therapy, and nasal discharge and tracheal sensitivity were clinically evident.

The main-stem bronchi and a portion of the distal trachea of the two-month old male Staffordshire bull terrier were fixed in buffered formalin and processed for transmission electron microscopy by standard techniques. Ultra-thin sections were stained with uranyl acetate and lead citrate, and examined in a Philips CM10 transmission electron microscope operated at 80 kV.

Thin sections were screened for cross-sectional profiles of cilia (n = 401) which were then classified according to structural criteria defined by Pizzi *et al.* 2003 as normal, or displaying primary ciliary dyskinesia (PCD) or secondary ciliary dyskinesia (SCD). The main defects encountered, indicative of PCD, were varying combinations of missing dynein arms, radial spokes and central microtubules, and accounted for 62% of the ciliary profiles examined. Secondary ciliary abnormalities (compound cilia, swollen cilia, addition/deletion of peripheral doublets and disorganized axonemes) suggesting SCD, were identified in 26% of ciliary profiles. Twelve per cent of the cilia examined displayed normal axonemal structure.

Transmission electron microscopy is ideally suited for the diagnosis of PCD, as demonstrated by this first description of this condition in a dog in South Africa. The various ultrastructural anomalies associated with this condition are easily identified, using this technique. PCD may be more common in this country than is generally reported because affected dogs may be misdiagnosed as having fading puppy syndrome, aspiration pneumonia or pneumonia caused by infective agents. Clinicians should therefore also consider PCD as a possible causative factor in young puppies presenting with recurrent respiratory conditions. The electron microscopic evaluation of the ciliary ultrastructure of nasal or respiratory biopsies and nasal brushings forms the basis for a definite diagnosis of primary ciliary dyskinesia.

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## The importance of the vomeronasal organ in modulating the “buck effect” in does

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The introduction of bucks to a group of does is known to synchronize their oestrus cycles even in the non-breeding season. This “buck effect” is mediated through the secretion of LH, which induces ovulation. GnRH released from the hypothalamus controls the secretion of LH. Since GnRH neurons, in the embryo, are known to migrate to the hypothalamus from the developing vomeronasal organ via the caudal vomeronasal nerves, it is possible that the vomeronasal organ may modulate the “buck effect” in adult does.

Nineteen matured, indigenous does were randomly assigned to either a treatment group (n = 10) or a control group (n = 9). The vomeronasal organ of each doe in the treatment group was rendered non-functional by means of cauterization of the nasopalatine canal. After estrus synchronization, bucks were introduced to the previously buck-isolated does. Blood samples were collected for five days before and five days after the introduction of bucks. The samples were analysed for estradiol and LH concentration. Conception rates were determined by ultrasonography ten weeks after buck introduction.

The results demonstrated that none of the does in the treatment group became pregnant, while five of the nine does (55%) in the control group became pregnant during this non-breeding season trial. Statistical analysis of LH concentrations demonstrated a significant absence ( $p < 0.05$ ) of the pre-ovulation surge in LH (necessary for ovulation) in the treatment group versus the control group. Therefore, it does appear that the vomeronasal organ modulates the pre-ovulation surge in LH that occurs with the “buck effect”.

## Detection of bovine papillomavirus DNA in sarcoids in the Cape Mountain Zebra (*Equus zebra zebra*)

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Bovine papillomaviruses (BPV) are small DNA viruses of the Family *Papillomaviridae*. There are 6 subtypes of BPV of which BPV types 1 and 2 are associated with tumors<sup>1,2</sup>. BPV types 1 and 2 are associated with sarcoids in horses<sup>2,3</sup> but the virus has not been demonstrated in sarcoids in the zebra. Two Cape Mountain Zebra (*Equus zebra zebra*) herds, at Gariep Nature Reserve and at Bontebok National Park, have a very high incidence of sarcoids.

Sarcoid biopsies (n=18), normal skin as well as blood samples were obtained from these animals. DNA was extracted from these samples using the QIAamp<sup>®</sup> DNA extraction kit (Southern Cross Biotechnologies) and were analyzed using PCR<sup>3,4</sup>. A segment of 300 bp was amplified that was further analyzed by restriction digestion. These amplicons contain an endonuclease site (BPV 1), or not (BPV 2), to differentiate between the two BPV types.

BPV was detected in all 18 sarcoid samples. Restriction enzyme digestion showed that both single and mixed infections occurred and this needs to be characterized further. These results provided evidence that sarcoid in the zebra are also associated with the papillomavirus as already been shown in other equidae<sup>1,2</sup>.

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## Withdrawal periods and tissue tolerance of intramammary antibiotics in dairy goats: preliminary results

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Limited research has shown that withdrawal times for antibiotic residues are longer in goat milk than in milk from cows. The standard safe withdrawal time is determined in cows. Antibiotic residues in goat milk may cause: anaphylactic and allergic reactions in humans (especially infants); the development of resistant strains of bacteria and inhibition of starter cultures during the cheese making process. Dairy goats rather than cows are often more appropriate for subsistence milk production and the demand for goat milk is increasing. In addition, mastitis can also be a major disease in dairy goats. The main aim of this study was to determine withdrawal periods of antibiotic residues in goat milk.

