

Faculty of Veterinary Science

Faculty Day

27 August 2009



Prof Peter Doherty
Nobel Laureate 1996



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1908 - 2008



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Faculty of Veterinary Science

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 showcasing 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 1980s, the staff, and later students, were involved in the activities of the "Academic Day" which

was aimed at highlighting the research activities of the University as well as exposing young researchers to a conference environment. The Faculty of Veterinary Science on 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 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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Faculty Day

Faculty of Veterinary Science

University of Pretoria

27 August 2009





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- 08:15 – 08:30 Welcoming and Opening Address
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- 08:30 – 09:30 First Session - Research Programme: Oral Presentations

Research Programme: Oral Presentations

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- 09:35 – 10:25 **Sir Arnold Theiler Memorial Lecture:**
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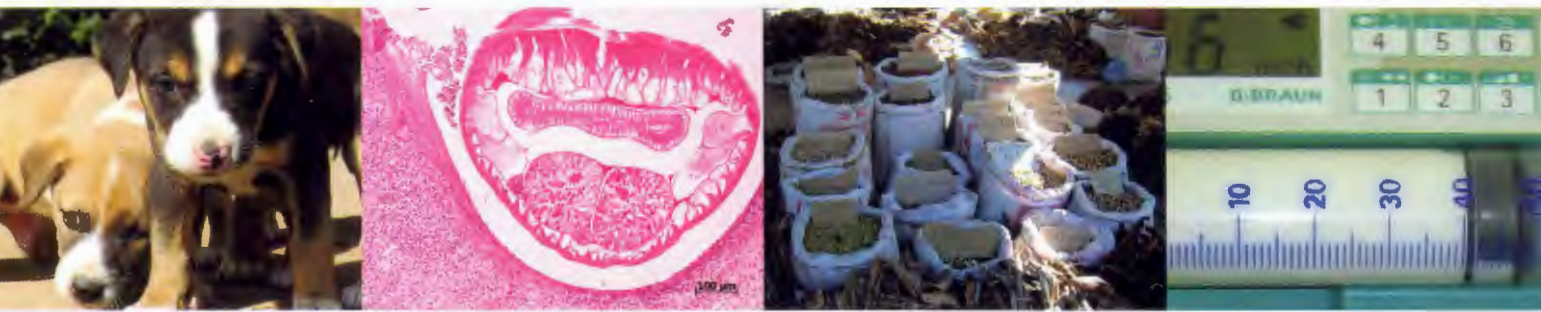
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1 Message from the Dean

For the past 25 years Faculty Day has represented the focal point of our academic year serving as a platform for showcasing the research activities within the Faculty.

It has provided an opportunity over this period for staff and students to share in their research achievements. I would like to honour all those who have been instrumental in establishing and arranging this important event over these many years. Faculty Day has no doubt contributed enormously in establishing a culture of research within the Faculty. Over the past 5 years our research output has virtually doubled and the number of postgraduate students grown by more than 10 % per annum. Postgraduate students presently represent just less than 30 % of our total student body. Indeed a great achievement. Increasing numbers of our staff are also becoming NRF-rated.



Prof Gerry Swan, Dean

The Sir Arnold Theiler Memorial Lecture reflects the spirit of Faculty Day. This year we could not have chosen a more esteemed veterinary scholar and researcher than Prof Peter Doherty. Prof Doherty is the first and only veterinarian to have been awarded the Nobel Prize. The fact that he was awarded the Nobel Prize in physiology or medicine which he shared with Dr Rolf Zinkermagel for their discovery of how the immune system recognizes virus infected cells, makes it even more remarkable. He has shown that his training as veterinarian provided him with a sound scientific foundation from where he was able to launch his remarkable scientific career. This should serve as an inspiration to all veterinarians.

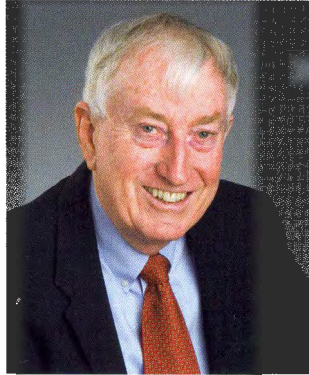
Welcome to Faculty Day and congratulations to the 2009 teaching and research award winners. You can be very proud of your achievement. My sincere thanks go to the organisers and sponsors of the day. Your contributions and commitment are instrumental in making this day possible.

Prof Gerry Swan
Dean



Curriculum Vitae: Professor Peter Doherty

Peter Doherty was born in Australia in 1940. He obtained his BVSc degree in 1962 from the University of Queensland.



Prof Peter Doherty

After qualifying as a veterinarian, he worked for 4 years as a Veterinary Officer at the Animal Research Institute in Brisbane. In 1967 Peter Doherty joined the Department of Experimental Pathology at Moredun Research Institute in Scotland. He received his PhD from the University of Edinburgh Medical School in 1970. He returned to Australia in 1972, and worked as a Research Fellow in the Department of Microbiology, Australian National University. In 1975 he was appointed an Associate Professor at the Wistar Institute in Philadelphia, USA. He returned to Australia in 1982, and served as Professor and Head in the Department of Experimental Pathology at the Australian National University.

Peter Doherty shared the Nobel Prize in Physiology or Medicine in 1996 with Swiss colleague Rolf Zinkernagel, for their discovery of how the immune system recognizes virus-infected cells. Peter Doherty is the first person with a veterinary qualification to win a Nobel Prize. The research conducted by Peter Doherty and Rolf Zinkernagel has laid a foundation for an understanding of general mechanisms used by the cellular immune system to recognize both foreign microorganisms and self molecules. This discovery relates both to efforts to strengthen the immune response against invading microorganisms and certain forms of cancer. In addition, their findings have been used in research on the effects of autoimmune reactions in inflammatory diseases, such as rheumatic conditions, multiple sclerosis and diabetes.

In 1997 Peter Doherty was named "Australian of the Year". He currently commutes between St Jude Children's Research Hospital in Memphis and the Department of Microbiology and Immunology at the University of Melbourne. His research is mainly in the area of defence against viruses. He regularly devotes time to delivering public lectures, writing articles for newspapers and magazines and participating in radio discussions. Peter is also the author of several books, including "A Light History of Hot Air" and "The Beginner's Guide to Winning the Nobel Prize".

Sir Arnold Theiler Memorial Lecture

Adventures in Infection and Immunity

Prof Peter Doherty, (pcd@unimelb.edu.au)

Department of Microbiology and Immunology, University of Melbourne, Australia; and Department of Immunology, St Jude Children's Research Hospital, Memphis, USA.

Beginning with the Swiss-born Arnold Theiler, many veterinarians have made major contributions to the understanding of infection and immunity. Sir Arnold first came to prominence when he produced a smallpox vaccine to protect mine workers, then went on to found the great South African tradition in veterinary infectious disease research. His son Max was awarded the 1951 Nobel Prize for developing the yellow fever vaccine that is still used today.

That back and forth between veterinary and human medicine has, of course, been a long tradition, particularly when it comes to pathogens. My personal scientific journey began at age 17 when I started at the University of Queensland School of Veterinary Science and, at least in the public sense, peaked almost 40 years later when the Nobel Foundation recognized my Swiss colleague Rolf Zinkernagel and I for discovering the basis of cell-mediated immunity. At least in those distant days when I was an undergraduate, veterinarians weren't too interested in geriatrics and degenerative conditions, but were well trained to deal with infections. My interest in viral pathogenesis and immunity began as an undergraduate and remains fundamental to what I do in science today. I'll relate some of that personal journey from student, to veterinary scientist, to experimental pathologist, to research immunologist to being a public advocate for rational enquiry and cultural values that emphasize an evidence-based view of the world.

Now as then, we may begin our professional lives with one focus, but may end up somewhere very different. A training in veterinary science gives a respect for reality, a knowledge that the world can never be a totally safe place, a set of practical skills, a solid scientific grounding and an understanding of ecological balance and sustainable production systems. That can be a pretty good place to start as we seek to do our part in dealing with the all too real problems that face humanity through this coming century. Apart from anything else, the challenge of feeding people will be very much to the fore. The future belongs to the young. My bet is that those who start out as veterinarians will continue to play a substantial part, and in a great diversity of roles.

Prof Peter Doherty
Nobel Laureate

Osteology of the thoracic limb of the suricate (*Suricata suricatta*): Selected features and correlative radiological anatomy

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There is limited osteological data available on wild carnivores. This study was performed to establish the normal osteology and correlative radiological anatomy of the thoracic limb of the suricate and provide comparisons to domestic carnivores.

Bone specimens of 19 animals (17 mature) were studied. Individual bones of the *Manus* region were studied *in situ* in preserved specimens. Radiographs were made of the thoracic limbs in 7 animals. Selected features of the osteology and the correlative radiological anatomy were described and compared to domestic carnivores (dog and cat).

A *Processus suprahamatus* is present on the scapula spine. Both supratrochlear and supracondylar foramina are present in the distal humerus, with a small *Fossa coronoidea* present cranially. The medial epicondyle is markedly larger than the lateral epicondyle. The *Tuberositas radii* is located caudally, and the *Trochlea radii* has distinct grooves cranially for tendons. The proximal end of the *Olecranon* is most prominent medially. The large *Processus coronoideus medialis* has an extensive proximal articulation facet for the humeral *Trochlea*, but a relatively small cranial facet for the radial head. The *Processus styloideus* (ulna) articulates with the ulnar- and accessory carpal bones. The *Manus* of the suricate has similar characteristics to the domestic carnivores, however *Os metacarpalia I* is markedly reduced with the complete absence of Digit I. There are 8 carpal bones. *Os carpi radiale* is the largest, with a small sesamoid bone present medially. *Os metacarpalia II-V* and corresponding phalanges are slender, with a markedly elongated *Processus unguicularis* (*Phalanx distales*) present. Paired proximal sesamoid bones are present palmarly at the metacarpophalangeal joints. Pertinent aspects of the correlative radiological anatomy are described using digital radiographic images. On the cranio-caudal view of the humerus and elbow joint, the distal radius and *Manus* are consistently rotated in a supinated position.

The unique osteology and radiological anatomy of the suricate's thoracic limb, indicates morphological changes for supination of the antebrachium and *Manus*, and superior digging ability. These findings constitute the first description of the normal osteology and radiological anatomy of the thoracic limb of the suricate, which will serve as a reference in clinical zoological medicine and paleozoological studies.

"The unique osteology and radiological anatomy of the suricate's thoracic limb, indicates morphological changes for supination of the antebrachium and *Manus*, and superior digging ability."



Comparative popliteal and mesenteric computed tomographic lymphography of the caudal canine thoracic duct

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Canine chylothorax is a problematic condition to manage due to poor response rates with both medical and surgical management. Surgical ligation of the thoracic duct (TD) has been the most frequently reported treatment; however success rates with TD ligation alone have been reported to be between 53% and 59%. Failure to ligate all of the TD branches has been described as the most common cause of surgical failure. Pre and post ligation lymphography have been used to help identify thoracic duct branches and ensure their successful ligation. Lymphography has conventionally been conducted by catheterization of a mesenteric lymphatic via a coeliotomy. Lymphography has recently been described using the less invasive technique of percutaneous injection into a popliteal lymph node. Computed tomography (CT) has been shown to be superior to radiographs in detecting TD branches and providing TD topographical information. This study assessed the comparability of the two lymphography techniques and determines the equivalency of helical and sequential CT modalities for TD lymphography.

Seven beagles underwent both mesenteric lymphography (ML) and ultrasound guided percutaneous popliteal lymphography (PPL). Iohexol 300 mg/mL (Omnipaque, Nycomed Inc., New York, USA) was administered at 1mL/kg for both techniques. For the PPL, contrast was administered at 100 mL/hr where as the ML contrast was given as a bolus over a one minute period. Helical CT was initiated immediately after completion of contrast injection followed by sequential CT of the same area. For both contrast administration techniques and CT modalities, images were taken at the mid vertebral bodies of thoracic vertebra nine to lumbar vertebra one. Images were assessed for the total

TD number and position of TD branches relative to the aorta as well as the maximal width, cross sectional area and mean Hounsfield units (HU) for the largest TD branch. The effects of administration technique, CT modality, vertebral site and animal on the observed TD number were assessed using zero-truncated Poisson regression, while their effects on maximum TD diameter, cross sectional area and mean HU, were analyzed using multiple linear regression .

“This study assessed the comparability of the two lymphography techniques and determines the equivalency of helical and sequential CT modalities for TD lymphography.”

The number of TD branches detected did not differ significantly between the two contrast administration techniques ($P=0.256$) or CT modalities ($P=0.417$), although the count ratio (CR) indicated a slight trend for the PPL administration technique ($CR=0.830$) and the helical CT modality ($CR=0.876$) to detect slightly fewer TD branches. ML and helical CT both resulted in significantly greater maximum TD diameter, cross sectional area and mean HU ($P<0.001$).

This study suggests that PPL is an acceptable alternative to ML for identifying TD branches when combined with either helical or sequential CT.



Ultrasonographic appearance of canine parvoviral enteritis in puppies

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Canine parvoviral enteritis is a significant disease of high prevalence in South Africa causing acute vomiting, haemorrhagic diarrhoea, anorexia and depression predominantly in young puppies. Without treatment, which is primarily supportive and symptomatic, mortality rates have been reported to be as high as 91%.

Ultrasonography of the gastrointestinal tract has become increasingly important in the diagnosis of numerous gastrointestinal diseases such as neoplasia, foreign bodies and enteropathies. The ultrasonographic appearance of canine parvoviral enteritis has not been documented. Canine parvovirus targets tissues with rapid cell turnover such as the lymphoid tissues and intestinal epithelium. The aim of this study was to describe the gastrointestinal ultrasonographic changes associated with canine parvovirus infection and compare these changes to the ultrasonographic appearance of the normal puppy gastrointestinal tract and established reference values.

