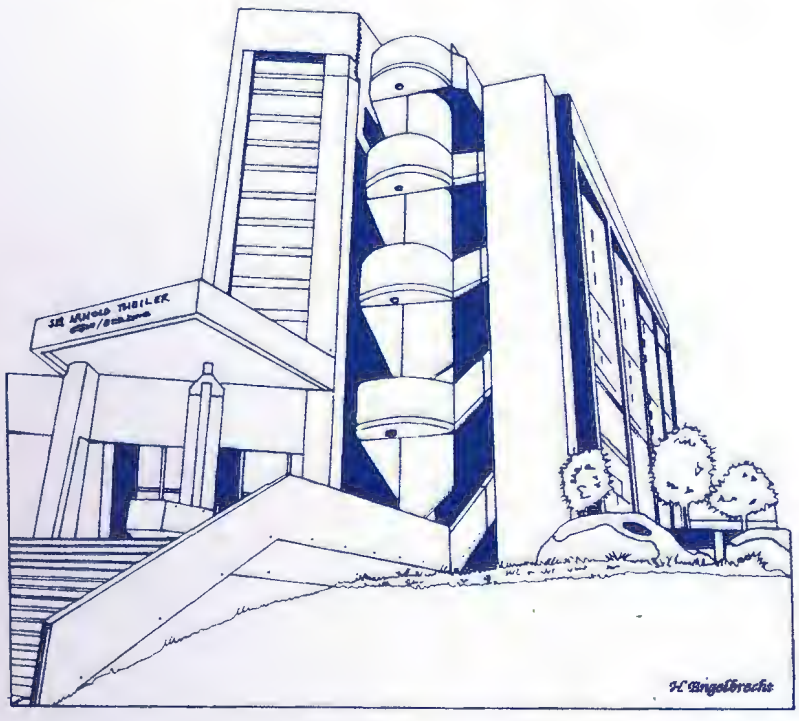
UNIVERSITY OF PRETORIA

FACULTY OF VETERINARY SCIENCE

18th Faculty Day

September 19, 2002

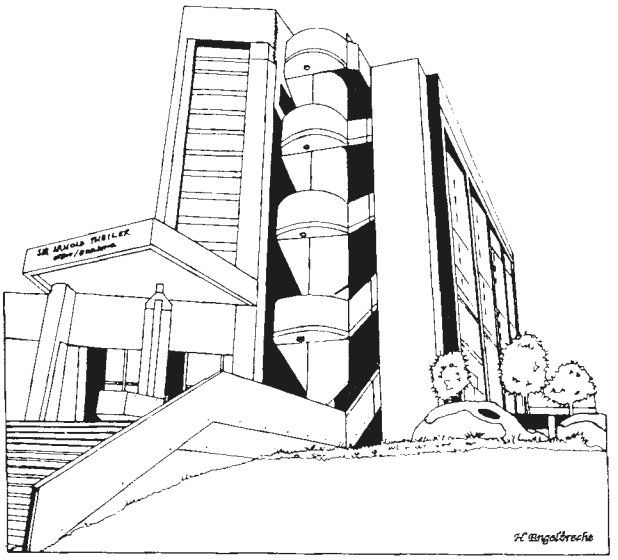
PROGRAMME AND SUMMARIES



FACULTY OF VETERINARY SCIENCE, UNIVERSITY OF PRETORIA

18th FACULTY DAY

19 SEPTEMBER 2002



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MESSAGE FROM THE DEAN

Prof. NPJ Kriek

It is my pleasure to welcome you all to Faculty Day 2002, which is one of the highlights of the academic year in this Faculty. On this day we not only strive to provide an insight into some of the more recent research activities in the Faculty, but we also give recognition to members of Faculty who have excelled in training and research.

As in the past, on this day we too give recognition to an excellent researcher, this time from within our institution, who has been invited to present the Arnold Theiler Memorial Lecture in recognition of a lifelong involvement in research. We honour Prof I van Horak who for many years has untiringly pursued the joys of research in his chosen field.

For many years now, recognition has been given to excellence in teaching by awarding the *Lecturer of the Year* award. This year, for the first time, recognition will also be given to a person who has excelled in research during the course of the preceding year. This award has been created to stimulate research in the Faculty and to again emphasise the importance of research in the University and in the Faculty of Veterinary Science.

We strive to become truly competitive internationally, but we lack the status as a research faculty. By rewarding excellence in research, we trust that we will be able to stimulate a research ethos in this institution to such an extent that it will allow us to subject ourselves successfully to international accreditation processes. Only then would we be internationally competitive and a successful faculty.

I am looking forward to seeing you all at Faculty Day: be sure to enjoy both the academic and the social programmes of the day.

NPJ KRIEK
DEAN

Prof. Ivan Horak

PROFESSOR IVAN HORAK

Born in Cape Town in 1934 Ivan Horak, matriculated from Maritzburg College in 1952. He was awarded his BVSc from the University of Pretoria in 1957. After a short stint as ranch veterinarian on Quest Ranch in KwaZulu-Natal, Professor Horak returned as a State Veterinarian to the Onderstepoort Veterinary Institute (1961-1966). He took up an appointment as research and development veterinarian at Hennops River Research Centre of MSD (Pty) Ltd. in 1966, a position he held until 1973. In 1974 he was appointed as a Senior Lecturer in Entomology at the Faculty of Veterinary Science at the University of Pretoria, later being promoted to Associate Professor. In 1984 he was appointed as Deputy Director of the Tick Research Unit at Rhodes University. At the time of his appointment as Professor in Ectoparisitolgy at the Faculty of Veterinary Science, University of Pretoria in 1987, he had been promoted to the position of Director at the Rhodes Tick Research Unit. While Professor Horak retired from his official duties in 1996, he has continued to pursue his research interests as Professor Emeritus in the Department of Veterinary Tropical Diseases at the Faculty until the present time. In 1966 Professor Horak was awarded the first of his three doctorates, a DVSc from the University of Pretoria. This was followed by a PhD from the University of Natal in 1980, with the pinnacle being attained on his being awarded a second DVSc (on publications) from the University of Pretoria in 1989. Professor Horak's prowess as a researcher has been recognised by his receipt if many prestigious awards, including the Elsdon-Dew Medal for service to Parasitology in Africa presented by the Parasitological Society of Southern Africa (1990), the Gold Medal of the South African Veterinary Association (1996) and in 2001 his election as an Honorary Member of the World Association for the Advancement of Veterinary Parasitology. Professor Horak has published widely with 189 full-length articles in refereed scientific journals as well as being co-author of two books.

FACULTY OF VETERINARY SCIENCE, UNIVERSITY OF PRETORIA

18TH FACULTY DAY

THURSDAY 19TH SEPTEMBER 2002

PROGRAMME

07:45-08:15 **Registration and Coffee**
Master of Ceremonies: *Professor F Reyers*

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

08:30-09:30 **RESEARCH PROGRAMME: SHORT COMMUNICATIONS**
SESSION CHAIRMAN: *Professor F Reyers*

Evaluation of radiographic film identification and possible legal implications of inadequate identification at Onderstepoort Small Animal Diagnostic Imaging Section
C R Makanjee,

Radiology and ultrasonography of the equine hyoid apparatus
T C Spotswood

Blood lactate as a prognostic indicator in canine babesiosis
M Nel, R Lobetti , N Keller

Hypoglycaemia in babesiosis: common, catastrophic and curable
N Keller, L S Jacobson, M Nel, J P Schoeman

Evaluation of the effect of vitamin B_6 on serum AlT activity in dogs with canine babesiosis
E Myburgh , F Reyers

09:30-10:20 **Sir Arnold Theiler Memorial Lecture : "The Joy of Research"**
Professor Ivan Horak