Trial 1: Eight goats were treated intramammary with Curaclox LC and six goats were untreated controls. All goats, except one, were in early lactation.

Trial 2: Seven goats were treated intramammary with Spectrazol and seven goats were untreated controls. All goats were in mid to late lactation. Milk production of each goat udder half was measured 12 hourly and samples were taken and tested for: conductivity, California Milk Cell Test (CMCT), Somatic Cell Counts (SCC) (Fossomatic 90), the presence of micro-organisms and for the presence of antibiotic residues, using the Thermo-Resistant Inhibitory Substances (TRIS) test.

Trial 1: The withdrawal periods for Trial 1 ranged from 2 days (48h) to 4 days (96h). Blue dye was visible for 2.5 days (60h) to 4.5 days (108h). The withdrawal periods of Curaclox LC according to TRIS were shorter than the withdrawal periods indicated by the disappearance of the blue dye in the milk.

Trial 2: The withdrawal periods for Trial 2 ranged from 2.5 days (60h) to 4.5 days (108h), and were therefore longer than those found during Trial 1. The withdrawal periods for Trial 2 were longer than those for Trial 1.

Antibiotic	Withdrawal period in hours: goats	Withdrawal period in hours:cows
Curaclox LC	48 to 96	72
Spectrazol	60 to 108	60

The withdrawal periods for goats were longer than those indicated for use in cattle. Dye disappearance was a good indicator of the absence of Curaclox LC in goat milk, therefore confirming that the milk was safe for human consumption. The withdrawal periods were on average longer for Spectrazol and did show larger variation than for Curaclox LC. This was a preliminary study and more data will be collected for reliable statistical tests. In late lactation (Trial 2), SCC remained high and thus were not a true indication of milk quality.

## Assessing the level of knowledge of Animal Health Technicians involved in extension to control internal and external parasites of cattle

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The main goal of agricultural development should be to increase sustainable farming productivity and welfare of the farming communities and stakeholders. Livestock health is a major factor in food security.

In terms of the Animal Health Act of 2000, the main role of state veterinary services is the prevention and control of major livestock diseases. Vector transmitted diseases and the direct effect on animal health of heavy parasite burdens therefore forms part of the job description of Animal Health Technicians (AHTs) employed at provincial level. The state employs AHTs who qualified at different institutions. The recognition and control of cattle parasites forms part of the curriculum in all of these qualifications. In addition, state veterinarians do in-service training.

The aim of this investigation was to assess the knowledge level of AHTs concerning internal and external parasites of cattle. Two groups of AHTs were assessed. The first group (n=15) came from North-West Province and the second group (n=29) from Limpopo Province. They were all given the same evaluation before and after a short refresher course on the recognition and control of parasites in cattle. Extension material supplied by the Onderstepoort Veterinary Institute and the Veterinary Faculty of the University of Pretoria was used for training.

As in a test performed to assess student knowledge at a tertiary institution, 50% was taken as an acceptable mark. The mean score before the course for AHTs from NWP was 35.64%, which was not acceptable. Only one respondent scored above 50%, with a range of 1.82% to 54.55%. After the course the mean improved significantly to 54.50%, with a range of 7.27% to 82.93%. Five of the respondents out of 15 still scored below 50% in the second test. It was found that none of the AHTs from Limpopo Province passed with a score of 50% or higher before the course and, although there was a significant improvement, only 17 of the 26 participants who wrote the second test (three did not write the second test), passed. The mean score before the course was 27.50% (range 7.32-43.90%) and after the course it was 49.72% (range 19.51-68.29%). The level of knowledge of the AHTs involved in this evaluation was not considered to be adequate to do farmer extension, and therefore further training was given.

## The influence of rearing systems on gut development of kids

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Ruminants begin to graze in the first week of life when given access to pasture<sup>1,2</sup>. Field observations in communal areas of North-West Province of South Africa have shown that kids are kept in closed kraals to reduce the risk of predation of young kids but there is no exposure to the roughage until they are about five months old<sup>3</sup>. The aim of the study was to evaluate the effect of this husbandry practice on gut development.

Crosses of Saanen and South African Indigenous goat kids, 4 to 7 days old, were assigned to two rearing groups. One group was fed milk only (M group) until four months of age and thereafter was given poor roughage (kikuyu) *ad libitum*. The other group was fed milk, lucerne and concentrates (MRCC group). Kids were sacrificed at two months, four months and six months of age. Tissue samples were collected from the stomach for microscopy. Ultrastructural differences of the fore-stomach were investigated using scanning electron microscopy (SEM) and Computer AnalySIS (SIS Extended Pro) Software Image Processing System,.

MRCC kids were bigger at slaughter when compared to the M kids. Differences in the omasum/body weight ratios were statistically significant ( $p < 0.05$ ). The papillae were less developed and less numerous for the goats in the M group when compared to those in the MRCC group. Poor growth that was noted in the group fed with milk only was consistent with earlier reports in calf<sup>1</sup> and sheep<sup>4</sup>. The findings showed differences between the groups, emphasizing a key role for adequate roughage in gut development in the rearing of goat kids. The adoption of early roughage in the diets of kids is recommended for small-scale farmers as a method of improving the productivity of their stocks.

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