A prospective clinical study was performed. Forty puppies between six and 24 weeks of age were examined ultrasonographically within 24 hours of admission for canine parvoviral enteritis confirmed on faecal electron microscopy. Sonographic findings included fluid filled small intestines in 92.5% of cases, and stomach and colon in 80% and 62.5% of cases respectively. Generalized atony was

seen in 30 cases and weak peristaltic contractions indicative of functional ileus observed in the remaining 10 cases. The duodenal and jejunal mucosal layer thicknesses were significantly reduced when compared to values obtained in normal puppies with mean duodenal mucosal layer measuring 1.7 mm and jejunal mucosal layer 1.0 mm. Additionally, a mucosal layer with diffuse hyperechoic speckles was seen in the duodenum (15% of cases) and the jejunum (50% of cases). The luminal surface of the duodenal mucosa was irregular in 22.5% of cases and the jejunal mucosa in 42.5% of cases. In all of these puppies, changes were accompanied by generalized indistinct wall layering. Small intestinal corrugations were seen within the duodenum in 35% of cases and within the jejunum in 7.5%. A mild amount of anechoic free peritoneal fluid was observed in 26 cases and was considered within normal limits for puppies and a moderate amount of anechoic free peritoneal fluid was observed in six cases. The jejunal lymph node size was within normal limits for puppies.

“Ultrasonography of the gastrointestinal tract has become increasingly important in the diagnosis of numerous gastrointestinal diseases such as neoplasia, foreign bodies and enteropathies.”

Each of the above described changes cannot be considered pathognomonic for canine parvoviral enteritis but in combination are highly suggestive of the disease. Further studies are needed to document the ultrasonographic appearance of other paediatric gastrointestinal diseases such as severe verminosis, giardiasis and corona or distemper viral infection before further conclusions can be drawn from this study.

An evaluation of changes over time in serum creatine kinase activity and C-reactive protein concentration in dogs undergoing hemilaminectomy or ovariohysterectomy

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Trauma of diverse origins is a common reason for presentation of pets for treatment. It is often difficult clinically to objectively measure the severity of any trauma to an animal. One approach is to measure the changes in the various serum parameters which are known to alter in response to trauma or inflammation. If the changes over time of relevant and easily measurable parameters can be established under two controlled but different conditions of surgical trauma, it may provide the foundation for evaluating their future use in establishing the severity of trauma in a patient.

A prospective study was performed on animals presented to the Onderstepoort Veterinary Academic Hospital for either thoracolumbar disc disease or for elective ovariohysterectomy. The two surgical procedures chosen for the study involved significant surgical trauma, particularly to muscle, in the case of thoracolumbar decompression and relatively minor surgical trauma in the case of ovariohysterectomy. Serial evaluation of creatine kinase (CK) and C-reactive protein

(CRP) were performed both pre- and post-operatively on two sets of patients derived from the two surgical categories. CK is an enzyme found predominantly in skeletal muscle and significantly elevated serum activity is largely associated with muscle damage. CRP is an acute phase protein which shows elevated serum concentration in response to a broad range of inflammatory stimuli.

Analysis of the data showed a very wide range of results at each time point for both CK and CRP. There were no significant differences between the two surgical groups for either analyte preoperatively. Thereafter CK results were markedly and significantly different between the two groups. CRP results were very similar in the two groups with no statistical difference at any time point. The results of this study suggest that the evaluation of CK and CRP at any one time point in a traumatized animal is of limited value. However the evaluation of the trend of these two analytes, even over a relatively short time period, may allow for useful prognostication in clinical cases.



"Trauma of diverse origins is a common reason for presentation of pets for treatment..."

Case report: Urinary steroid analysis in a female adult Nile crocodile (*Crocodylus niloticus*).

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Common neutral steroids are excreted in urine as end products of steroids that were secreted by the glands and metabolized in various body tissues. Interference by endogenous or exogenous substances with either synthesis, secretion, metabolism or excretion of the steroids or their metabolites may change the patterns of excretion of the metabolites.

This study was aimed at obtaining results from a crocodilian urine sample. Urine was collected from a fertile female adult Nile crocodile, using a urine catheter. A well-established gas chromatographic-mass spectrometric method for determination of human urinary steroid metabolites was employed for the validation of steroid analysis in the Nile crocodile.

In the total ion chromatogram 23 peaks were observed. Nine of these peaks were positively identified by comparison of retention times and m/z mass spectra with those of standard derivatives.

The analytes that could be identified and their retention times (RT) were (steroid metabolite/RT): Androsterone (5 α -androstan-3 α -ol-17-one)/7.375 min, Etiocholanolone (5 β -androstan-3 α -ol-17-one)/ 7.742 min, Han (5 α -androstan-3 α ,11 β -diol-17-one)/10.558 min, Pregnanediol (5 β -pregnan-3 α ,20 α -diol)/11.925 min, Pregnanetriol (5 β -pregnan-3 α ,17 α ,20 α -triol)/12.208 min, THE (5 β -pregnan-3 α ,17 α ,21-triol-11,20-dione)/17.075 min, THF (5 β -pregnan-3 α ,11 β ,17 α ,21-tetrol-20-one)/17.933 min, α -THF (5 α -pregnan-3 α ,11 β ,17 α ,21-tetrol-20-one)/18.433 min, and corticosterone/21.117 min.



Prominent peaks of the androgen metabolites (androsterone and etiocholanolone), the metabolite of progesterone (pregnanetriol), cortisone and cortisol metabolites (THE, THF and α -THF), as well as corticosterone, were observed. The 14 unidentified peaks need to be investigated further by more selective mass spectrometric techniques. The employment of detectors that are capable of MSⁿ would enable structure elucidation of these unknowns, and help to determine whether they are metabolites of interest.

Although quantification was not the aim of this study, the concentrations of all steroid metabolites found in this profile can be calculated and expressed as μ mol steroid/mmol urine creatinine.

More urine samples should be collected from healthy farm crocodiles and analyzed in order to establish reference ranges for urinary steroid metabolites, as well as the steroid metabolite ratios. Future investigations will include urine samples of crocodilians from polluted areas, to evaluate the significance of urine steroid profiling in the investigation of endocrine disruption.



"Urine was collected from a fertile female adult Nile crocodile..."

Multimedia computer-assisted learning for veterinary anatomy

MR Crole¹, E Mostert², H Untiedt² (martina.crole@up.ac.za)

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The study of anatomy presents the undergraduate veterinary student with many challenges. The subject content is often overwhelming and the input required by the student to grasp and memorize the work is extremely time-consuming. The traditional practical-based course presented at the Faculty of Veterinary Science, University of Pretoria, although supplemented by study material in the form of a comprehensive dissection guide containing black and white illustrations, largely confines learning opportunities for the students to the anatomy dissection hall and museum. Additional study material such as colour text books, models and computer programmes, although recommended, are not provided on an individual basis due to the prohibitively high additional cost that this would add to the tuition fees. The aim of this project was to develop an affordable learning and teaching tool which would allow students to study and comprehend the subject matter in their own environment, and at their own pace. The visual contents of the programme were prepared using high quality photographs of the actual specimens dissected by the students and on which they are examined. The resulting 'Veterinary Anatomy' learning resource can be described as an electronic multimedia learning tool consisting of a combination of integrated text and graphic images. The use of multimedia opens up many new possibilities for teaching and learning, specifically in such a visual subject.

A team from the department for Education Innovation (EI), consisting of a photographer, graphic designer, instructional designer and a project manager, was involved in the design of the programme. A template based on the best practice in

multimedia design was developed. The subject specialist (anatomy lecturer) consequently developed the course contents within the template. Continuous quality assurance was conducted by both subject specialists and instructional designers. A survey to obtain the students' view on the value of the resource was conducted in 2008.

The majority of the students agreed that the programme, together with attendance of anatomy practicals, provided them with a better learning opportunity. The learning resource was used extensively by most of the students with some indicating that they spent up to 50% of their study time using the programme. 97% of respondents felt that they could apply what they had learned with the programme in a practical situation and that the content was easy to understand. 99% of the respondents agreed that it was good for self study while 93% used the programme to compliment their studies. Only 2% indicated that they found the programme boring. Most comments received were very positive and highlighted the ease of use of the programme, the excellent quality of the visual material, the inclusion of pertinent information and its value as a source of revision. Its versatility in allowing individuals to work freely in their own environment away from the confines of the busy anatomy hall filled with other students was also emphasized.

This multimedia tool promotes student-centred learning and reflects a high educational value for both the teaching and learning of veterinary anatomy. It also provides a platform with great potential for continuing development and enhancement.



A dedicated problem-based learning module in the preclinical veterinary curriculum: utilizing case-based, small-group cooperative learning as a bridge between basic science and clinical literacy

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In 1999 a dedicated problem-based learning (PBL) module was introduced into the lecture-based preclinical veterinary curriculum of the University of Pretoria. This module forms part of the Introduction to Clinical Studies Course, which combines traditional lectures, practical sessions, self-learning and guided tutorials. The self-directed component of the course utilizes case-based, small-group cooperative learning as an educational vehicle to integrate disciplines and to link basic science with clinical medicine. In the light of recent concerns expressed about problem-based learning as a pedagogic base and because the Faculty of Veterinary Science is embarking on a major curriculum redesign, we sought to obtain objective data to test the feasibility of its continuation. The aim of this paper is to describe the structure and objectives of the course and to report on the assessment of the students' perceptions on some aspects of the course.

After the final examination on the course, the students were requested to complete a questionnaire regarding their perceptions on the following aspects: (1) the ability of the course to enhance their problem-solving skills; (2) the workload of the course and (3) the clarity of the objectives of the course.

"Students indicated positive perceptions on the workload of the course with a mean score of 3.37/5."

Students reacted very positively on the ability of the course to equip them with problem-solving skills with a mean score of 4.04/5, which was significantly higher than the two other aspects respectively, ($p < 0.001$ for both). Students indicated positive perceptions on the workload of the course with a mean score of 3.37/5. There were significantly lower scores on the clarity of the course objectives, where a mean score of 2.95/5 was attained ($p < 0.001$).

A combination of pedagogic tools facilitates a sound introduction to clinical studies for preclinical veterinary students. The case-based, small-group cooperative component of this course utilizes positive aspects of problem-based learning combined with structured tutorials that is positively perceived by students. Although the study guide for the course is very comprehensive, the practice regarding the objectives is still uncertain.

It is imperative to set clear objectives in non-traditional, student-centered courses. The core and ancillary objectives have to be explained at the outset and reiterated throughout the course. Tutors should also communicate the rationale behind PBL as a pedagogical method to the students. One of the major strengths of our course is the fact that the tutors are subject experts, guiding the learning process during the tutorials.



Is community engagement a COPout? A report-back on the Calvinia experience

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The community of Calvinia, a small town in the Northern Cape, requested the Faculty of Veterinary Science, University of Pretoria, to assist with the sterilisation of dogs and cats. Calvinia has no private veterinarians, with the nearest private veterinary practice 170 km away. There is one state veterinarian servicing an area of 8336084 hectares (1597 farms). In the absence of accessible veterinary services, the dog and cat populations have been allowed to breed indiscriminately and stray dogs in particular have become a problem by attacking sheep belonging to local farmers, biting people, disturbing the peace and polluting the environment with faeces and by foraging in dustbins. The problems are most pronounced in the large indigent community in Calvinia West and the surrounding farms.

The Faculty agreed to assist the community and COPout (Calvinia Outreach Project) was born. A community-based approach to animal welfare was followed and included prior engagement with the community to obtain their support and determine their needs and assets; an educational programme; a primary health care clinic (vaccination, deworming, basic treatment of disease); a sterilisation clinic and a vaccination campaign a few weeks prior to the sterilisation clinic.

“The team comprised of 16 people in total and processed 310 animals of which 148 were sterilised and 82 vaccinated...”

In July 2009, a team of veterinarians, veterinary nurses, a veterinary technologist and veterinary support staff visited Calvinia and set up a primary health care and sterilisation clinic for four days in collaboration with the local state veterinarian, private volunteer veterinarians and the community itself. The team comprised of 16 people in total and processed 310 animals of which 148 were sterilised and 82 vaccinated (150 animals had been vaccinated by the state veterinary services prior to the visit). A client-centred approach was followed with emphasis on clear communication with clients. Educational literature on basic animal health care was provided to all clients. All the schools in town except one were visited by a team member and over 1500 learners were introduced to basic concepts of animal welfare, dog bite prevention and careers in veterinary science. Data on animals and people in the community was collected for research purposes. Reflections of team members were documented to assist with formulating a standard operating procedure for future similar community engagement activities. Community engagement is an excellent vehicle for simultaneously helping communities, facilitating teaching, and motivating and stimulating Faculty staff.

Sedative and cardio-pulmonary effects of acepromazine, midazolam, butorphanol, acepromazine-butorphanol and midazolam-butorphanol on propofol anaesthesia in goats

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Goats are seldom anaesthetised resulting in a paucity of information on available anaesthetic approaches, even on details about effects of commonly used sedative agents. The sedative, propofol-sparing and cardiopulmonary effects of acepromazine, midazolam, butorphanol and combinations of butorphanol with acepromazine or midazolam in goats were evaluated.