10:20-10:30 **Pfizer** "Lecturer of the Year Award"
OPVSC Representative

10:30-11:00 **TEA** and Viewing of Posters, Commercial Exhibits and Photographic Exhibition

11:00-12:30 **RESEARCH PROGRAMME: SHORT COMMUNICATIONS**
SESSION CHAIRMAN: *Professor G E Swan*

Radiographic gastrointestinal contrast study in the ostrich (*Struthio camelus*) *W M Wagner, R M Kirberger*

Evaluation of the effects of rearing systems on gut morphology and development of goat kids: preliminary report
T Songabe, E F Donkin, E D Green

Isolation and identification of the infectious agents primarily associated with ulcerative balanoposthitis and vulvovaginitis in sheep in Northern Cape province in South Africa and determination of *in vitro* antimicrobial sensitivity
A Kidanemariam, M van Vuuren, B Gummow, J Gouws, M van Aardt

The effect of different protein supplements on production economics and nematode resilience in Merino ewes
A Janse van Rensburg, G B Bath

In *vitro* cholinergic effects of crude extracts of *Gunnera perpensa* L. on isolated rat uteri
R Gehring, L C Katsoulis, G E Swan

Isolation of a combretatstatin from *Combretum woodii* leaves by bioassay guided fractionation
J O Famakin, D R P Katerere, J N Eloff

Delivery of veterinary services in remote rural areas of South Africa; the role of the "local expert": A short communication
T Songabe

Molecular epidemiology of serotype O foot-and-mouth disease viruses in Ethiopia and neighbouring countries
M. Sahle, R M Dwarka, E. Venter, W. Vosloo

12:30-13:15 **RESEARCH PROGRAMME: PRESENTATION OF POSTERS**
SESSION CHAIRMAN: *Professor EH Venter*

P1. **Screening test for a congenital myasthenic syndrome in cattle**
P N Thompson, O K Steinlein, E van Dyk, S Kraner, C K Harper, A J Guthrie, A Nel, E Bell

P2. **A scanning electron microscopic study of the magnum of the immature ostrich(*Struthio camelus*)**
M-C Madekurozwa

P3. **Serological survey to confirm the foot and mouth disease free status of South Africa after the 2000/2001 outbreaks of the disease**
W Vosloo, C J Dickason, B Gummow

P4. **A stochastic decision tree model to assess the impact of groundwater pollution on livestock**
B Gummow

P5. **A field evaluation of three trypanosomosis control strategies, in Kwazulu-Natal, South Africa**
F.R.Emslie, B.Gummow, J.R.Nel

P6. **Reverse Line Blot: A diagnostic tool to detect blood parasites**
A-M Bosman, V Pillay, A Nijhof, E H Venter, B L Penzhorn, F Jongejan

P7. **The fine structure of the rete testis in the ostrich (*Struthio camelus*)**
T A Aire, J T Soley

P8. **New recognition of an old enzyme: the antibacterial effect of the lactoperoxidase system in goat milk**
E Seifu, E F Donkin, G B H Bester, E M Buys

P9. **Fine structure of *Neospora caninum* in a white rhinoceros calf**
J H Williams, E van Wilpe

P10. **Breed prevalence of Dog Erythrocyte Antigen 1.1 in the Onderstepoort area: significance in donor selection**
L L van der Merwe, L S Jacobson

P11. **The use of planar chromatography to evaluate traditional medicine**
J V Manana, J N Eloff

P12. **Herbarium specimens can be used to bioprospect for some antibacterial compounds**
J N Eloff

P13. **Selection of *Combretaceae* spp for the isolation of antibacterial compounds based on biological activity, chemical composition and taxonomic information**
J N Eloff

P14. **Can extractants be used to selectively enrich antibacterial compounds in complex *Combretum microphyllum* leaf extracts?**
M Kotze, J N Eloff,

13:15-14:15 **LUNCH:**
During lunch the awards for the best scientific paper, the best scientific poster, the Roche Clinical Pathology Award, Photography prizes and the Researcher of the Year Award will be presented

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

1 PHOTOGRAPHIC EXHIBITION
An exhibition of photographs taken by staff and students
Organisor: Dr E van Dyk

2 EXHIBITS BY SPONSORS

3 SCIENTIFIC POSTERS

Evaluation of radiographic film identification and possible legal implications of inadequate identification at the Onderstepoort Small Animal Diagnostic Imaging Section

CR Makanjee

Diagnostic Imaging Section, Department of Companion Animal Clinical Studies, Faculty of Veterinary Science, University of Pretoria, Private Bag X04, Onderstepoort, 0110

Radiographic film identification (RFI) data is one of the criteria evaluated in determining if the radiograph meets the requirements of acceptable diagnostic quality. At the Onderstepoort Veterinary Academic Hospital it is the clinician/ veterinary nurse who makes this decision. The RFI data is comprised of two components, namely identification and technical data.

A total of 385 radiographs were prospectively evaluated for adequacy of RFI. Identification data consists of the name of the institution, owner's name, X-ray reference number and date of examination. Technical data consists of anatomical identification, ie "R" and "L" and position identification eg dorso-ventral.

Radiographs with inadequate data (that is incomplete identification or technical data) revealed a result of 52.7% of the total radiographs evaluated. 8.6% of the total radiographs evaluated had no identification or technical data. Position identification data as a component of technical data was one of the highest contributing factors to inadequate data. The date of examination category was the second highest contributing category to inadequate identification data.

Criteria	Name of institution	Owner's name	Reference number	Date of examination	Anatomical identification	Position identification
Readable	331	324	331	192	150	42
Wrong	0	0	0	0	2	2
Written	8	9	8	9	10	3
No data	46	52	46	184	100	76
Total	385	385	385	385	262	123

No information on the legal implication of inadequate RFI could be found in the literature. According to the South African Veterinary Council code of ethics, each radiograph must have a permanent identification legibly exposed in the film emulsion. Despite the code of ethics prescribed by the professional body the court reserves the right to make its own decision.

The veterinary clinician/ veterinary nurse may still be summoned to substantiate their role in making the radiograph should a charge of malpractice arise. The court can find the above guilty of negligence if the incorrectly labelled radiograph is part of the chain of evidence presented.

Radiology and ultrasonography of the equine hyoid apparatus

T C Spotswood

Department of Companion Animal Clinical Studies, Section Diagnostic Imaging, Faculty of Veterinary Science, University of Pretoria, Private Bag X04, Onderstepoort, 0110

The objective of this study was to provide reference radiology and ultrasound images of the equine hyoid apparatus. The radiological and ultrasonographic anatomy of the laryngeal and pharyngeal region of the horse has been reviewed, but with little emphasis on the hyoid apparatus. Few conditions affecting the hyoid apparatus are described: most clinically important and best reviewed are fractures and infection of the stylohyoid bones. Hyoid disease can be challenging to diagnose, compounded by the complicated radiological anatomy of region. There are a few reports of lingual abscessation in horses due to foreign bodies that include ultrasonography as an imaging modality for clinical evaluation.

Standard radiographs (Dorso-ventral (DV) and lateral views) of the hyoid region were made with high output and low output (portable) machines on three cadaver specimens and four live thoroughbred horses of various ages. Metallic markers were placed in the cadaver specimens to help define the various hyoid bones. Ultrasonographic images of the hyoid apparatus and laryngeal area were made using 5 and 7.5 MHz curvilinear array (Aloka SSD-630 Echo Camera) and multifrequency linear array 7.5 MHz (Siemens Omnia Sonoline) transducers. The images were acquired from ventrally between the rami of the mandible in transverse and sagittal planes and stored on a magneto-optical diskette (MOD). Colour flow Doppler was used to identify and highlight the vascular structures.

On DV radiographs, in addition to the stylohyoid bones, the thyrohyoid bones were seen in most horses: basihyoid and lingual process was not seen. With ultrasound the basihyoid and lingual process, tongue root, linguofacial vessels, thyrohyoid bones, thyroid cartilage and laryngeal gas were easily identified. The stylohyoid bones could not be visualized with ultrasound due to the surrounding guttural pouch gas. In the clinical case of Actinomycosis of the basihyoid, extensive mixed osteoproliferative and osteolytic changes to the lingual process were seen on radiographs.

A horse with confirmed Actinomycotic osteomyelitis of the basihyoid bone was evaluated. Ultrasonography showed a midline fistulous tract extending from the skin surface to the lingual process. The whole ventral surface of the basihyoid bone and lingual process showed a very irregular bone surface echo and numerous separate clean-shadowing mineralised fragments. Histopathology performed on biopsy samples acquired during surgery confirmed these to be bony sequestra: PAS staining revealed clumps of branching filaments, identified as *Actinomyces* organisms on culture.

Easily recognisable anatomical landmarks and accessibility facilitate ultrasonography of the ventral hyoid apparatus and adjacent ventral laryngeal region. Ultrasonography provided superior detail of this region compared to radiography and proved indispensable in diagnosing a rare case of basihyoid Actinomycotic osteomyelitis.

Blood lactate as a prognostic indicator in canine babesiosis

M Nel[a], R Lobetti[b], N Keller[a]

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[b] Bryanston Veterinary Hospital, PO Box 67092, Bryanston, 2021

Babesiosis is a tick-borne disease caused by the haemoprotozoan parasite *Babesia canis rossi.* Canine babesiosis can be classified as complicated or uncomplicated, the latter classified as mild or severe. Severe uncomplicated and complicated cases of babesiosis require intensive therapy including blood transfusions, fluid therapy, oxygen supplementation and treatment for the various complications. These treatments can be very costly and it was found that approximately 10% of these patients do not survive in spite of intensive therapy.

It would be ideal if the clinician could predict the outcome in these cases in order to give the owner a prognosis before starting expensive intensive therapy in these patients. The search for prognostic indicators continues, and recently blood lactate measurements have been shown to be of value in both humans and animals.

In dogs, the normal value for blood lactate ranges between 0.2 and 2.5mmol/L. We examined 24-hour serial blood lactate measurements in 90 dogs that were admitted to the Onderstepoort Veterinary Academic Hospital with severe or complicated canine babesiosis. Blood lactate was measured every 8 hours and the outcome (death or survival) was recorded for each patient.

Results showed that 48.8% of patients with severe or complicated babesiosis presented with hyperlactataemia at admission. Once appropriate therapy was instituted, blood lactate values dropped dramatically, and by 8 hours post-admission most patients had blood lactate values within the normal range. These patients all survived. However, patients who did not show the dramatic drop in blood lactate, but had persistently high blood lactate measurements, did not survive. Patients who did not survive showed blood lactate values that were persistently above 4 mmol/L for a 24-hour period.

We concluded that blood lactate values taken over a period of 24 hours, gives a good estimate of possible outcome. Patients who do not respond to therapy show no significant changes in blood lactate. Patients who have the ability to clear lactate within 24 hours show good survival rates. Serial lactate measurements over a 24 hour period seems to be an accurate method of predicting outcome in patients suffering from severe or complicated canine babesiosis.

Hypoglycaemia in babesiosis: Common, catastrophic and curable

N Keller[a], LS Jacobson[a], M Nel[a], JP Schoeman[a]

[a]Department of Companion Animal Clinical Studies, Faculty of Veterinary Science, University of Pretoria, Private Bag X04, Onderstepoort, 0110

Hypoglycaemia in dogs with severe, debilitating disease is not uncommon. However, very little information regarding hypoglycaemia is found in the literature. Canine babesiosis shares numerous complications with human falciparum malaria, in which hypoglycaemia is a known complication. For this reason, and because both diseases share many characteristics with sepsis, it was suspected that hypoglycaemia might occur in babesiosis. The pathogenesis of hypoglycaemia in falciparum malaria is unknown and likely to be complex. Hypoglycaemia in human malaria is consistently seen as a poor prognostic indicator. It is more common in children and pregnant women. Glucose therapy does not appear to improve the prognosis in malaria. A recent study was performed at Onderstepoort to determine if, and how many dogs with babesiosis are hypoglycaemic.

Twenty-three (9.2%) of the 250 dogs studied had hypoglycaemia at admission. All of these dogs were severely ill and were admitted. The prevalence of hypoglycaemia in admitted dogs was thus 20.5%. Sixteen dogs (14%) had blood glucose levels below 2.2 mmol/l (the cut-off used in malaria literature - this is severe hypoglycaemia and requires urgent treatment). Five patients (4.5%) in this study had blood glucose readings below 1.0 mmol/l. Three dogs were normoglycaemic at admission, but developed hypoglycaemia subsequently. All three became severely hypoglycaemic within 8 hours of admission (2.3mmol/l, 1mmol/l and 1.8mmol/l respectively). Puppies were more at risk and 29.4% of puppies admitted to the Intensive Care Unit were hypoglycaemic. 30% of the dogs with hypoglycaemia presented with icterus. Five pregnant bitches were admitted and none was hypoglycaemic. This appears to differ from malaria, in which pregnancy is an important risk factor for hypoglycaemia. Glucose concentrations below 2.8 mmol/l are considered clinically significant in the veterinary literature. In this study most of the dogs with hypoglycaemia presented in a stupor and were unresponsive to their surroundings. Two puppies presented with seizures. In the past, cerebral babesiosis would probably have been diagnosed in these patients. However, in the light of these new findings, it is vital to test blood glucose levels in semi-comatose patients and those showing other suggestive neurological signs, and to correct hypoglycaemia as soon as possible if it is present. Diagnosis is quick, simple and relatively inexpensive. Treatment consists of a bolus of dextrose (0.5ml/kg of 50% dextrose i.v.), followed by addition of dextrose to the intravenous fluids. Blood glucose should be monitored closely - at least every 2 hours. Neurological signs rapidly resolve following a bolus of i.v. glucose, but can recur if plasma glucose concentrations are not monitored and are allowed to drop again. Some patients with hypoglycaemia needed several boluses before normoglycaemia was accomplished.

Clinical outcome is good in hypoglycaemic patients that show a quick and early response to the treatment. Over 80% of dogs with hypoglycaemia in this study responded well to treatment. This is in contrast to the malaria situation, where hypoglycaemic children frequently die despite glucose infusions.

Evaluation of the effect of vitamin B_6 on serum A*l*T activity in dogs with canine babesiosis

E Myburgh, F Reyers

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

Serum alanine transaminase (A*l*T) and aspartate transaminase (AsT) are enzymes that are often used to help in the diagnosis of liver pathology. For optimal activity, they require Vitamin B_6 (pyridoxal-phoshate) (Vit B_6) as "activator". In most patients, there is sufficient Vit B_6 in serum to allow the reaction to proceed optimally. There are reagent kits available that use added Vit B_6 in order to overcome this obstacle. These methods are, however, more difficult to automate. It has been reported that in cirrhosis, in man, the Vit B_6 levels drop sufficiently low to cause a serious "under-read" of the serum transaminase activity (up to 50% lower than the true activity). It has also been suggested that toxicology trials using rats may be compromised by prolonged anorexia leading to low Vit B_6 levels which, in turn, would lead to serum liver enzyme activity underestimates. Canine babesiosis cases are often presented with anorexia of a few days' duration and therefore it is possible that they might be sufficiently Vit B_6 depleted to lead to clinically significant A*l*T underestimates. This has fairly serious implications for the proper care of these patients.

A trial was conducted to evaluate whether the serum A*l*T activity in canine babesiosis cases was underestimated when an analytical method that did not use additional Vit B_6, was used, compared to a method using added Vit B_6. Serum from 105 consecutive canine babesiosis cases (where the period of anorexia was known) was harvested and analysed by the two methods (one without and one with added Vit B_6).