Six healthy Boer - Indigenous African crossbreed goats were by randomised cross-over design to six groups: Group SAL that received saline, Group ACE that received acepromazine, Group MID that received midazolam, Group BUT that received butorphanol, Group ACEBUT that received acepromazine and butorphanol and Group MIDBUT that received midazolam and butorphanol as premedication agents intramuscularly on different occasions at least 3 weeks apart. The degree of sedation was assessed twenty minutes after administration of the premedication agents. Thirty minutes after premedication, the dose of propofol required for induction of anaesthesia adequate to allow placement of an endotracheal tube was determined. Cardiovascular, respiratory and arterial blood-gas parameters were assessed for up to thirty minutes after induction of general anaesthesia. The Friedman rank sum test adjusted for ties was used to test differences between groups for non-repeatedly measured data. Where statistically

significant differences between groups were observed, a pair-wise Wilcoxon test followed by a Bonferroni adjustment for multiple testing was used to identify which groups were different. Repeatedly measured data was analysed for statistically significant differences between and within groups using a repeated measures analysis of variance test followed by a Tukey test for multiple comparison of means. A value of $P < 0.05$ was considered significant.

Acepromazine and midazolam produced significant sedation when administered alone, but premedication regimens incorporating butorphanol produced inconsistent results. The dose of propofol required for induction of anaesthesia was significantly reduced in goats that received midazolam alone, or midazolam combined with either acepromazine or butorphanol. The quality of induction of anaesthesia was good in all groups including the control group. Cardiovascular and respiratory and blood-gas parameters were within normal limits in all groups and not significantly different between or within all groups.

In conclusion, sedation with midazolam alone, or midazolam combined with either acepromazine or butorphanol significantly reduces the induction dose of propofol with minimal cardiopulmonary effects in goats.



Evaluation of butorphanol as an adjunct to etorphine in the immobilization of white rhinoceros (*Ceratotherium simum*)

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Researchers have reported no improvement in ventilation when butorphanol, at a total dose of 10 to 20 mg is added to an immobilizing mixture consisting of etorphine, azaperone, detomidine and hyaluronidase for free ranging white rhinoceros. However, it was recommended that higher doses of butorphanol be investigated. The aim of this study was to determine whether higher doses of butorphanol may be of benefit in improving ventilation in the etorphine/azaperone immobilization combination. In a double blinded study 19 white rhinoceros were randomly divided into three groups. The control group (n=8) received etorphine (mean = 1.94mg; \pm 0.98 mg) and azaperone (mean = 50 mg; \pm 3.78 mg); the second group (B₁₀, n=6) received etorphine (mean = 3.58 mg, \pm 0.27 mg), azaperone (mean = 40 mg) and butorphanol at 10 times the etorphine dose; the third group (B₂₀, n=5) received etorphine (mean = 3.50 mg, \pm 0.31 mg) and azaperone (mean = 40 mg) and butorphanol at 20 times the etorphine dose. Drug doses, time to first effect of drug, time to immobilization and distance ran were recorded. At 5, 10 and 15 minutes post-immobilization blood pressure, rectal temperature and respiratory rate were recorded. At 10 minutes post immobilization an arterial blood

sample was taken from the ear and pO₂, pCO₂, sO₂, pH and HCO₃⁻ were recorded. Compared to published normal values for the species the animals in all the groups were acidaemic, hypercapnic and hypoxic. pO₂ was significantly higher (p<0.05) in the B₂₀ group than in the control group. pCO₂ was significantly higher in the B₁₀ group than in the control, but significantly lower in the B₂₀ group than in the B₁₀ group. There was no difference in the lactate between the two treatment groups, but both were significantly lower than the control group. There was no significant difference in sO₂ between the control and B₁₀ group but the haemoglobin saturation was significantly improved in the B₂₀ group. Time to first effect was shorter in both the treatment groups when compared to the control group, but no difference was noted in time to first effect between the treatment groups. No difference was seen in the time to immobilization between any of the groups. Although improvement in pO₂, sO₂ and CO₂ is seen in the B₂₀ group it could not be shown that butorphanol rectifies the problems of acidaemia, hypercapnia and hypoxia a dose of 20 times the etorphine dose.

Antibacterial, antioxidant and cytotoxic activities of different extracts of selected South African medicinal plants

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Leaf extracts of seven South African plants used as traditional remedies, extracted using acetone, hexane, dichloromethane and methanol were evaluated for antibacterial activity using bioautography and the serial micro plate dilution method against two Gram-positive and Gram-negative bacteria. Qualitative antioxidant was evaluated by the DPPH method and cytotoxicity using the MTT assay on Vero cells.

Generally, the extract yield was highest in methanol followed by acetone. The acetone and dichloromethane extracts showed good antibacterial activity against tested pathogens with MIC = 0.08 mg/ml. Some plants extracts depending on the polarity of the eluent systems, showed activity with same R_f values active against one or more test organisms.

The cytotoxic effects of plant extracts varied with the polarity of solvents. More viable cells were observed in all the plants with hexane and dichloromethane extracts at higher concentration of 0.1 mg/ml than acetone and methanol extracts. Four of the acetone extracts and one hexane extract at 0.001mg/ml showed an increase in viable cells. Increase in cell viability in some extracts with or without antioxidant activity, suggests the presence of constituents with mitogenic effect. These results suggest a diversity of constituents in crude extracts of the same plant with differences in antimicrobial and cellular response associated with the type of solvent used for extraction.



Gousiekte: the mode of cardiac damage in H9c2 cells and rat neonatal cardiomyocytes, exposed to pavetamine, a novel polyamine.

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Gousiekte is a disease of ruminants characterized by acute heart failure without any premonitory signs four to eight weeks after ingestion of certain rubiaceous plants. The active principle, pavetamine, was isolated from *Pavetta harborii*, which is structurally related to the polyamine group. Ultrastructural examination of sheep hearts revealed that the myofibres became disintegrated, had a frayed appearance and this was accompanied by replacement fibrosis. Pavetamine inhibited protein synthesis in rat hearts. This study was undertaken in two *in vitro* models, namely H9c2 cells and rat neonatal cardiomyocytes (RNCM), to investigate the mode of cell death and damage to the cardiomyocytes.

The mode of programmed cell death (apoptosis, autophagy and necrosis) was investigated by means of different techniques. Immunofluorescent staining was performed in RNCM with antibodies to the three major contractile proteins and the cytoskeleton proteins, F-actin and the microtubules. Two of the three proteolytic systems existing in cells, were evaluated, namely the proteasome and some of the lysosomal enzymes.

The primary mode of cell death in H9c2 cells exposed to 20 μ M pavetamine for 72h was necrosis. Autophagy may play a role as numerous vacuoles were present in cells exposed to 20 μ M pavetamine for 48h. All three contractile proteins (actin, myosin and titin) had an abnormal morphology, compared to the untreated RNCM. The well-organized striated pattern disappeared and it seemed that myosin and titin were being degraded by a protease. Pavetamine at a concentration of 20 μ M, also caused damage to F-actin of H9c2 cells exposed for 24h and 48h, and in some cells were even absent. The proteasomal activity was not significantly activated in H9c2 cells exposed to 20 μ M pavetamine. Of significance was the increased activity of the lysosomal hexosaminidase enzyme and acid phosphatase. Pavetamine may thus act as a lysosomotropic compound and cause increased lysosomal permeability.

Is using anti-infective plant products to increase animal health and productivity a third world pipe dream or a realistic commercial possibility?

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One of the five research focus areas of our Faculty is Ethnoveterinary and Phytomedicine. Most of the work in this field is within or at least in association with the Phytomedicine Programme. This programme focuses on protecting animals against infections by using plant products. It is active in two main areas the first is related to the industrial production of animals and the second is related to developing products that can be used by resource poor rural farmers.

The Phytomedicine Programme is probably one of the leading centres in this area in Africa based on many accolades. Funding from sources within Africa and from Europe over the past eight years totalled approximately R20 million. Current applications from within South Africa and from Europe to European funders total about R17 million.

Since the Phytomedicine Programme moved to Onderstepoort from the Faculty of Health Science eight years ago, it has delivered 68 research papers, mainly in ISI rated journals, 20 MSc students and 12 PhD students. Currently there are 19 PhD and 13 MSc students in the Programme. These students originate from all over Africa and Europe. Yet there was only one student from our Faculty that continued the study in the Phytomedicine Programme. The reasons for this situation will be discussed.

Examples will be presented on the delivery of products that have been patented with one product already in the market and other exciting developments in this area.

The oesophageal nodule in canine spirocercosis is not a granuloma

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In both the primary and secondary literature dealing with canine spirocercosis, the nodule inhabited by the adult worms in the wall of the oesophagus of the host is invariably referred to as a granuloma. This is not the correct term for this lesion. A granuloma is a more or less spherical focus of granulomatous inflammation. Granulomatous inflammation, according to Slauson & Cooper (*Mechanisms of Disease*) is "an inflammatory response characterized by the presence of numerous macrophages, with a variable number of lymphocytes and with or without plasma cells; the predominant cell is the macrophage." At no stage in the histogenesis of the spirocercal nodule are macrophages predominant. The oesophageal lesion in canine spirocercosis is therefore not constituted by granulomatous inflammation and cannot be designated as a granuloma.

We have been unable to find any good histological descriptions of the nodule dating from the second half of the 19th to the middle of the 20th Centuries. More recently, Bailey stated that the histopathology of the nodule is well known, but did not provide any references in support of this statement. Ribelin & Bailey appear to be the first investigators to consider the temporal development of the nodule, in the process making a number of seminal observations. They noted that the initial lesion is constituted by highly vascular loose connective tissue, associated with oedema and fibrin accumulation. Later, fibroblasts predominate. They observed that these fibroblasts have a prominently

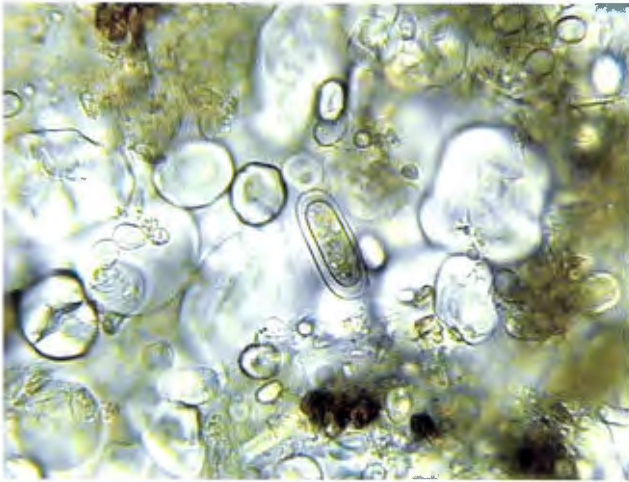
embryonal appearance, suggestive of a preneoplastic state. Still later these fibroblasts form small, scattered tumour foci in which mitoses are common. They also describe the development of areas of osseous and chondrous metaplasia within the nodule, and both fibrosarcoma and osteosarcoma, some of which had metastasized. Unfortunately, because they erroneously consider the nodule to be a granuloma, they make the statement that: "It appears that neoplasia thus is a sequel to development of the granulomatous lesion."

Based on our own unpublished histological observations on nodules in various stages of development, we have noted the following: Early nodules are characterized by necrosis of the muscular tunic of the oesophagus and by acute inflammation, with the exudation of large numbers of neutrophils and moderate amounts of protein. Sometimes numerous lymphocytes and plasma cells are also present. This exudate is gradually replaced by highly vascular granulation (repair) tissue, proliferating fibroblasts and scattered lymphocytes and plasma cells. Later still the nodule consists predominantly of fibroblasts, some of which have an embryonal appearance. Eventually, some of these fibroblasts may undergo metaplastic change to chondro- or osteoblasts or they may become neoplastic, giving rise to fibrosarcomas, osteosarcomas or anaplastic (undifferentiated) sarcomas. For most of their existence the nodules consist of proliferating fibroblasts for which we suggest the term 'fibroblastic nodule'.

The sensitivity of direct faecal examination, faecal flotation, modified centrifugal flotation and centrifugal sedimentation/flotation in the diagnosis of canine spirocercosis

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Spirocerca lupi (*S. lupi*) has an indirect life cycle, with the dog as the predominant definitive host. Canine spirocercosis can be subclinical or clinical. A variety of faecal examination methods have shown varying sensitivity in identifying larvated *S. lupi* eggs. The goal of this prospective study was to determine which faecal examination method, including a novel modified centrifugal flotation method, was most sensitive in the diagnosis of this disease

Faeces were collected from 33 dogs diagnosed with spirocercosis by oesophageal endoscopy at the Onderstepoort Veterinary Academic Hospital. Ten faecal examinations using 1g aliquots of faeces were performed for each dog. Four faecal examination methods were evaluated; direct faecal examination using saline, routine

faecal flotation, a modified faecal centrifugal flotation and a laboratory performed faecal sedimentation/flotation. The routine and modified centrifugal flotation methods were each performed using four faecal flotation solutions; NaNO₃ (SG 1.22), MgSO₄ (SG 1.29), ZnSO₄ (SG 1.30) and saturated sugar (SG 1.27). The sedimentation/ flotation method utilised MgSO₄ (SG 1.29). The modified centrifugal flotation method required centrifugation (1400G) of a prepared faecal solution (1g faeces mixed with 5mls of flotation solution) for 10 minutes after which 0.1ml of the supernatant was aspirated from the surface, using an adjustable volume micropipette, for microscopic examination. The 10 methods were statistically analysed using the Friedman test (non-parametric equivalent of analysis of variance) $p=0.000$, z value = 0.05.

The sensitivity ranged between 42% and 67%, with the NaNO₃ solution in both the routine and modified centrifugal flotation methods showing the highest sensitivity.

The modified NaNO₃ centrifugal method ranked first with the highest mean egg cell count (45.24 ± 83). The modified centrifugal NaNO₃ method was found to be superior (i.e. higher egg counts) and significantly different ($p<0.001$) compared to the routine saturated sugar, ZnSO₄ and MgSO₄ flotation methods. The routine NaNO₃ flotation method was found to be superior and significantly different ($p<0.001$) compared to the routine ZnSO₄ and MgSO₄ flotation methods.

The results show that faecal examination using a NaNO₃ solution is the most sensitive in the diagnosis of spirocercosis. The modified centrifugal flotation faecal method using this solution has the highest egg count. This method would be an effective simple screening test for dogs in *S. lupi* endemic areas.

"Spirocerca lupi (S. lupi) has an indirect life cycle, with the dog as the predominant definitive host..."

ALP as a possible screening test for neoplastic transformation in canine spirocercosis

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Spirocerca lupi is a nematode of canidae, which infests the oesophagus, forming a nodule that can undergo neoplastic transformation into a sarcoma. The diagnosis of malignant transformation is based on histopathology. Histopathology samples obtained by endoscopy, however, showed a high degree of false negative results, therefore, there is a need for a reliable and convenient way to predict neoplastic transformation. Alkaline phosphatase (ALP) has been found to be elevated in a variety of neoplastic conditions, especially appendicular osteosarcoma, where the bone-specific ALP isoenzyme is elevated. Certain malignancies in humans excrete a tumour derived ALP, identical to the placental ALP isoenzyme, that has been used for diagnostic and prognostic purposes. The purpose of this study was to determine if total serum ALP could be used as a screening tool for neoplastic transformation in spirocercosis.

Medical records of dogs diagnosed with spirocercosis at the University of Pretoria, from 1998 to 2008 were reviewed and 24 benign cases and 20 malignant cases were selected. Serum total ALP activity was determined on day of admission. Because the samples were collected in two time periods with two different normal ranges, the results for the ALP were analysed as a ratio between the case result and the mean

reference range. The mean ratio was compared between the malignant and benign groups using the T-test. Of the 20 malignant cases, 10 were osteosarcomas, 8 fibrosarcomas and 2 anaplastic sarcomas. There was a significant age difference between the two groups ($p=0.018$) with 4.5 ± 2.66 years in the benign and 6.2 ± 2.26 years in the malignant group. The mean ratio of ALP activity/reference range in the benign group was 0.51 ± 0.47 (0.1-1.7). The mean ratio of ALP activity/reference range for the malignant group was 1.01 ± 1.02 (0.13-3.99), significantly higher ($p=0.048$) compared to the benign group. There were no differences in serum ALP levels between the tumour types, however, the number of cases was too small for meaningful analysis. Despite being significantly higher in the malignant group, ALP activity is a poor indicator for neoplastic transformation in dogs with spirocercosis, as there was a marked overlap between benign and malignant cases. The mean ALP ratio was also not clinically different from the normal range in either group. Possible reasons for the wide range of ALP levels in malignant spirocercosis are the variety of sarcoma types and the fact that the osteosarcomas are also not appendicular. Further identification of serum ALP isoenzymes activity expressed in spirocercosis in its various stages is warranted.



Causes of gastrointestinal colic at a university referral hospital (1998 – 2007)

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The most common causes of gastrointestinal colic at an equine referral hospital in South Africa was determined following retrieval of the medical records of horses admitted during a 10 year study period.

Of the 1201 horses admitted with gastrointestinal colic, 1042 records were available and 107 (10%) cases were horses admitted more than once. The study included 935 horses of which 28% were admitted after-hours. Most horses were Thoroughbreds (54%), male (57%), with a median age of 8 years and from the Northern region (93%). Overall, an admission peak occurred during March (12%). Thirty-six percent of admissions were horses aged 5 to 10 years while 34% were horses < 5 years.

Heart rate (98%), mucous membrane colour (95%) and auscultation of the abdomen (91%) were the clinical data commonly obtained at admission. Packed cell volume, total serum protein and white cell count was recorded in 78%, 75% and 44% of horses respectively. Transrectal palpation (93%), nasogastric intubation (84%), intravenous catheterization (74%) and abdominocentesis (53%) were the most frequently performed procedures.

“Overall, medical intervention was successful in 93% of horses...”

Medical intervention was performed in 558 horses (60%). Overall the most common causes of medical colic were impactions (39%), tympany (7%) and anterior enteritis (6%). An exploratory laparotomy was performed in 331 horses (35%). A second laparotomy was performed on 10 horses of which 5 horses survived. Overall, the most common causes of surgical colic were impaction (22%), displacement (18%) and torsion (12%). Only 11 horses (3%) underwent surgical resection of which 4 horses survived to discharge.

Overall, medical intervention was successful in 93% of horses and 67% in horses managed surgically. Death occurred in 3% of horses, while euthanasia before medical intervention was performed in 4%. The median length of stay and cost of hospitalisation per day was 4 days and ZAR 716 respectively.

In conclusion, the majority of horses with gastrointestinal colic at a university referral hospital in South Africa responded to medical therapy. The recovery rate for horses receiving both medical and surgical intervention was good.



Small intestine entrapped within a mesenteric rent in an American Saddlebred horse



Incarcerated small intestine entrapped in the epiploic foramen in a Thoroughbred horse.

The effect of milk volume and group size on the growth and health of dairy calves

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Most dairy producers feed restricted quantities of milk to calves because of cost and the perception that increased milk intake may lead to a higher incidence of diarrhoea, and reduced intake of dry calf starter feed resulting in reduced weight gains. The aim of the study was to test the hypothesis that the feeding of high volumes of milk will increase growth rate and reduce cross-sucking behaviour in group fed calves without compromising their health.

One hundred and twenty 3-day old Jersey heifer calves in a seasonal calving herd were randomly assigned to one of four treatment groups of 30 calves each. Two groups received unrestricted (high) volumes of milk (HMV), while two groups received restricted volumes of milk (RMV) during the pre-weaning period. One each of the HMV and RMV groups was subdivided into two

smaller sub-groups of 15 calves. Calves were weaned at 42 days and were monitored for feed intake (liquid feed and calf starter), cross-sucking behaviour, average daily mass gain (ADG), and health until 60 days of age.

The HMV calves drank 72% more milk and gained 154 g/d more than the RMV calves ($p < 0.001$). Using multiple regression, adjusting for birth mass, birth date, dam parity and sire, ADG (d0-42 and d0-60) was higher in HMV than RMV calves ($p < 0.001$). After weaning growth rates showed no differences and on d 60 the HMV calves maintained an advantage in mean body weight. The mean intake of starter pellets was higher in the RMV groups than in the HMV groups. Overall feed conversion rate of HMV calves was 9.6% better than RMV calves. However, the cost per kg weight gain was 12% higher for HMV calves. There was no significant effect of group size on the growth rate.

In the RMV groups 75% of calves showed cross-sucking behaviour pre-weaning and 18% post-weaning, whereas in HMV calves the proportions were 2% and 7% respectively. There was no significant effect of milk volume on the incidence of diarrhoea, although smaller groups showed a significantly higher incidence of diarrhoea during the pre-weaning period. The occurrence of calf scours depends more on the load of pathogens in the environment and the degree of environmental stress on calves.

The faster pre-weaning growth rate of the HMV calves confirmed that the growth of milk-fed calves is proportional to the amount of milk provided. As might be expected the starter intake of the HMV calves was negatively influenced by greater milk intake. Although restricted milk feeding encourages earlier intake of starter feeds, which in turn promotes rumen development, smoother weaning transition and earlier weaning, results of this study demonstrated no difference in the smoothness of weaning between groups. There was also no evidence that reduced intake of solid feed of the HMV group before weaning led to lower intakes after weaning. The feeding of high volumes of milk to Jersey calves can therefore have a significant positive effect on growth rates, without compromising health or reducing the intake of solid food after weaning.

“Overall feed conversion rate of HMV calves was 9.6% better than RMV calves.”

The detection of *Babesia* spp. in domestic felids (*Felis domesticus*) using DNA probes and phylogenetic analysis.

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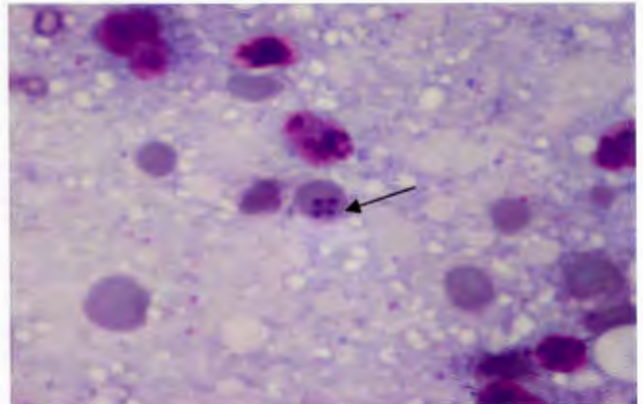
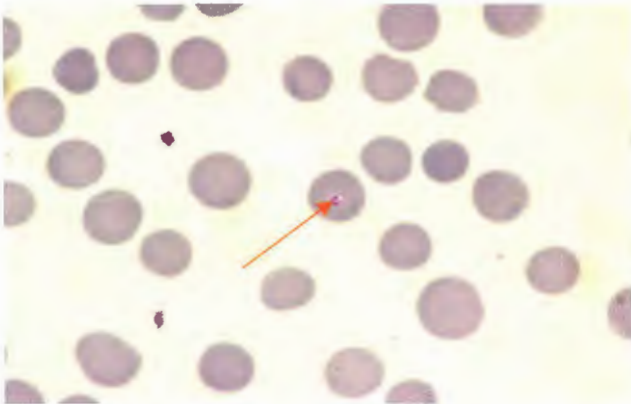
Babesia is an intracellular erythrocytic haemoprotozoan of mammals and it has also been reported in reptiles and birds. The two most frequently reported *Babesia* species in felids are *B. felis*, which causes clinical babesiosis in domestic cats, and *B. leo*, primarily reported from asymptomatic lions.

"*Babesia* is an intracellular erythrocytic haemoprotozoan of mammals..."

In this study, DNA was extracted from blood collected from 480 domestic cats (*Felis domesticus*) and the hypervariable region of the 18S rRNA gene was amplified. The PCR products were analysed using the Reverse Line Blot (RLB)

hybridization assay, a technique that simultaneously detects and differentiates between *Babesia* and *Theileria* spp. RLB probes to detect *B. felis*, *B. leo* and *Babesia* sp. (cheetah) were designed, using the 18S rRNA gene sequence data, and used to screen samples collected from domestic cats.

Results showed that *B. felis*, *B. leo* and *Babesia* sp. (cheetah) occur in domestic cats either as single or as mixed infections. However, some samples tested positive only with the genus-specific *Babesia/Theileria* probe. This suggested the presence of a novel species or variant of a species. The full-length 18S rRNA gene of these unknown samples was subsequently amplified, cloned and sequenced. Sequence and phylogenetic analysis confirmed that a novel *Babesia* spp. was present.



Molecular characterization of *Theileria* species of the African Buffalo (*Syncerus caffer*) by 18S rRNA gene sequence analysis

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The African buffalo (*Syncerus caffer*) is the natural reservoir host of both pathogenic and non-pathogenic *Theileria* species. Corridor disease, caused by *Theileria parva*, is a controlled disease in South Africa. *Theileria* parasites usually occur as mixed infections in infected animals, and although the non-pathogenic forms do not have any significant economic importance, their presence interferes with the diagnosis of *T. parva*. In this study, the phylogenetic relationship of pathogenic and non-pathogenic *Theileria* species obtained from buffalo blood samples originating from different geographical regions in South Africa were investigated using 18S rRNA gene sequences analysis.

DNA was extracted, the V4 hypervariable region of the 18S rRNA gene was amplified and subjected to the Reverse Line Blot (RLB) hybridization assay using *Babesia* and *Theileria* genus- and species-specific probes. Results of the RLB revealed the presence of the pathogenic *T. parva*, benign *T. mutans*, and the non-pathogenic *T. velifera*, *T. buffeli* and *Theileria* sp. (buffalo). In some samples, the PCR products hybridized only with the genus-specific probes, and not

with any of the species-specific probes, suggesting the presence of novel species or genotypes. The full length 18S rRNA gene of selected samples was amplified, cloned and the recombinants sequenced. Sequence and phylogenetic analyses indicated that novel *T. mutans*, *T. velifera* and *Theileria* sp. (buffalo) genotypes occur in buffalo. This could have serious implications, since such sequence variants could compromise the specificity of the real-time PCR test currently used to detect *T. parva* infections in buffalo and cattle in South Africa.

(Acknowledgements: This research was supported by the SA National Research Foundation and UP Research Development Programme. We thank Dr Roy Bengis, Dr Dave Cooper and Dr Fred Potgieter for providing buffalo blood samples.)



Immune status assessment in lions (*Panthera leo*) during *Mycobacterium bovis* infection: development of a real-time qPCR for lion IFN- γ , TNF- α , IL-4 and IL-10

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Mycobacterium bovis (*M. bovis*) infection rapidly spreads amongst the lion population in the Kruger National Park (KNP). Since the infection ultimately results in severe clinical signs of tuberculosis, concern is raised about the future of these animals, a main tourist attraction of the park. It is generally agreed that Th1 type cell-mediated immunity is of major importance in controlling the infection. Assessment of cytokine expression along with other immune response parameters, will offer insight in (changes in) immune responsiveness during progression of *M. bovis* infection in lions. For this purpose, four cytokines were chosen: TNF- α and IFN- γ considered to be (pro-)inflammatory, and IL-4 and IL-10 with anti-inflammatory characteristics.