The results revealed that the method without added Vit B_6 gave serum A*l*T activity readings that were statistically significantly lower ($p < 0.001$) than the method with added Vit B_6. There was no correlation between the period of recorded anorexia and the degree of underestimate.The actual size of the underestimate, however, was not considered to be clinically significant as no cases of secondary hepatopathy would have been misdiagnosed.

There appears to be no need to change the method currently in use (no added Vit B_6) for the sake of canine babesiosis cases. Furthermore, previous data recorded in canine babesiosis cases that resort in the Department's data base can be considered to be reliable estimates of the actual serum A*l*T activity in these cases. The effect of Vit B_6 depletion in patients with other diseases producing prolonged anorexia and serious malnutrition cases can, however, not be deduced from these data.

This Study was approved by the Faculty Animal Use and Care Committee (Project No. 36-5-481) and was generously funded by Technikon Pretoria and Bayer Healthcare SA.

Radiographic gastrointestinal contrast study in the ostrich (*Struthio camelus*)

WM Wagner, RM Kirberger

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In the beginning of the 1980's the limits of radiography to diagnose internal abnormalities of soft tissues in pet birds was recognised, and contrast medium was added as a tool to avian diagnostics. But to the best of the authors' knowledge there has been no work published concerning gastrointestinal contrast studies in the ostrich (*Struthio camelus*), or ratites in general. The object of this study was to describe the appearance of the normal gastrointestinal tract with contrast radiography and to provide a guideline for optimal dosage and concentration of liquid barium sulphate and a reliable protocol for frequency of radiographs post barium administration.

Ten contrast studies were performed on seven clinically healthy ostriches. They were starved 16 h prior to contrast administration per stomach tube. Barium dosages varied from 7 - 10 ml/kg and concentration of the liquid barium sulphate from 25 - 50%. Radiographic examination was performed as described previously[1] using a 6-frame technique for left-to-right lateral views in standing and a 3-frame technique for the dorsoventral view in sternal recumbent adult ostriches. Ostrich chicks had whole body radiographs made.[1]

There was substantial variability in visibility and possibility of identifying components of the gastrointestinal tract as well as filling and emptying times. However, the recommended time intervals for taking radiographs were: survey, immediately post contrast administration and 20 min, 40 min, 1 h, 2 h, 3-4 h, 8-14 h and 24 h after that. In adult ostriches the kVp had to be increased 2 h post contrast administration to avoid underexposed radiographs, however no compensation was necessary for ostrich chicks. Structures that were consistently identified included the oesophagus, proventriculus, ventriculus, duodenum and proximal and distal rectum. Due to the superimposition of the remainder of the small intestine, individual parts were difficult to differentiate. The caecae could not consistently be highlighted and when identified, it was only for a short time. The ventral pouch of the coprodeum never filled with contrast.

The authors believe that the radiographic contrast study in the ostrich can be a useful part of a diagnostic work up and can answer anatomic and physiologic questions as set out in the above paragraph. However, for diagnostic purposes, the veterinarian must be aware that this procedure is time consuming, costly and contrast administration may be stressful.

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Evaluation of the effects of rearing systems on gut morphology and development of goat kids: preliminary report

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Goats are a valuable component of rural economy of South Africa and many parts of the world. A major constraint in goat farming is kid mortality, and an important pre-requisite for kid survival and development is the rapid attainment of ruminant status[2]. Ingestion of food other than milk is linked to rapid physical growth, establishment of microbial population, fermentation, formation of volatile fatty acids and maturation of the rumen epithelium[1,4 & 5]. This study evaluates differences in gut morphology in three groups of kids reared on various diets.

Crosses of Saanen and Indigenous goat kids were assigned to rearing groups identified as M, MR and MRCC according to diets in which milk only (M) or milk and poor quality roughage (MR) or milk, creep feed, high quality roughage and concentrates (MRCC) were fed. The M group was given poor quality roughage at four months of age. In each group, animals were sacrificed at two months, four months and six months of age for evaluation. Differences in gut morphology (length, size and volume) were evaluated. Ultrastructural differences of rumen papillae (height and shape) were investigated and compared using calibrated eye-piece micrometer, light microscopy and scanning electron microscopy.

These preliminary results indicate that the goats in the M group appear to have had less gut development than those in the MRCC group. Gut length and size were smaller, and rumen papillae size and development were less for the goats in the M group compared to those in the MRCC group. Statistical evaluation will be carried out when further results are available. These findings have shown differences between the groups emphasising the key role of adequate nutrition in the survival of goat kids.

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Isolation and identification of the infectious agents primarily associated with ulcerative balanopothitis and vulvovaginitis in sheep in Northern Cape province in South Africa and determination of *in vitro* antimicrobial sensitivity

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Ulcerative balanoposthitis and vulvovaginitis is a disease characterized by erosion and ulceration of the glans penis, mucosa of the prepuce, vulva and vagina of sheep. The disease was first recognized in South Africa in 1976 in the Calvinia district and later spread all over the country affecting mainly of Dorper sheep. It is now a major concern for Dorper sheep breeders and the Association has committed significant funding to support this project.

Flocks of sheep in the Northern Cape province that experienced an outbreak of ulcerative balanoposthitis and vulvovaginitis were examined. The study was carried out with the aim of isolating and identifying the pathogenic microorganism/s that contribute to the disease in sheep and to determine the antimicrobial susceptibility of the isolates. Although the study is still in-progress, this paper reports interim results on the microbiological findings and an overview on the disease situation in South Africa.

The microbial flora of 100 clinically unaffected sheep and 100 affected sheep with the characteristic ulcerative lesions of the disease from 15 different sheep studs was examined. Swabs from both rams and ewes and sheath washes from rams were collected aseptically, and put into cryovials consisting of transport medium for bacterial, mycoplasmal and viral maintenance. Swabs for chlamydophila antigen detection were placed into tubes without transport medium. Twenty-one preputial mucosal biopsies were also taken for electron microscopic investigation.

All specimens have been processed for virus isolation in cell culture, chlamydophilal antigen detection by ELISA and bacterial isolation on ordinary culture media. No viruses were isolated and all specimens tested for chlamydophila were negative. Results of bacterial isolations indicated that the predominant organisms present in clinically affected animals were Gram-positive rods, mainly *Arcanobacterium pyogenes* and other *Corynebacterium* species. In clinically healthy sheep, the predominant isolates were Gram-negative rods.

Preliminary results of attempts to isolate *Mycoplasma* from clinically affected sheep have yielded positive results in 43 % of the specimens. Identification of the isolates by means of indirect immunofluorescence test (IFAT) is in progress.

Provisional results point towards *Mycoplasma* species as an important aetiological agent of ulcerative balanoposthitis and vulvovagintis, most likely in association with Gram-positive bacteria.

The effect of different protein supplements on production economics and nematode resilience in Merino ewes.

A Janse van Rensburg, GF Bath

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Protein is a vital factor in sheep nutrition since it affects production, reproduction and resilience to nematodes. Animals kept on natural grazing are often exposed to insufficient protein levels especially during periods of high demand eg lactation. Animals are often fed supplements during these periods. However protein is expensive and the improvement in profitability has to be sufficient to justify the higher input costs.