Reverse transcriptase Real-Time PCRs based on primers and probe sequences described in literature for cat cytokine genes and the housekeeping gene GAPDH were adopted and showed to replicate lion cytokine cDNA i.e. to detect cytokine expression at mRNA level in peripheral blood mononuclear cells (PBMC). A total of 30 PBMC samples were obtained from lions in the KNP. Twenty-two animals showed a positive PPD skin test, indicating infection with *M. bovis*, whereas eight animals tested negative.

PCR results were recorded as Ct values. In case no signal was observed after 40 replication cycles Ct was assumed to be 40. Test samples were normalized to GAPDH control samples and the Δ Ct was calculated: Δ Ct = GAPDH Ct - target Ct. To calculate the difference between the skin test positive group and the skin test negative group ($\Delta\Delta$ Ct), the mean was taken of all Δ Ct's of the different groups: $\Delta\Delta$ Ct = Δ Ct mean negatives - Δ Ct mean positives. Finally, the cytokine expression in test samples was expressed as n-fold difference relative to the calibrator (i.e. the negative skin test group): amplification rate (AR) ^{$\Delta\Delta$ Ct}.

Comparison between skin test positive lions and skin test negative lions, showed a 3-fold increase in IFN- γ gene expression in the *M. bovis* infected lions. TNF- α was

expressed 2-fold higher in *M. bovis* positive animals as compared to negative animals. The difference in IL-10 expression is minimal. Expression of IL-4 was almost 4-fold lower in *M. bovis* positive animals than in *M. bovis* negative animals. In general a bias towards an inflammatory Th1 type immune responsiveness was noted. However, results

presented should be considered as very preliminary since for 50% of the samples of the negative skin test lions Ct values could not be determined (i.e. were assumed to be 40); sample collection circumstances were suboptimal, and PCR efficiencies were not optimal for all cytokines.

Availability of reverse transcriptase Real-Time PCRs for lion cytokines IFN- γ , TNF- α ,

IL-4, and IL-10 constitutes a new possibility to define the *M. bovis* infection status in lions. They may give insight in the contribution of Th1 and Th2 responses to protective immunity and pathology in the course of *M. bovis* infection, might aid in the assessment of potential effective vaccines for lions and contribute to development of new diagnostic methods for *M. bovis* infection in lions.

"Since the infection ultimately results in severe clinical signs of tuberculosis, concern is raised about the future of these animals..."

Biodegradable microspheres as a single dose delivery system for *Ehrlichia ruminantium* vaccines

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Four 1H12 *E. ruminantium* open reading frames used as a recombinant DNA vaccine against heartwater protected 100% of sheep (using a cocktail for the individual ORFs) in a laboratory needle challenge, while 20% of sheep were protected after a natural tick challenge. The lack of protection under natural field conditions could be attributed to the delivery strategy used and therefore there is a need to investigate other delivery methods. Polymeric nanoparticles based on PLGA polymers have been used extensively to target the delivery of vaccine to antigen presenting cells, play a role in the induction of cellular immunity and can be used as single dose vaccine mimicking prime/boost vaccination.

In this study the four *E. ruminantium* ORFs and their respective recombinant proteins were either encapsulated into or adsorbed onto nanoparticles using a modified double emulsion solvent evaporation technique. The particles were formulated to release DNA on day 0 and day 21 and recombinant proteins on day 42, thus mimicking 3 x DNA prime/recombinant protein-boost immunization strategy. Encapsulation did not have any detrimental effects on

the stability of the recombinant proteins as determined by gel electrophoresis and western blotting. The *in vitro* incubation of nanoparticles in either the Float-A-Lyzer dialyzer or the eppendorf tube showed the potential use of nanoparticles as a vaccine due to their release profiles that mimic a heterologous prime/boost immunization strategy. Nanoparticles formulated using polymers with low glycolide ratios released 80% of the encapsulated proteins within the first week of *in vitro* incubation with most of the proteins being released on day 1. Nanoparticles formulated using polymers with 50:50 monomer ratios released the recombinant proteins during week 1 and 3 of *in vitro* incubation. These nanoparticles did not release any protein in week 2 (day 7-14). Nanoparticles with 0.5% CTAB on their surfaces adsorbed DNA and released more than 40% of DNA on day 1 with 100% release by day 14. RG502H nanoparticles formed with PVA as the internal phase viscosity enhancer released intact DNA only from day 12 to day 21. A cocktail of these nanoparticles could therefore be used as an auto-booster vaccine thus reducing the need for repeated immunizations needed to obtain protective immunity.

"The lack of protection under natural field conditions could be attributed to the delivery strategy used..."



Immunohistochemical study to determine the distribution of lentogenic vaccine and virulent NDV in the reproductive tract of laying hens

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Newcastle disease (ND) is a devastating disease of poultry which is reportable to the World Animal Health Organization (OIE). Since 2002, the South African poultry industry has experienced outbreaks of ND caused by a recently introduced NDV strain from the Far East. These viruses belong to lineage 5d/VIId of NDV and are locally known as “goose paramyxovirus” (GPMV). This strain of virus has proved to be more persistent, causing disease even in waterfowl which other strains were not known to do. Control of the disease has proved difficult with commercially available vaccines appearing not to be fully effective as declines in egg production are observed in vaccinated pullets. This has led to concerns being raised about the efficacy of commercially available ND vaccines against this strain. This study was conducted to determine the pattern of NDV viral antigen distribution in the oviduct of laying hens by immunohistochemistry (IHC). This study also compared the efficacy of cloacal and ocular routes of vaccination against challenge, following reports that cloacal vaccination offered a better protection against egg production losses than the oronasal route of vaccination.

Groups of specific pathogen-free ($n = 40$) and commercial ($n = 40$) hens in lay were vaccinated with a field dose of La Sota vaccine ($10^{6.0}$ EID₅₀) and challenged 12 days later, together with 10 unvaccinated SPF birds with a virulent NDV strain (GPMV; Genbank Reference # FJ985978) at a dose of $10^{7.0}$ EID₅₀/0.1ml/bird via eye-drop. Hens were fed and watered *ad libitum*, monitored and euthanased at intervals of 2, 4, 6, 8 and 10 days post-vaccination and post-challenge. Birds were

necropsied and the oviducts sampled, processed and stained with H & E and immunohistochemistry using a monoclonal antibody to NDV (Anti-NDV P pure ascites 10-5E6, 1985) directed against the Phosphoprotein (P) genome.

No clear difference could be demonstrated in the protection of the oviduct from challenge with GPMV by either the cloacal or ocular routes of vaccination. The vaccine gave 100% protection from clinical disease against the challenge virus but not against infection and replication of the virus, as birds showed varying degrees of macropathology and numerous stained viral antigens were demonstrated by IHC in the oviducts. The susceptibility and colonisation of the oviduct of laying hens by both the lentogenic La Sota and the virulent NDV isolates was confirmed, with the uterus being more susceptible than the magnum and the isthmus. Positive NDV-specific staining was demonstrated in the cytoplasm of the epithelial cells, subepithelial cells and different mononuclear cells in the interstitium of the various sections of the oviduct as target cells. Necrosis and apoptosis of cells of the oviduct were not detected but cellular infiltration, gland dilatation and interstitial oedema were observed. Both vaccination and challenge affected egg production and egg quality in this trial. The study concluded that other causes such as stress-induced reproductive hormonal changes and biochemical changes in affected cells and organs, in addition to the structural damage reported by other workers, may be responsible for the drop in production as well as the production of poor quality eggs encountered in this study.

Ultrastructural features of the microvasculature and lymphatics of the ostrich (*Struthio camelus*) epididymis

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Previous studies on the ostrich epididymis have shown that spermatozoa are concentrated in the connecting ducts and epididymal ducts, implying the removal of fluid from the rete testis and efferent ducts. As no information is available on the mechanism of fluid absorption in this species, this study describes the ultrastructure of the epididymal microvasculature and lymphatics in an attempt to determine the possible role of these structures in this process.

Samples of the testis and epididymis were obtained from six sexually active, mature male ostriches slaughtered at a commercial abattoir and routinely processed for transmission electron microscopy. The ultrastructural features of blood and lymphatic vessels located in the epididymal stroma were described and digitally recorded.

The parenchyma of the epididymis displayed numerous capillaries, some arterioles and venules as well as some larger vessels. Some capillary profiles revealed an attenuated endothelium, some were thick-walled, while others displayed both thick and thin regions. In some profiles the endothelium appeared continuous while in others numerous fenestrations were observed, particularly in capillaries close to the efferent ducts. Numerous cytoplasmic processes projected from

the endothelium into the lumen and large micropinocytotic vesicles were present in some profiles. Adjacent endothelial cells were connected by slender cytoplasmic processes displaying *zonulae adherents*. Capillaries were surrounded by a prominent basal lamina and occasionally by pericytes and fibroblasts.

Occasional lymphatic capillaries with an attenuated endothelium were present in the epididymal stroma, either adjacent to blood capillaries or isolated within the stroma. The presence of micropinocytotic vesicles was variable, but when present these structures were smaller than those observed in vascular capillaries. Attenuated regions of the endothelium occasionally displayed fenestrations fitted with a membranous diaphragm.

The morphological characteristics of the vascular capillaries (fenestrations, stretches of attenuated endothelium and micropinocytotic vesicles) in the region of the efferent ducts of the ostrich epididymis are consistent with fluid absorption. Lymphatic capillaries apparently perform a similar function. This finding compliments the observation that the epithelial lining of the proximal efferent ducts in this species is adapted for fluid absorption.



Growth patterns of the giraffe vertebral column

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Although the extraordinary shape of the giraffe skeleton has fascinated researchers for years, published data on its morphology are scarce, and of these, data tend to be inconsistent and sample sizes small. Giraffe height has been attributed to neck and limb elongation with the rest of the skeleton fairly similar to other large ruminants. Because height is the main feature of giraffe we have investigated the way in which height develops from birth to maturity, and if there are any differences relating to gender. The present study focused on the vertebral column.

Proportions of body parts tend to change as an animal grows. This is referred to as allometric growth (as opposed to isometric growth).

Allometric growth can generally be described through power law equations of the type $y = bx^k$. Where the exponent k is larger than 1, the growth is positively allometric and yields a concave curve; where it is smaller than 1 growth is negatively allometric and yields a convex curve. In our study we derived allometric equations by measuring body mass (kg) and vertebral body length (mm) (and the longest part of the ventral arch of C1) of each vertebra in 25 males and 23 females ranging from foetuses to old mature animals. The data were log transformed in order to perform a linear regression analysis. From these analyses, we obtained predictive allometric equations for each vertebra ($y = bx^k$). In turn, this was used to predict vertebral body length at 100 kg intervals for male and female giraffes.

In both males and females for every vertebra plotted against body weight, we obtained a convex curve (thus without exception the vertebrae displayed negative allometric growth). The mean differences between males and females were found to be small. For example, differences in the cervical vertebrae (mm) are shown in the table below:

Differences were evaluated using the F test and we concluded that there were no significant differences between genders: vertebral body length male = vertebral body length female in same mass individuals ($P > 0.5$) for any of the vertebrae



measured. An analysis of covariance was also performed with \ln (vertebral length) as response variable, sex as classification variable and \ln (mass) as covariate. From this analysis we could only find differences between T3, T12, T14, L2 ($P < 0.05$). However these differences were scattered at random intervals within the vertebral column (cervical to lumbar) and, as none of them were situated in a significant area, such as neck or first thoracic vertebra, they were assumed to be chance occurrences.

This study confirms our previous study on giraffe external morphology (Mitchell *et al*, 2009) that showed no significant differences in growth pattern between male and female same mass giraffes. Consequently we have concluded that the selective pressures that resulted in the evolution of their shape are not gender specific. A practical outcome is that in terms of vertebral length, male and female data can be pooled to obtain a bigger sample size, a valuable tool in allometric studies as giraffe specimens can be difficult to obtain. Future research should expand the data analysis to long bones and other species.

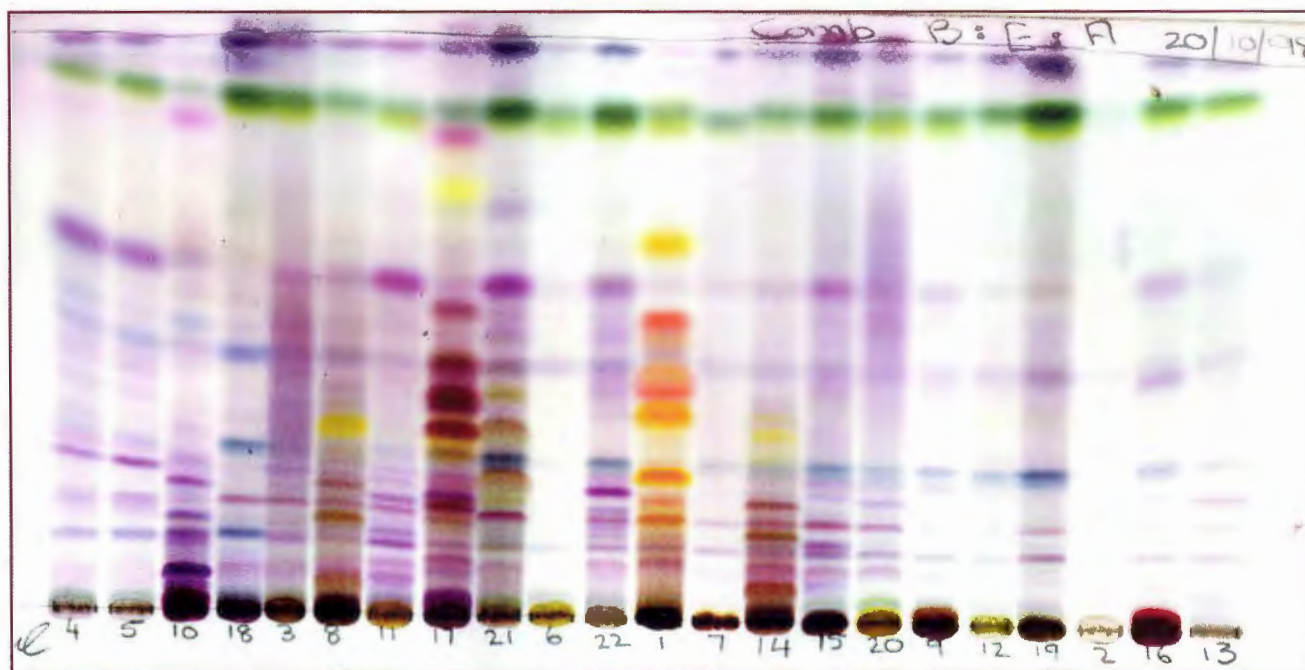
Reference: Mitchell G, van Sittert SJ, Skinner JD. Sexual selection is not the origin of a long neck in giraffes. J Zool (Lond), in press, 2009.