In our trial, 90 fine wool Merino ewes were kept on natural grazing in the eastern highveld of Mpumalanga. The ewes were divided into three groups of 30 each; two groups received commercial protein supplements. The third group (control) received a mineral supplement *ad lib*. All ewes were kept as a single group except when they were given supplements. During these periods they were rotated in equivalent camps. The nutritional value of the veld was estimated by analyzing hand-cut samples for crude protein, crude fibre, Ca and P content. Two types of protein supplements were used; a rumen undegradable protein supplement (RUP) and a rumen degradable protein supplement (RDP). Ewes were supplemented for two weeks prior to breeding and for 14 weeks starting three weeks before lambing. Supplements were given at 250 g/ewe/day prior to breeding, at 300 g/ewe/day for six weeks starting three weeks before lambing and at 500 g/ewe/day for eight weeks starting three weeks after lambing had started. Body weight (BW), body condition scores (BCS) and FAMACHA (F©) scores were recorded every two weeks during periods when the ewes were kept together and weekly when supplements were given. Faecal egg counts were done monthly when the ewes were kept as one group and fortnightly when supplements were given. The groups were compared with respect to lambing percentage, wool production and resilience to nematodes.

The groups receiving the supplements displayed superior production compared to the control group. Lambing percentages were 96% and 89% for the RUP and RDP groups, compared to 83% for the control group. Weaning percentages were 75% and 67% for the RUP and RDP groups, compared to 55% for the control group. Wool production was similar for all the groups: 2.5 kg per ewe. Nematode resilience was also similar for all groups. Income per ewe was the highest for the RUP group (R162.14) and lowest for the control group (R135.06). (RDP = R143.06). However, the profit per ewe was the highest for the control group (R131.33) and lowest for the RUP group (R87.30) with the RDP yielding R99.94. Thus, in this trial, providing protein supplements did improve production but the additional income was not sufficient to cover the increased costs under the prevailing conditions. Neither supplement could therefore be economically justified.

In vitro cholinergic effects of crude extracts of *Gunnera perpensa* L. on isolated rat uteri

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Infusions or decoctions of the fleshy rhizome of *Gunnera perpensa* L. are used by black South African women to induce or augment labour and as a postnatal medication to expedite the expulsion of the after-birth[1,2,3]. According to anecdotal reports, this herbal remedy is also used in cattle by subsistence and commercial farmers of the Underberg region of Kwazulu Natal (South Africa) to assist in the expulsion of foetal membranes and in the treatment and prevention of endometritis (Dr T Collins, Underberg, pers. comm.).

The pharmacological effects of a crude extract of *Gunnera perpensa* L. were investigated using isolated rat uteri from virgin female Sprague-Dawley rats (250-300 g) injected with stilboestrol. The organs were challenged with cumulative doses of a reference agonist (acetylcholine) (n=29) or herbal extract (n=12). Organs were also pre-incubated with selected antagonists (atropine (n=8) and indomethacin (n=10)) before adding cumulative doses of the herbal extract, or pre-incubated with the herbal extract before adding cumulative doses of the reference agonist (n=10). Isotonic contractions of the organs were measured and recorded electronically using labographs and semi-logarithmic dose-response curves were drawn using GraphPad Prism®.

The crude extract of *Gunnera perpensa* L. caused direct smooth muscle contraction of the isolated rat uterus. The maximal response to the herbal extract was approximately 60% of the contractions caused by the reference agonist, acetylcholine. These contractions were depressed by pre-incubation with both indomethacin and atropine. Pre-incubation with the herbal extract followed by cumulative addition of acetylcholine, caused the semi-logarithmic dose-response curve of the reference agonist to be shifted towards the right. This suggests that the pharmacological action of *Gunnera perpensa* L. on uterine smooth muscle is mediated through binding to muscarinic cholinergic receptors.

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Isolation of a combretatstatin from *Combretum woodii* leaves by bioassay guided fractionation

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Preliminary screening indicated that *Combretum woodii* had substantial antibacterial activity and that the chemical fingerprint of this plant differed substantially from other *Combretum* species.

Several extractants were tested to determine if any preferentially extracted antibacterial compounds[1]. There were not major differences, but for several reasons acetone was selected as the extractant. Extracts were simplified by solvent-solvent extraction [2] Antibacterial activity was determined by a serial dilution microplate assay [3]. According to bioautography results [4] there were two antibacterial compounds present. These compounds were the only compounds with a distinct red-brown colour on the chromatogram. The chloroform soluble fraction appeared to have the highest activity. This fraction was analyzed by open column silica gel column chromatography. The most important antibacterial compound was isolated and characterized by Nuclear Magnetic Resonance Spectroscopy (NMR) and Mass Spectroscopy (MS). It was Combretastatin B5 previously isolated from *C. kraussii* seed. Related combretastatins were previously isolated from *C. caffra* roots and some of these are important anticancer agents currently undergoing clinical trials.

Combretastatin B5 had significant antibacterial activity against *Staphylococcus. aureus*, *Pseudomonas aeruginosa*, *Enterococcus faecalis* and slight activity against *Echerichia. coli*. The Minimal Inhibitory Concentration (MIC) value for *Staphylococcus aureus* was 16 (g/ml, which compares favourably to the MIC values of 80 (g/ml and 160 (g/ml for ampicillin and chloramphenicol, respectively. This is the first report of antibacterial activity of a combretastatin and also the first report of the presence of combretastatins in leaves.

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Delivery of veterinary services in remote rural areas of South Africa; the role of the "local expert": A short communication

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The productivity of livestock owned by small-scale, poorly resourced subsistence farmers could be increased by veterinary intervention in the form of disease prevention, treatment and extension advice about management techniques[2,6]. Where veterinary personnel are unable to meet all demands for veterinary service, farmers look elsewhere, to "local experts"[6]. This study was conducted to investigate: the extent of use of local experts by the farmers, training of local experts, negative and positive effects of the use of local experts.

Structured interviews and questionnaires were used to elicit responses from farmers (n=87) and local experts (n=11) gathered at the dipping area.

All people interviewed had consulted a local expert for advice, treatment and routine management activities such as castrations. Only 3% had also sought professional advice. In cases attended by the local experts 57% had been successfully resolved. The success rate of major conditions such as dystocias and major wounds attended by local experts was 2%. Local experts had had no professional training: 67% had learned from their fathers or a close relative while 16% had worked on a farm and 17% had attended some agricultural shows or farmer's days. All local experts used both indigenous and commercially available veterinary medicines. Commercially available medicines used by local experts were evaluated: 60% of medicines in stock had expired by more than two years. Administration of medicines was evaluated: 75% of local experts demonstrated inappropriate knowledge of dosages and storage. The use of old needles was common amongst all local experts.

The full extent of the use and efficacy of indigenous veterinary medicines could not be evaluated. It can be concluded, however, that local experts play a much greater role in the delivery of veterinary services than professional veterinary personnel. The potential benefits of basic training and of integrating local experts in rendering primary health care in veterinary services should be investigated.

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Molecular epidemiology of serotype O foot-and-mouth disease viruses in Ethiopia and neighboring countries

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Foot and mouth disease (FMD) is a highly contagious viral vesicular disease of cloven-hoofed domestic and wild animal species and is characterized by fever and vesicular eruption on the feet and mouth. FMD is the most commonly encountered viral infection in cattle in East African countries and serotype O is the most wide-spread. Due to the disease, strict export regulations are in place, and unless effective control measures can be implemented, no progress will be made in international trade. This, however, will largely depend on a better understanding of the epidemiology of diseases in the region.

A total of 10 outbreaks of FMD occurred in cattle in Ethiopia during 2000-2001. Virus isolates obtained from individual outbreaks were confirmed to be serotype O. The genetic diversity of these viruses, together with 60 isolates from East African countries (Somalia 4, Kenya 7, Sudan 11, Uganda 15, Ethiopia 16, Tanzania 5 & Eritrea 2) isolated between 1974 -2001 and 5 reference strains were investigated in this study. Partial VP1 nucleotide sequences (400 bp) of these viruses were determined and compared. Phylogenetic analysis of the VP1 sequences indicated that the recent O type isolates from Ethiopia shared 91 % similarities to virus strains isolated from Kenya, Ethiopia and Eritrea, over the period 1990 to 1998. Three separate lineages were observed for the East African isolates. Lineage I comprised of 4 genotypes, lineage II of 3, while lineage III comprised of only 1 genotype. The geographical distribution of isolates in each genotype was as follows:

Lineage I: Ethiopia Kenya, Sudan Tanzania, Uganda,
Lineage II: Eritrea Ethiopia, Kenya, Somalia,
Lineage III: Eritrea, Ethiopia, Sudan,

Ethiopia has isolates from each genotype and the close genetic relationship indicates that cross border movement is a major cause for disease dissemination in the East African region.

Screening test for a congenital myasthenic syndrome in cattle

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A congenital myasthenic syndrome was first reported in cattle in 1998, when progressive muscular weakness was observed in four half-sibling Red Brahman calves [1]. The genetic basis for the disease is a homozygous 20 base pair (bp) deletion (470del20) in exon 5 of the gene (bov*CHRNE*) coding for the epsilon subunit of the nicotinic acetylcholine receptor (AChR) (2). This mutation is predicted to result in a non-functional allele and the inability to produce functional adult-type AChR. The mutation is inherited in an autosomal recessive manner.

Blood samples were collected in EDTA from 40 Red Brahmans and eight cattle of other breeds. These included several known carrier animals, close relatives of the carriers and unrelated animals. The blood was stored and the DNA fixed on FTA® paper. Exon 5 of the bov*CHRNE* gene was amplified using specific forward and reverse labelled primers. Amplification products were then sized by capillary electrophoresis on an ABI 310 Genetic Analyser. A single 211 bp peak indicated a homozygous normal animal; two peaks at 211 bp and 191 bp indicated a heterozygous carrier animal. For verification, DNA was extracted from 30 of the blood samples and screened at the Institute for Human Genetics, Bonn. Screening was done by amplification of exon 5 and separation of fragment sizes by polyacrylamide-gel electrophoresis. The structures of the two different-sized fragments were confirmed by sequencing. There was 100% agreement between the results obtained from both tests.

The validation of this screening test provides a rapid, inexpensive and accurate means of identifying carriers of the mutation responsible for this congenital myasthenic syndrome. This will allow future studies of the prevalence of carrier animals and of the clinical and economic importance of the condition. In particular, investigation into the possible involvement of this mutation in cases of idiopathic calf mortality are warranted.

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A scanning electron microscopic study of the magnum of the immature ostrich (*Struthio camelus*)

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In the oviduct of birds, the magnum is responsible for the production of several egg proteins including avidin, ovomucoid and conalbumin[1,2]. A recent study on the magnum of the immature ostrich has indicated seasonal morphological changes, which appear to be related to the presence of an active ovary and changes in daylength[3]. In comparison to other avian species there is a paucity of information on the maturation of the magnum in the ostrich. This paper describes changes in the surface morphology of the magnum, in the immature ostrich, during periods of ovarian activity and inactivity.

In the present study oviducts were collected from a total of 40 immature ostriches during periods of short daylength (April to June), long daylength (September to December) and decreasing daylength (February). Twenty-five birds sampled from September to February had active ovaries, whilst 15 birds sampled from April to June had inactive ovaries. All the birds were slaughtered at a commercial ostrich abattoir. Tissue samples from the magnum were pinned to the paraffin-covered bottom of a Petri dish containing Karnovsky's fixative. The fixed tissue was then prepared for light and scanning electron microscopy using standard techniques.

The magnum region in all the birds studied was lined by a pseudostratified columnar epithelium. Non-ciliated cells were observed in birds with inactive ovaries, whilst both ciliated and non-ciliated cells were seen in birds with active ovaries. Furthermore, well-defined tubular glands were evident only in birds with active ovaries. Scanning electron microscopy (SEM) revealed that the mucosa in ostriches with inactive ovaries was arranged in longitudinally orientated furrows and primary folds. In contrast the magnum mucosa in birds with active ovaries had tortuous, branching folds. SEM confirmed that the magnum in birds with inactive ovaries was lined by only non-ciliated cells, whilst ciliated and non-ciliated cells occurred in birds with active ovaries. SEM of the magnum of birds sampled during the period of decreasing daylength showed that a combination of ciliated and non-ciliated cells lined the surface. The non-ciliated cells many of which lacked microvilli appeared to be sloughing.

The findings of the present study indicate that the magnum in the immature ostrich undergoes seasonal changes in morphology, which appear to be photoperiod-dependent.

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Serological survey to confirm the foot and mouth disease free status of South Africa after the 2000/2001 outbreaks of the disease

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During 2000/2001 South Africa experienced its first outbreaks of foot and mouth disease (FMD) outside the FMD control zone since 1957. The first outbreak was caused by serotype O, which had never previously been recorded in the country. This outbreak in KwaZulu-Natal affected a small part of the Camperdown district. An outbreak of SAT-1 subsequently occurred in a feedlot in the Middelburg district of Mpumalanga. Both outbreaks were efficiently controlled and no spread beyond the outbreak foci occurred.

However, it was necessary to perform a countrywide serological survey to confirm that the disease had not spread to other parts of the country in order to regain OIE FMD-free status without vaccination and to regain trust by trade partners. The survey was planned using a two-stage sampling strategy with stratification according to provinces. The FMD control zone, and land parcels where animals would normally not occur, such as mining areas, water masses, urban areas, etc., were not included in the sampling frame. Only cattle were sampled, as it was assumed that they were the most susceptible domestic animal species to FMD infection and because they show clinical disease more readily. In regions where cattle numbers were extremely low sheep were sampled in place of cattle.

Several assumptions were made for each stage of the sampling strategy. Based on these 62 land parcels per province were randomly chosen according to a grid reference system and 20 animals were systematically randomly bled on each farm. Since the communal farming areas were perceived as an unknown risk, more of these areas were included in the final study.

A total of 10 826 animals were sampled on 634 farms and no animals tested positive for antibodies to SAT-1, SAT-2 and serotype O. Results from an independent pig survey, buffalo tested upon movement, as well as results generated for imports and exports were added to the survey to increase the validity of the survey.

The findings of the survey resulted in the reinstatement of the country's previous OIE zoned disease-free status without vaccination on 31 May 2002.

A stochastic decision tree model to assess the impact of groundwater pollution on livestock

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Due to concerns expressed by residents in the vicinity of a large steelworks that the steelworks was polluting their groundwater, a pollution forum steering committee was set up by local authorities to investigate the situation. Since the districts concerned comprised numerous small holdings and several farms, a subcommittee was set up to investigate what livestock were in the area and whether there was any significant impact on livestock health in the districts as a result of groundwater pollution. The investigation had to be completed within six weeks, with limited manpower and within a limited budget.

Six districts (Rietspruit, Drakeville, Rietkuil, Louisrus, Steelvalley and Linkholm) were included in the study. A hazard assessment based on retrospective data from previous studies, established that livestock were at risk due to excess concentrations of sulphates, chlorides, iron, nitrates, phenols, *E. coli* and total dissolved solids. An exposure assessment was carried out using a door-to-door interview questionnaire census. One hundred and ninety one property owners were interviewed, which comprised approximately 85 % of the properties in the districts being investigated. The survey provided baseline data on the proportion of properties with animals, the species of animals found on these properties, sources of water and feed, and the perceived health status of the animals in the various districts. Risk characterisation was carried out using a decision tree model to establish the potential impact of exposure. The dose response assessment was quantified using economic measures as the response. The inputs for the model were based on the questionnaire survey results. To deal with the uncertainty inherent in the survey results, Latin-hypercube simulation techniques were applied at the various probability nodes in the decision tree model. This allowed for better risk characterisation. The study therefore combined Bayesian and stochastic principles to provide a novel approach for doing livestock impact studies when investigators are faced with limited information.