Vertebra	C1	C2	C3	C4	C5	C6	C7
mean difference (M -F, mm)	-0.4	-2.3	-0.6	0.2	-1.2	-0.8	-7.0
standard deviation	0.21	0.48	3.29	3.87	2.70	3.17	4.00

Thin layer chromatographic screening of ethanolic crude extracts of six indigenous South African Plants for acetylcholinesterase inhibitory activity.

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Alzheimer's disease is a chronic neurodegenerative disorder characterized by loss of memory, behavioural abnormalities and death. It affects individuals older than sixty-five years of age. The prevalence of this disease is expected to reach 11-16 million people by 2050. Most of the drugs currently in use are alkaloids with acetylcholinesterase inhibitory activity. These drugs have side-effects, such as gastrointestinal disturbances, muscle cramps and hepatotoxicity. In view of these, ethanolic extracts of six indigenous South African plants (*Eythrina latissima*, *E. lysistemom*, *E. caffra*, *E. humeana*, *E. abyssinica* (Fabaceae) and *Ammocharis coranica* (Amaryllidaceae), with psychoactive properties

used in traditional medicine to treat mental illness, were tested for acetylcholinesterase inhibitory activity. The thin layer chromatographic plate was developed and stained with Ellman's reagent, 5,5'-dithiobis-(2-nitrobenzoic acid) to detect inhibitory activity qualitatively. The extracts of leaves and seed of none of the *Eythrina* species inhibited the enzyme at concentrations as high as 250 µg/ml. The crude extract of *Ammocharis coranica* inhibited acetylcholinesterase at 25 µg/ml.

There were no false positives. Further work is in progress to determine the inhibitory concentration 50 (IC₅₀) of the crude extracts of *Ammocharis coranica*, and to isolate and characterize the active constituents.

"The prevalence of this disease is expected to reach 11-16 million people by 2050."

Comparative antibacterial activity of five *Ochna* species

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The acetone leaf extracts of *Ochna natalitia*, *Ochna pretoriensis*, *Ochna pulchra*, *Ochna gamostigmata*, and *Ochna serullata*, were quantitatively and qualitatively investigated for antibacterial activity using the serial microplate dilution assay and bioautography respectively against *Staphylococcus aureus*, *Escherichia coli*, *Enterococcus faecalis* and *Pseudomonas aeruginosa*. The percentage yields of the extracts were: *O. gamostigmata* (8%), *O. pulchra* (7.5%), *O. serullata* (7%), *O. pretoriensis* (6%) and *O. natalitia* (2.5%). The MIC values of the five plants ranged from 0.039 mg/ml to 1.25 mg/ml. Bioautogram

inspection showed five active bands against *S. aureus*. *E. coli* was sensitive to all the extracts. *O. pretoriensis* was the most active with MIC values of 0.065 mg/ml and 0.039 mg/ml against *E. coli* and *E. faecalis* respectively; it also had the highest total activity. Bioautographic fingerprint showed that the two most active species *O. pretoriensis* and *O. pulchra* contain similar antibacterial compounds. TLC pattern and reaction to phytochemical spray reagents suggest that the active compounds in the acetone extracts of these plants are mainly flavonoids.



Antibacterial activity of nine South African medicinal plants

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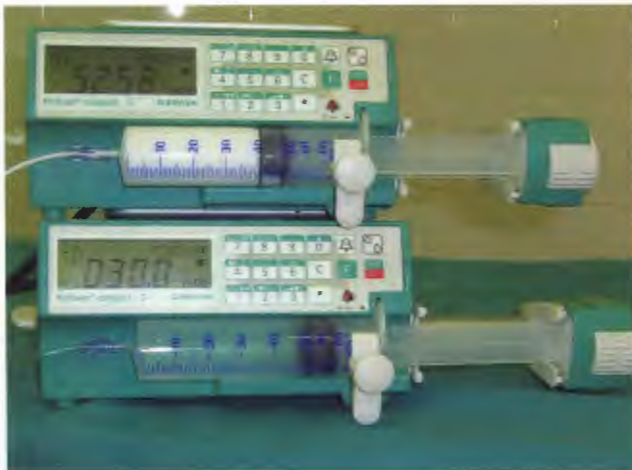
An estimated 27 million South Africans depend on traditional medicine for their primary health care needs. Some of the threatened South African indigenous plant species may become extinct before investigated for their biological activity. Acetone extracts on leaves of nine plant species (*Acalypha sonderana*, *Androstachys jonsonnii*, *Draceana mannii*, *Drypetes natalensis*, *Funtumia africana*, *Necepsia casteneifolia*, *Oncinotus tenuiloba*, *Turrea floribunda* and

Xylia torreana) were investigated against four bacterial strains (*Staphylococcus aureus*, *Enterococcus faecalis*, *Escherichia coli* and *Pseudomonas aureginosa*) using a microdilution method and direct bioautography. *F. africana* and *O. tenuiloba* showed the best activity against the four bacteria with minimal inhibitory concentration as low as 0.08mg/ml against *S. aureus*. Further work is in progress to isolate and characterize the bioactive compounds of *Funtumia africana*.

Total Intravenous Anaesthesia (TIVA) with propofol-fentanyl and propofol-midazolam combinations in spontaneously-breathing, oxygen-supplemented goats

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Total intravenous anaesthesia techniques have developed rapidly over the last few years in human, canine and equine anaesthesia, but little has been documented about these techniques in goats. The potential of combinations of propofol with either fentanyl or midazolam for total intravenous anaesthesia in goats were evaluated.

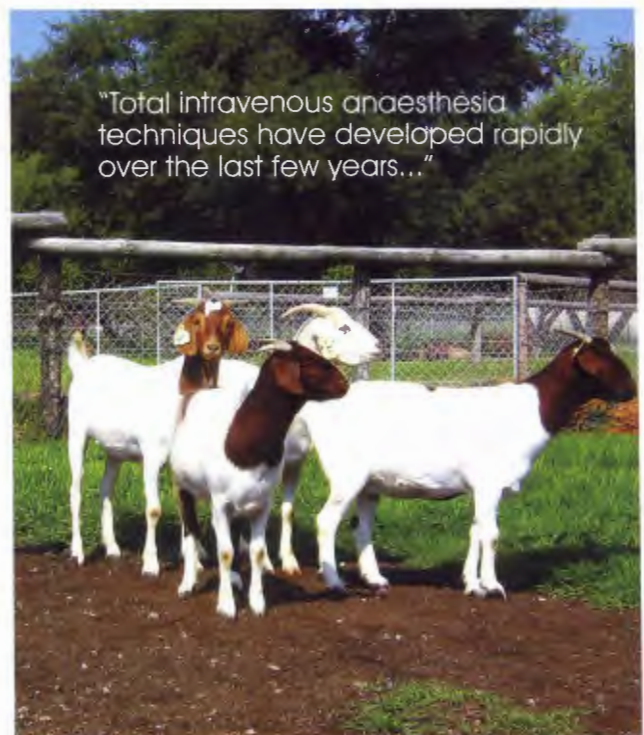
In a prospective, randomized, crossover experimental study, six, mature Boer - Indigenous African crossbred goats, 3 castrated males and 3 females were assigned to two groups. General anaesthesia was induced with fentanyl at 0.02 mg/kg (Group PF), or midazolam at 0.3 mg/kg (Group PM) followed a minute later by propofol at about 4 mg/kg titrated to effect, all administered intravenously. After placement of an endotracheal tube, general anaesthesia was maintained by constant rate infusion (CRI) of propofol 0.2 mg/kg/min combined with fentanyl 0.02 mg/kg/hr (Group PF) or midazolam 0.3 mg/kg/hr (Group PM) for 90 minutes, while providing oxygen at 2 L min⁻¹ and allowing the goats to breath spontaneously. Depth of anaesthesia was assessed every 10 minutes and the propofol CRI dosage adjusted accordingly, while keeping the fentanyl or midazolam CRI dosage constant. Cardiopulmonary parameters were monitored throughout the anaesthetic period. Arterial blood-gas analysis was regularly performed. Quality of recovery was scored.

The Wilcoxon signed-ranked test was used to test for differences in dosages of propofol required for induction and maintenance of anaesthesia, while the analysis of variance (ANOVA) for repeated measures followed by a Tukey test for

multiple comparison of means was used to test for differences between cardiopulmonary parameters and blood-gas analysis data ($p < 0.05$).

The dose of propofol required for induction was exactly 4.0 mg/kg for both groups. The quality of induction in both groups was smooth without any signs of excitement. The CRI dose required for maintenance was statistically significantly higher ($p = 0.004$) in Group MP (0.3 ± 0.1 mg/kg/min) than in Group FP (0.2 ± 0.0 mg/kg/min). Cardiovascular and respiratory function were minimally affected although lower respiratory rates were observed in both groups, were more pronounced in Group FP. Quality of recovery was good for Group MP, but was marginally poorer for Group FP.

Total intravenous anaesthesia (TIVA) achieved by co-administration of propofol and either fentanyl or midazolam for induction and maintenance of anaesthesia in spontaneously-breathing, oxygen-supplemented goats is satisfactory, although caution must be exercised with the fentanyl-propofol combination as recovery from anaesthesia might be rough.



The spirocercosis-induced oesophageal nodule: progression from inflammation to sarcoma

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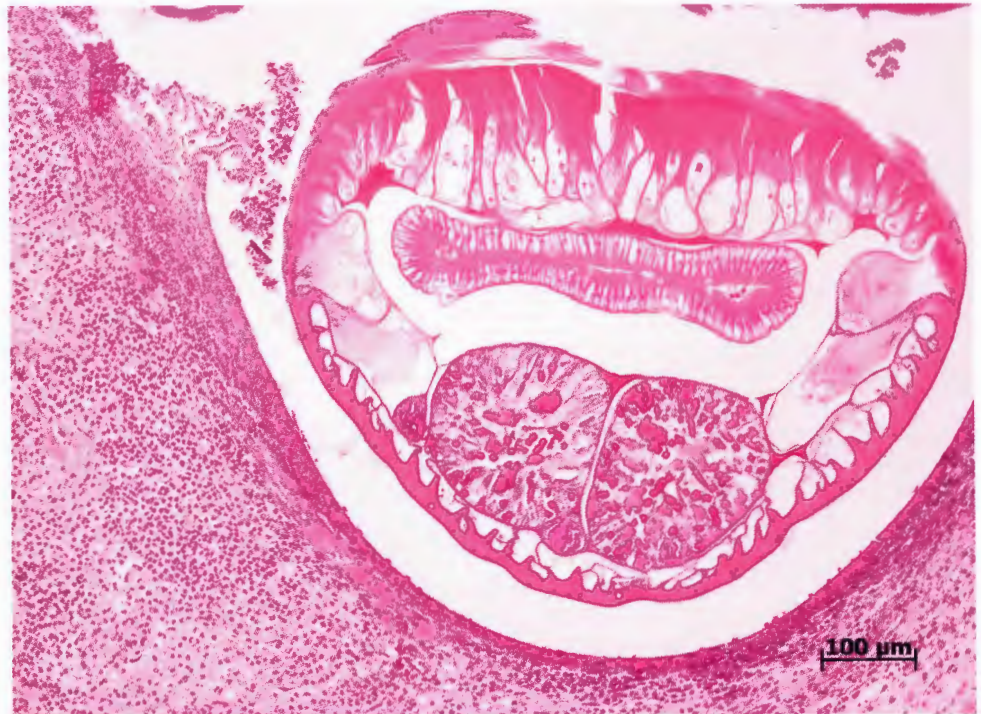
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Spirocerca lupi is a nematode that infects the oesophagus of dogs, where it forms a nodule that may undergo malignant transformation. This study aims to outline the histological progression of the nodule from infection to sarcoma.

Sixty two spirocercosis-induced nodules, 42 benign and 20 malignant, were stained with HE. Ten non-overlapping high power fields were examined per nodule. Chi-square and T tests were used to compare categorical and continuous parameters, respectively. Inflammation was scored 0-3 (0 = absent or scant, 1 = less than fibrocytes, 2 = roughly equal to the fibrocytes, 3 = predominant) and revealed a score of 1.91 ± 0.52 in the benign and 0.97 ± 0.5 in the malignant cases ($p < 0.01$).

In 40% of benign cases the inflammatory infiltrate was lymphoplasmacytic, in 24% of cases lymphocytes and neutrophils were mixed, and in 21% of cases, neutrophils predominated, compared to 25%, 5% and 70%, respectively in the malignant cases ($p = 0.02$).