Comparison of the models for each district showed that the greatest impact on livestock was likely to occur in Rietspruit followed by Drakeville, Rietkuil, Louisrus, Steelvalley and finally Linkholm. This made intuitive sense, as these were the areas where commercial farming occurred and livestock formed the sole or larger portion of the owner's income. The worst predicted effect of pollution was a possible loss of 45 % in livestock value in Rietspruit (< 0.001 % probability). On average, however, a 16.22 % loss in value could be expected in Rietspruit. With the exception of Drakeville (at 6.25 %), all the other suburbs were predicted to have less than a 5 % drop in livestock value, on average. Hence, it would not seem that pollutants, in general, were likely to have a major impact on livestock health within the study area as a whole.

A field evaluation of three trypanosomosis control strategies, in KwaZulu-Natal, South Africa

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Rural subsistence farming practices are the primary agricultural activity in northeastern KwaZulu-Natal, South Africa. Since Bruce first identified *Trypanosoma.brucei brucei* in the late 1800's, cattle in this area have been affected by tsetse-borne trypanosome infections. Approximately 120000 cattle fall within a tsetse (*Glossina.austeni* and *Glossina.brevipalpis*) belt common to Mozambique and South Africa. Between 1991 and 1994 cattle in this area were treated with homidium bromide, and dipped with cyhalothrin, in an attempt to control trypanosomosis. However, since 1994 no control measures were implemented and trypanosomosis re-emerged as a threat to animal health.

In order to determine the optimum control measure available, a longitudinal incidence study was conducted to evaluate three possible control options.

Four sentinel herds were selected from populations exposed to similar trypanosome challenges. The baseline trypanosome incidence rate was determined for each herd, after which each herd was subjected to a different trypanosome control measure. Two of the herds were subjected to topical pyrethroid treatment (Cyfluthrin pour-on and Flumethrin plunge-dip) as a vector-control measure, one herd was treated six-weekly with an injectable trypanocidal drug (isometamidium hydrochloride), and one herd served as an untreated control group. Monthly incidence rates were determined using the 'dark-ground buffy smear technique'.

The monthly incidence rates were standardized in order to account for variation in trypanosomosis challenge between the four herds. The standardized rates were then compared and the impact of the control strategies was quantified using the Area Under The Curve method.

The cost efficacy of each control strategy was evaluated based on a partial budget system.

Both the cyfluthrin pour-on and the injectable trypanocide were cost effective and had a dramatic trypanosomosis control effect with the pour-on having the greater impact/ control.

The flumethrin plunge-dip displayed moderate trypanosomosis control properties, but proved not to be cost effective.

Reverse Line Blot: A diagnostic tool to detect blood parasites

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The Reverse Line Blot (RLB) is a versatile tool for the simultaneous detection and differentiation of blood parasites. It is based on the hybridization of specific amplified DNA to oligonucleotides (probes). These probes are specific for the group of organisms of interest and will not hybridize to mammalian DNA. Samples of various animal species were obtained and blood was collected in citrate-, EDTA- and heparin-buffered tubes, and stored at -20 °C. Filter paper with blood spots was also collected and stored in a dry place at room temperature. Ticks fixed in 70% ethanol and un-fixated ticks were also collected. The DNA was extracted from whole blood and ticks as described by Gubbels *et al.* (1999)[1]. The DNA was also extracted from blood collected on filter paper using the FTA extraction reagent (Whatman® Bioscience, Laboratory Specialist Services). Polymerase chain reaction (PCR) was performed on these samples and analysed using the RLB hybridization technique, as described by Gubbels *et al.* (1999)[1]. Probes are sensitive at genus (*Babesia* and *Theileria*) and species level.

Host	Sample type	Samples tested			Positive	
		Pos	Neg	Total	Genus	Species
African Buffalo	Blood (EDTA)	437	525	962	437	437
Kudu	Unstained blood smears	19	0	19	19	16
Carnivores	Blood (citrate and EDTA)	4	0	4	4	3
Other	Cell cultures, Ticks, Unstained blood smears Blood (citrate, EDTA and heparin)	66	12	77	66	21
TOTAL		526	537	**1063**	526	477

The RLB technique is extremely versatile and opens a new research and diagnostic tool to detect and characterize blood parasites in animals and vectors (ticks). The results can lead to identification of new parasites and the information from a RLB can be applied in nanotechnology techniques such as micro arrays. This technique is used routinely in our laboratories for diagnostic tests.

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The fine structure of the rete testis in the ostrich (*Struthio camelus*)

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The rete testis is a network of lacunae that link the seminiferous tubules with the rest of the excurrent ducts of the testis. It is considered that the rete epithelium is capable of modifying the fluid in which spermatozoa are suspended and transported because the composition of the rete fluid is different from that of the seminiferous tubules. The rete epithelium is also capable of uptake of substances from the lumen and their disposal by the lysosomal system, as well as transporting certain macromolecules from the lumen to the laterobasal surfaces of the cells. The rete testis has been studied in only a few birds in which it constitutes between 10 % and 13 % of the epididymal volume in some galliformes. This paper describes the fine structure of the rete testis in the ostrich whose reproductive biology, in health and disease, is still largely unknown.

Peri-epididymal testicular tissue, capsular tissue and strands of tissue conducting rete ducts, as well as epididymal tissues, were taken from five abattoir carcasses and fixed by immersion in 4% glutaraldehyde buffered in Millonig's phosphate buffer. The tissues were subsequently processed by standard methods for both light and transmission electron microscopy.

The rete testis of the ostrich displays intratesticular, capsular and epididymal segments. The intratesticular portion is minor but the capsular segment is prominent and runs medially within the capsule towards the epididymis, and then traverses the testiculo-epididymal space within a number of strands of connective tissue to enter the epididymis. The intra-epididymal portion is distributed throughout the organ, where each rete focus is surrounded by a set of more distal excurrent ducts, such as the efferent and connecting ducts.

There are no notable segmental differences in cell structure, and the entire rete testis is lined by a simple columnar to low cuboidal epithelium. The apical surface of the epithelial cells extends into short, regular microvilli and a solitary cilium. The nuclei are generally regular in outline. The cytoplasm contains numerous mitochondria that may be aggregated basally, a moderately developed Golgi complex, numerous strands of rough endoplasmic reticulum, some sparsely granulated endoplasmic reticulum, as well as a relatively abundant free ribosomes and rosettes of ribosomes. A single, large lipid-like droplet uniquely occurs in the supranuclear region. Adjacent lateral plasmalemmae interdigitate complexly.

The rete testis cell of the ostrich contains a greater volume of organelles than has been reported for other avian and mammalian species. The more numerous mitochondria and their basal aggregation in several cells may indicate a more metabolically active cell in the ostrich than that ascribed to other birds in previous reports. Generally, this cell appears capable of limited synthesis of protein-like material, and transportation of luminal substances to the basal part of the cell.

New recognition of an old enzyme: the antibacterial effect of the lactoperoxidase system in goat milk

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Milk contains several antibacterial factors such as immunoglobulins, lysozyme, lactoferrin and lactoperoxidase. Lactoperoxidase is the most abundant enzyme in milk. It catalyzes the oxidation of thiocyanate by hydrogen peroxide and produces an intermediate product which has an antibacterial effect. This is called the lactoperoxidase system. The objective of this research is to examine the effect of the lactoperoxidase system on the growth and survival of some food-borne pathogens in goat milk.

Milk from Saanen goats was pasteurized at 63° C for 30 minutes, cooled to room temperature and inoculated with a pathogen. Each milk sample was divided into two portions. The first was activated with lactoperoxidase by the addition of sodium thiocyanate (14mg per litre), followed sodium percarbonate (30mg per litre) to produce hydrogen peroxide. The second portion of milk was untreated. Both were incubated at 30° C for six hours, and the colonies of micro-organisms were counted.