Necrosis was scored 0-3 (0 = no, 1 = small areas, or scant single cell necrosis, 2 = obvious but $< 50\%$, 3 = $> 50\%$) and revealed a score of 0.88 ± 0.41 in the benign and 1.47 ± 0.5 in the malignant cases ($p < 0.01$). The average mitotic index per field was 1.31 ± 1.55 in the benign compared to 42.85 ± 30.79 in the malignant cases ($p < 0.01$). The average number of multinucleated giant cells per field was 0.9 ± 1.45 in the benign compared to 13.9 ± 14.66 in the malignant cases ($p < 0.01$). In the malignant cases, purulent inflammation seemed to be particularly associated with ulceration and necrosis. These differences warrant further investigation, especially the role of the lymphoplasmacytic infiltrate in tumor progression.

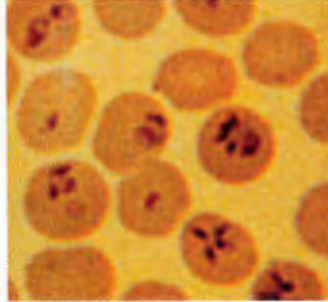
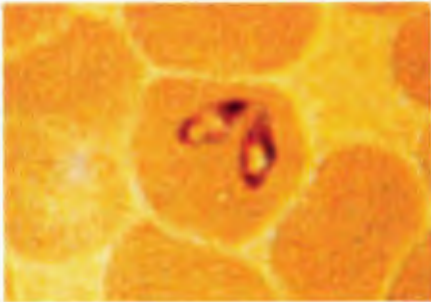


In the benign cases, collagen and immature fibroblasts were scored (collagen: 0 = no or normal amount, 1 = less than cells, 2 = roughly equal to cells, 3 = more than cells, immature fibroblasts: 0 = no or scant, 1 = $< 50\%$ of fibrocytes, 2 = roughly equal to fibrocytes, 3 = $> 50\%$ of fibrocytes). If the fibroblasts looked activated (excessively plump euchromatic nuclei with multiple/prominent nucleoli), the intensity of activated fibroblasts was scored using the same scheme. A combined score of fibroblasts plus activation score minus collagen was calculated. The benign cases were divided into cases with a combined score of ≤ 1 ($n = 15$) or > 1 ($n = 27$), respectively. These 2 groups were significantly different ($p < 0.01$) in their collagen (2.66 ± 0.52 vs. 1.51 ± 0.36), fibroblasts (1.48 ± 0.67 vs. 2.52 ± 0.35) and activity scores (0.45 ± 0.6 vs. 1.02 ± 0.41). The 2 groups had similar scores for inflammation and necrosis, but were significantly different ($p < 0.01$) in mitotic index (0.26 ± 0.46 vs. 1.89 ± 1.65) and number of multinucleated cells (0 vs. 1.4 ± 1.6). These results indicate 2 stages in benign nodules: Early inflammation, characterized by fibrocytes and abundant collagen, and a pre-neoplastic stage, characterized by activated fibroblasts and reduced collagen.

Investigating the Rhopty Associated Protein-1 (RAP-1) gene of *Babesia caballi*

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Babesia caballi is a tick-borne haemoprotozoan parasite, and is one of the causes of equine piroplasmiasis. Infections caused by *B. caballi*, which are characterized by fever and anemia, are considered less severe than *T. equi* infections, which are the more consistent cause of haemoglobinuria and death. These apicomplexan parasites secrete proteins from their apical organelles, which are thought to play pivotal roles in attachment, invasion, expansion and maintenance within the host cell. Among these proteins secreted by *B. caballi* is the rhopty-associated protein-1 (RAP-1), which contains several immunogenic epitopes, and antibodies directed against these proteins have been shown to inhibit merozoite invasion. A monoclonal antibody to the recombinant RAP-1 gene has thus been used in the development of a cELISA for the detection of *B. caballi* antibodies in infected horses.

The cELISA developed for the detection of equine antibodies specific for *B. caballi* was tested on 107 South African equine field samples. None of these samples tested positive using the cELISA, although ten samples tested positive for *B. caballi* antibodies using the indirect fluorescent antibody test (IFAT). We therefore characterized the *B. caballi* rhopty associated protein (RAP-1) gene, which codes for the antigen used in the cELISA assay, by designing three sets of primers to amplify the

complete gene (~1800bp). However, only one set of primers yielded PCR products and we were able to amplify a region at the 5' end of the gene (615 bp) from ten South African *B. caballi* *in vitro*-cultured samples. We obtained sequence data from seven of these. BLASTN analysis revealed that the sequences showed between 79 and 81% identity to published *B. caballi* RAP-1 sequences.

The GenomeWalker Universal kit (Clonetech) was used to amplify the region flanking the 615 bp *B. caballi* RAP-1 fragment. Amplified products were cloned into the pGEM-T Easy vector and sequenced. The complete *B. caballi* RAP-1 gene sequence, comprising a single open reading frame of 1479 bp that encodes a protein of 493 amino acids, was obtained from two samples. This sequence data was used to design amplification primers and RAP-1 sequences were obtained from a further eight South African isolates. BLASTP analysis indicated 65% amino acid identity to published RAP-1 protein sequences, with most differences occurring at the C-terminal end of the sequence.

The monoclonal antibody used in the *B. caballi*-specific cELISA has been shown to bind to a peptide epitope within the C-terminal repeat region of the *B. caballi* RAP-1 amino acid sequence. Close inspection of the C-terminal region of the RAP-1 amino acid sequences obtained from the South African *B. caballi* isolates, clearly indicates the absence of this repeat region and thus the absence of the monoclonal antibody binding site. This observation probably explains the failure of the cELISA to detect antibody to *B. caballi* in sera of infected horses in South Africa. Redesigning the current cELISA assay using a more conserved protein as the target antigen may overcome this problem.

Serial daily adrenal function in critically ill puppies with parvoviral diarrhoea

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The adrenal response to critical illness and its role in prognostication is an important issue in human medicine. A positive association between high basal serum cortisol and adverse outcome has been demonstrated in human and canine illness. Although delta cortisol is the preferred parameter used in human sepsis, the association of delta cortisol and serum ACTH-stimulated cortisol concentrations with outcome in canine critical illness is less clear.

This prospective, longitudinal, case-controlled study was conducted on sixty-three puppies with parvoviral diarrhoea. The diagnosis was confirmed by detection of viral particles on faecal electron microscopy. Seventeen healthy puppies were used as controls. Blood samples were obtained in each dog at admission prior to treatment and daily thereafter until death or discharge from the hospital. Immediately after the basal samples were drawn, each dog was injected daily with 5 ug/kg of ACTH (tetracosactrin) intravenously. A second sample was taken 1 hour later for serum ACTH-stimulated cortisol measurement and the calculation of delta cortisol. Cortisol concentrations were determined by a commercial canine radioimmunoassay kit (Coat-a-count®, DPC, CA). Dogs were retrospectively assigned to two groups: survivors (n=50) and

non-survivors (n=13). Hormone concentrations between the survivors and non-survivors were compared with the Mann Whitney U test for non-parametric data. Significance was set at $p < 0.05$.

Median day 1 (D1) basal cortisol and ACTH-stimulated cortisol was significantly higher in patients than in controls (259 vs. 77 nmol/L) and (393 vs. 295 nmol/L); $P < 0.01$ for both. Median delta cortisol was lower in patients (68 vs. 203 nmol/L). In nonsurvivors vs. survivors, D1 basal cortisol was significantly higher (539 vs 234 nmol/L), $P < 0.05$, but ACTH-stimulated cortisol did not differ significantly (459 vs. 380 nmol/L); $P = 0.2$; therefore delta cortisol was significantly lower in nonsurvivors than in survivors (-60 vs 128 nmol/L); $P < 0.05$. On day 3 (D3), basal cortisol was still higher in non-survivors, but not significantly so (189 vs. 68 nmol/L); $P = 0.18$, yet ACTH-stimulated cortisol was significantly higher in nonsurvivors (417 vs. 345 nmol/L); $P < 0.05$. In contrast to D1, delta cortisol was not significantly lower in nonsurvivors on D3 (179 vs. 256 nmol/L); $P = 0.2$.

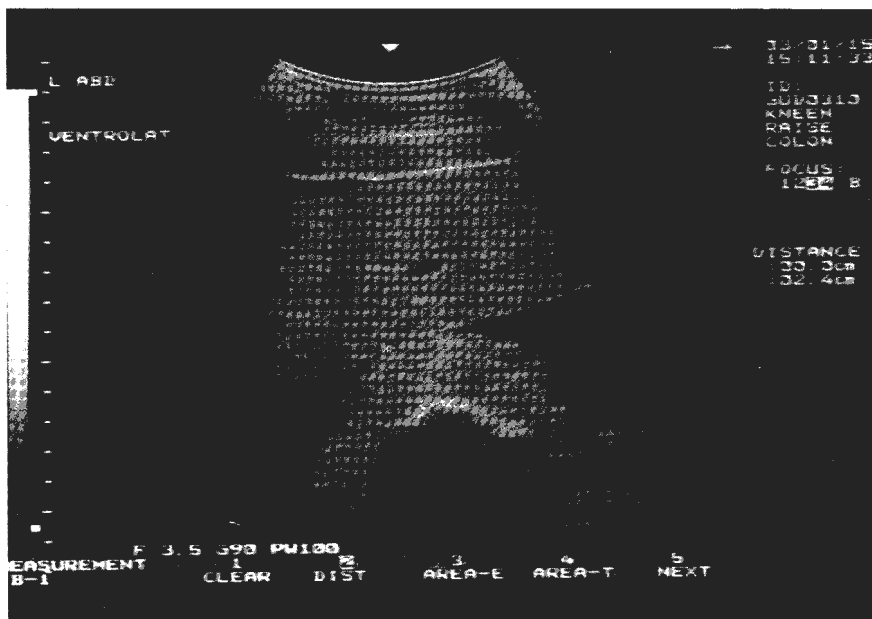
"This study confirmed the previously described association between high basal serum cortisol concentrations and mortality in parvoviral diarrhoea dogs."

This study confirmed the previously described association between high basal serum cortisol concentrations and mortality in parvoviral diarrhoea dogs. Low delta cortisol was also associated with mortality on D1. However, the D3 delta cortisol was not significantly lower in nonsurvivors compared to survivors. This study highlights the important limitation of designating a patient as adrenal insufficient on the basis of a one-off ACTH stimulation test in the early stages of acute illness. During this stage basal cortisol production is close to maximum, with a resultant low delta cortisol value, without necessarily indicating adrenal insufficiency. The longitudinal nature and daily serial sampling in this study provided novel insights into the adrenal response and re-enforces the need to consider basal-, delta- and ACTH-stimulated cortisol in the evaluation of adrenal reserve in canine critical illness. This study is the first to demonstrate serious shortcomings of the much-touted delta cortisol parameter and calls for ways of accessing glucocorticoids function at the receptor-, rather than serum level.

Clinical use and findings of after-hours diagnostic imaging evaluation: A retrospective study (1998-2007).

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Medical records of after-hour equine admissions were reviewed and horses that received diagnostic imaging assessment were identified. Data extracted included diagnostic imaging technique used, anatomical region imaged, body system affected, diagnosis based on imaging modality and final diagnosis. Ultrasonography and radiography was individually classified. Ultrasonographic small and large intestinal findings were recorded including wall thickness, motility and diameter. Admissions were classified according to the most significantly affected body system.

Eight hundred and twenty records were available of which 15% had diagnostic imaging performed, of which 88% was ultrasonography and 12% radiography. The gastrointestinal tract was the most commonly affected (67%), while abdominal ultrasonography was most commonly performed (62%). Admissions in which gastrointestinal tract ultrasonography was performed, most commonly presented with large intestinal related problems (64%) and 36% with small intestinal problems. Small and large intestinal

motility was recorded in 31% and 18% of admissions respectively, while small and large intestinal wall thickness was recorded in 35% and 19% of admissions. Small and large intestinal wall thickness abnormalities were recorded in 16% and 5% of admissions respectively, while motility abnormalities were recorded in 19% and 9% of admissions. An increase in small intestinal diameter was recorded in 26% of admissions. In 58% of admissions on which abdominal ultrasonography was performed, a diagnosis based on the findings of the imaging technique used, was reported by the after-hours clinician on duty.

Radiography was performed in 13% of admissions and was mostly taken for suspected fracture confirmation (67%). Ultrasonographic findings and abnormalities were recorded in 40% of admissions and in 75% of admissions which had undergone radiographic evaluation.

“Ultrasonography was the most commonly performed after-hour diagnostic imaging modality,”

Ultrasonography was the most commonly performed after-hour diagnostic imaging modality, however, a lack of complete reporting of abnormalities made accurate interpretation of admissions difficult. Accurate and systematic evaluation and record keeping, together with appropriate training of after-hours clinicians to gain essential experience and knowledge in the field of diagnostic imaging, will lead to improved utilisation of these modalities after-hours.

DNA extraction from horse bone samples for routine parentage testing

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Genotyping of animals for parentage verification and registration has become routine practice and many species, particularly horses, are genotyped from various samples including hair or blood samples. Cases do occur when an animal needs to be genotyped after it has died. This is usually necessary when a parent animal has not been DNA profiled and offspring of this animal are submitted for parentage verification for the purpose of registration. Bone is often the only remaining tissue. DNA can be found in bone, preserved and bound to inorganic crystals known as hydroxyapatite [HAp, $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$]. The electrostatic charges between the HAp and DNA are strong, making it difficult to release DNA from the bone. In addition, bone samples are often very old and have been exposed to fluctuating temperatures and soil contaminants, which all decrease the amount and quality of DNA present. These factors make DNA profiling and thus parentage verification from bone samples difficult and often inconclusive or inaccurate. A standard extraction protocol was required that could be used routinely with highly repeatable and unambiguous results. Several methods had been used previously by various researchers to genotype animals from bone samples mainly for evolutionary studies.

The Veterinary Genetics Laboratory (VGL) received bone samples of 3 different horse breeds from clients requesting parentage analyses. The samples had been exposed to a variety of conditions and ranged in age from a few months to 3 years old. All samples had been kept at -20°C from the date of receipt to the date of extraction. All consumables used in the extraction process were sterilized and bone samples were scrubbed with Terg-A-Zyme® in order to remove external contaminants. Large bone samples were cut into smaller segments using a metal hand saw. The segments were taken from as many different positions on the bone as possible. Alternatively, a hand drill (Einhell Bavaria) was used to produce bone powder from the samples. Bone fragments and powder were subjected to an EDTA wash. Washed bone fragments were placed into MagNaLyser Green Beads tubes (Roche) containing an EDTA-based lysis buffer and broken down on the MagNa Lyser (Roche). DNA was extracted from the lysate using a standard Phenol/Chloroform method with the addition of 15µl Dithiothreitol (DTT). This was followed by a cleanup step using the MSB® Spin PCRapace Invitek Kit (Invisorb®). Samples were PCR amplified in singleplex reactions using the standard equine microsatellite panel employed by the VGL, and capillary electrophoresis was carried out on the ABI 3100Genetic Analyzer.

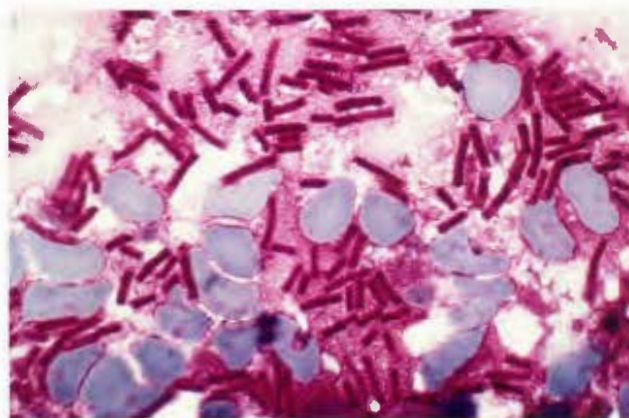
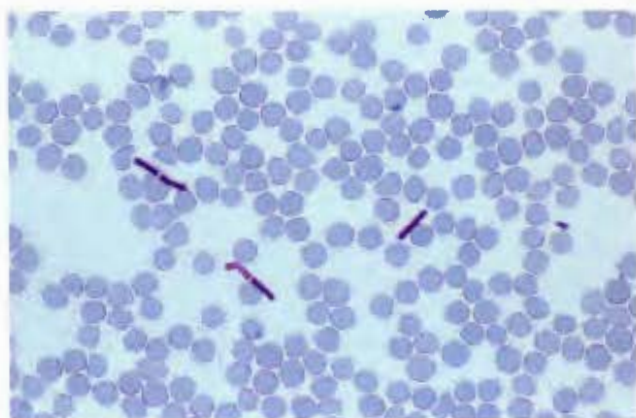
“There is no apparent correlation between the age of the bone, colour and dryness of the bone and the efficiency of DNA extraction.”

There is no apparent correlation between the age of the bone, colour and dryness of the bone and the efficiency of DNA extraction. It is evident, though, that certain bone types yield better quality DNA than others. Teeth and intact long bones give the best results. Factors that affect DNA quality and genotyping efficiency from bone include bone type, sterility of the extraction process, sample handling and cleanliness with the emphasis on the removal of all contaminants from the sample and environment. The EDTA wash decalcifies the bone and breaks the DNA, Hap bonds. The addition of DTT is also known to break strong bonds such as disulphide bonds, helping to release DNA from the sample. Allelic dropout due to low DNA concentration is addressed by performing singleplex PCR reactions and running each sample 8 times. This protocol provides a simple method of extracting DNA from bone that gives consistent and useful results and resulted in seven out of 11 parentage questions being answered.

Molecular characterisation of Southern African *Bacillus anthracis* strains using multiple locus variable number tandem repeats analysis

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Bacillus anthracis is a gram-positive, non-motile, spore forming bacterium and the causative agent of anthrax. Southern Africa has a documented history of sporadic epidemics of anthrax, chiefly amongst wildlife, resulting in widespread disease in the areas where they are concentrated. This highly monomorphic bacterium occurs globally, but Southern Africa is a candidate for being the geographic origin as it contains great genetic diversity. The molecular technique known as Multiple Locus Variable Number Tandem Repeat (VNTR) Analysis (MLVA) assay is the most frequently used to evaluate this diversity. Currently 31 tandem repeat loci have been described and validated. The MLVA assay for *B. anthracis* uses 8, 15 and 25 markers or all 31 loci depending on the expected relevancy. When the equipment is available, the most convenient typing approach is multiplexed PCR followed by capillary electrophoresis. When using monoplex PCR, 28 of the 31 markers can be distinguished using agarose

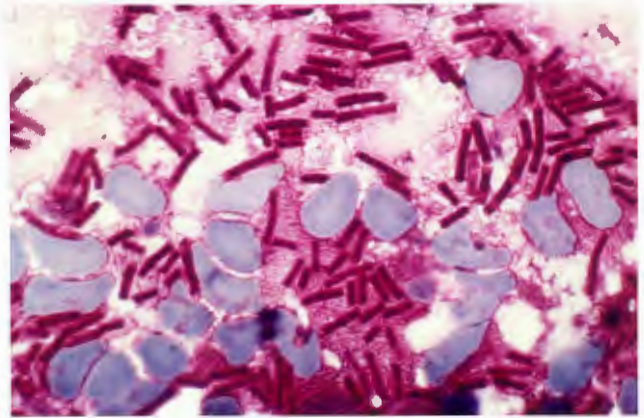
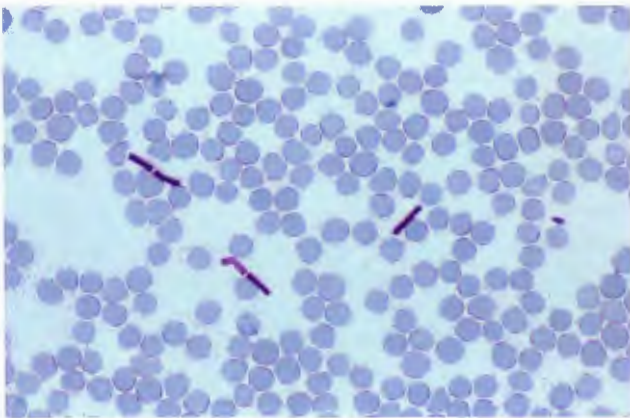
“Southern Africa has a documented history of sporadic epidemics of anthrax...”

electrophoresis with a high degree of accuracy. The 31 markers can be multiplexed into seven PCR reactions before analysis with capillary electrophoresis (agarose gel electrophoresis is not adapted for analysing multiplex PCRs). Agarose electrophoresis can also be used to analyse samples at a lower cost for 23 loci among the 25 of the MLVA25-UPSUD assay. The aim of this study was to use the 31 markers to determine whether multiplexing and discrimination by capillary and / or agarose electrophoresis is possible on 47 anthrax samples from southern Africa (42 isolates from the Kruger National Park, 3 from Rondebosch and 2 from Zambia). The typing results of the 47 strains using the MLVA23-UPSUD combination of loci demonstrate a relationship between geographically distant isolates and the predominance of a single genotype during an outbreak. Further analysis using the 31 MLVA assay will prove whether discrimination will necessitate the use of the additional 8 markers.

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"Southern Africa has a documented history of sporadic epidemics of anthrax..."

Proteome annotation and phenotype analysis of South African Territories type FMDV

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Although foot-and-mouth disease (FMD) is generally referred to as a single disease, the causative agent, FMD virus (FMDV), exists as seven distinct viral serotypes. These have different geographical distributions and epidemiologies globally, though they are clinically indistinguishable. Even within a serotype distinct genetic and antigenic variants exist in different geographical regions with serious implications for the control of the disease by vaccination. Comprehensive genome comparisons of FMDV isolates are essential to understand the evolution and biology of the virus, to predict whether a vaccine strain will protect against an emerging field strain or in the structural design of improved vaccine strains or virus-like particles. Currently there are limited studies on the complete proteome of South African Territories (SAT) serotypes, the variation within the proteome, a likely consequence of the high mutation rate of the virus, and the resulting viral plaque phenotypes on cultured cell lines. The current study was designed to investigate the variation observed within the structural and non-structural proteins and mapped the variation to the structure of SAT type viruses.

It has been noted that changes in proteins like the Leader protease and 3A influence virulence and host range. Similarly, point mutations in the structural proteins are associated with affinity to alternative heparan sulphate proteoglycan receptors

during adaptation of viruses on cultured cells, a feature commonly associated with a change in host range *in vitro*. Annotation of the proteome of 19 SAT-1, 15 SAT-2 and 3 SAT-3 isolates, revealed variation within serotype not only on a residue level, but also on a higher structural and potentially at a regulatory level in almost all the proteins. The replication of FMDV in cell culture is dependent on several factors, including cell entry, replication of the RNA genome, translation, the correct polyprotein processing by viral encoded proteases, and packaging of the RNA into virions. In spite of the variation observed within the proteome, the SAT types displayed a remarkable ability to accommodate the variation and maintain biological activity. The difference in the biological properties of SAT viruses was observed in the different plaque morphologies and host ranges on cultured cells. Linking changes in plaque morphologies and cell host range during adaptation of isolates on cultured cells to differences in amino acid residues provides a means to design FMDV that are fit for purpose.

“Although foot-and-mouth disease (FMD) is generally referred to as a single disease, the causative agent, FMD virus (FMDV), exists as seven distinct viral serotypes...”

A questionnaire survey assessing the risk factors associated with antimicrobial residues in commercial layer eggs in Khartoum State, Sudan

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A structured questionnaire survey was carried out in April 2008 in Khartoum State Sudan, to assess and collect data on risk factors associated with the presence of antimicrobial residues in table eggs. The questionnaire investigated antibiotic usage patterns for each layer farm, as well as the basic knowledge and understanding of farmers about public health concerns associated with antibiotic use in food producing animals. Questions were closed ended and data was obtained through direct interviews with farm owners and managers. Descriptive statistical analysis was carried out on the information captured; calculating frequencies, graphs and measures of association, using EpiInfo™ statistical package. Ninety-two farms were surveyed of which 98%

comprised open-sided houses. It was found that 48.9% of the farms surveyed were on antibiotic treatment when the survey was conducted, while 58.7% of the farms had used antibiotics within the last three months. There was a significant association between having disease on farm and using antibiotics ($P < 0.001$). The study showed that there is a serious lack of knowledge about the dangers of using antibiotics in animals and their potential impact on human health. In addition, Sudan lacks any type of formal control of veterinary drugs in terms of legislated residue limits or monitoring and surveillance programmes. This leads the authors to the conclusion that all Sudanese consumers are at risk.

"The questionnaire investigated antibiotic usage patterns for each layer farm..."



A primary assessment of problems and challenges related to cattle keeping in the Mnsi tribal area

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As rural engagement is an important aspect of the mission of this Veterinary faculty a community development project was created with the main objective being to increase the livelihood security and sustainability of rural people living in the Mnsi community. This tribal area is situated in the Mpumalanga province and covers 30.000 hectares, it contains a population of approximately 40.000 people. This community was chosen because of its challenging geographical position, behind the red line, the high importance of agricultural in local livelihoods, the widespread presence of well-developed veterinary services and the long-term good relationship between the tribal authorities and the University of Pretoria.

The project contains several pillars and research foci. In this poster we focus on the livestock production and animal health pillar. One of the main priorities of this pillar is to assess the most important challenges and problems faced by the livestock owners in the area. To identify these main challenges we used a qualitative research method called focus group discussions. From the 3rd to 8th March 2009, five diptanks in the area were visited. Every morning after dipping a discussion was held with the present cattle owners. Specific questions were asked to identify the main challenges and problems in cattle keeping and to learn about local people's perceptions towards constraints and opportunities in the area.

Problems with water and grazing were named in four of the five discussion groups while problems related to ticks or dipping procedures, cattle marketing, cattle diseases, drought, stock theft and veldfires were named in three out of the five discussions. Other problems were mentioned during only one discussion. Results are represented in Table 1.

Local people seemed to have positive attitudes towards installing a camp system. During the discussions several advantages were named for using a camp system instead of the current free ranging system: 1) less labour intensive, 2) better water management, 3) better management of grazing land, 4) more theft prevention opportunities, 5) better fire detection opportunities, 6) better overall cattle management, 7) ability to initiate a breeding season with marketing and nutritional advantages.

A next step in the project is to quantify and describe the issues related to the previously identified problems and challenges using a quantitative questionnaire. Results from this preliminary work have therefore provided an important source of information to fine-tune and focus the questionnaire. A thorough description of problems and challenges will allow for the creation and development of appropriate intervention activities.

Table 1: Identified problems and challenges during the five discussions

4 out of 5 discussion groups	3 out of 5 discussion groups	1 out of 5 discussion groups
Water	Ticks/Dipping	Vet. Availability
Grazing	Marketing	No camps
	Diseases	Quality bulls
	Drought	Dehorning
	Stock theft	Elephants
	Veldfire	

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