Activation of the lactoperoxidase system inhibited the growth of all pathogens tested.
The concentration of bacteria in the case of *Staphylococcus aureus*, *Listeria monocytogenes* and *Brucella melitensis* decreased at the end of the activation period compared to the initial concentration (a bactericidal effect). In contrast, with *Esherichia coli*, activation of the LP system only delayed its growth, and the concentration remained the same as its initial concentration (bacteriostatic effect). In almost all cases the concentration of bacteria in the LP treated milk was significantly lower than that of the untreated control at the end of the activation period.

Activation of the lactoperoxidase system will provide a great potential for preservation of goat milk at ambient temperature, especially in areas where it is not possible to use refrigeration.

Acknowledgement

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Fine structure of *Neospora caninum* in a white rhinoceros calf

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Neospora caninum, an apicomplexan protozoal parasite, is the causative agent of the multisystemic disease neosporosis. This disease affects a wide variety of animals[1], but had not previously been reported in the white rhinoceros (*Ceratotherium simum)* [2]. This paper illustrates the fine structure of the parasite in a 16-day-old white rhinoceros calf that died acutely while in excellent condition and showing no obvious previous clinical signs [2]. It was the eighth calf of a mature female free-ranging with 11 other rhino as well as various other game species on a 2000 hectare Game Breeding Centre adjacent to the town of Lichtenburg and outlying cattle farmlands in the Northern Province, South Africa. Post-mortem gross examination ascribed death to heart failure.

Formalin-fixed myocardial tissue was processed for both light microscopy and transmission electron microscopy. Positive identification of the organisms as *Neospora* species was made light microscopically by using the immunhistochemical avidin-biotin technique [3,4] employing both polyclonal and murine monoclonal *Neospora caninum* antibodies. Tissue for transmission electron microscopy was post-fixed in 1% osmium tetroxide and ultrathin sections prepared by standard electron microscopic techniques.

Ultrastructural examination revealed intracellular encysted bradyzoites as well as tachyzoites lying free within the host cell cytoplasm. The bradyzoite cyst wall was 0,34µm wide, generally evenly thick and consisted of a parasitophorous vacuolar membrane and a thick underlying granular layer. Cross-sections of bradyzoites revealed a nucleus, dense granules, rhoptries, amylopectin granules and micronemes which in some sections were arranged perpendicular to the zoite pellicle. Longitudinally sectioned tachyzoites measured 4,8 x 0,2µm and contained a subterminal nucleus, a conoid, moderately electron dense rhoptries, micronemes, dense granules, lipid bodies, mitochondria and vesico-membranous organelles in their cytoplasm. The sections also provided evidence that the tachyzoites multiply by endodyogeny.

Ultrastructural findings of the bradyzoite-containing cyst and the tachyzoites are largely consistent with those described for *Neospora caninum,* but also showed some overlap with the characteristics of *Toxoplasma gondii,* in particular the smooth nature and dimension of the cyst wall and the moderately electron dense rhoptries[4]. However, negative immunohistochemistry for *T. gondii* and the dark, indistinct internal structure of the rhoptries (as opposed to the labyrinthine internal rhoptry structure of *T. gondii)* suggests that the parasite is a Neospora species.

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Breed prevalence of dog erythrocyte antigen 1.1 in the Onderstepoort area: significance in donor selection

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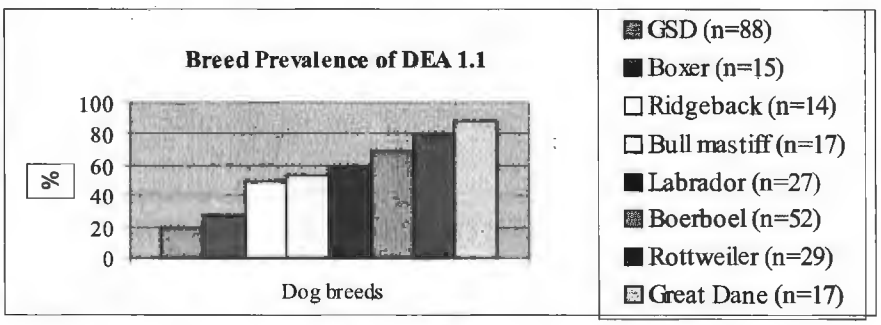
Six dog erythrocyte antigens, DEA 1.1, 1.2, 3, 4, 5, and 7 can be identified. DEA 1.1 is the most reactive and clinically important. Incompatibility causes acute haemolysis . As no pre-formed antibodies exist against DEA 1.1 a first transfusion with DEA 1.1 positive blood to a negative recipient will not result in a transfusion reaction. These animals will be sensitised to subsequent DEA 1.1 positive transfusions. Based on the reported prevalence of DEA 1.1 (42-46%) approximately 23% of random transfusions have the ability to sensitise recipients. The risk of a second random incompatible transfusion is 15%. This is especially relevant in South Africa where patients are more likely to receive multiple transfusions due to the prevalence of babesiosis. Prospective canine donors of the Onderstepoort Animal Blood Bank (n=373) were typed for DEA 1.1 using a monoclonal antibody kit: the RapidVet™-H (Canine 1.1) test kit (DMS Laboratories, USA). Overall prevalence of DEA 1.1 was 50%. Distinct breed differences were noted although the frequency in the entire population was similar to those previously reported.



Note: Number bars (Dog breeds) from left to right: 1. "GSD" to 8. "Great Dane"

These results strongly support the utility of seeking blood donors from specific breeds to obtain a DEA 1.1 negative donor population.

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The use of planar chromatography to evaluate traditional medicine

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With the development of analytical techniques such as HPLC and GC and especially the hyphenated techniques the arsenal in the discovery and application of chemical compounds present in plants have expanded dramatically. Many of these techniques are frequently too expensive to be used in developing countries where the bulk of uninvestigated plants and molecular diversity occur.

There are problems in the phytomedicine market with plants incorrectly identified by mistake or for financial reasons. Intoxication has resulted from this situation. It is very difficult to identify a plant from the piece of bark, root or ground leaves that is sold in the market. A chemical fingerprint may, however, have sufficient diagnostic characters to identify a plant species[1]. We did a survey of plants sold in Pretoria traditional medicine [muthi] markets. Criteria were developed to select the plants most likely to be adulterated. The scientific name was deduced from the African name and reference material of that species was collected from botanical gardens and from the ARC at Roodeplaat. Using standardized thin layer chromatography (TLC) techniques involving three solvent systems with different polarity and pH as well as two spray reagents we could show that that the bulk of plant material sold in muthi markets are the species they are purported to be. In the one case where there are clear discrepancies the reason is probably that the wrong species of the same genus is associated with the African name.

A major difficulty in using chemical fingerprinting is that environmental factors may influence the concentration of plant secondary compounds. Samples of *Artemisia afra* growing under widely different environmental and soil factors had a very similar chemical fingerprints, however

TLC analysis of bark of overexploited trees illegally collected can be identified from the chemical fingerprint. For nature conservation purposes, the bark of over exploited trees illegally collected can be identified from the chemical fingerprint using TLC analysis.

Reference

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Herbarium specimens can be used to bioprospect for some antibacterial compounds

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Extracts of different members of the Combretaceae display a surpising stability regarding their antibacterial and anti-inflammatory properties[1,2]. Several authors have examined herbarium specimens and found that compounds such as alkaloids, flavonoids, sweetening agents, volatile oils and amino acids can still be detected in herbarium specimens after many decades. By using a serial microplate dilution method[3]. The antibacterial activity of herbarium samples of *Combretum erythrophyllum* growing in the Pretoria area which had been collected between 92 and 12 years ago was compared with the antibacterial activity of freshly collected leaves.

There were no differences between herbarium and fresh specimens regarding the minimal inhibitory concentration [MIC] of the different specimens using *Staphylococcus aureus, Enterococcus faecalis, Escherichia coli* and *Pseudomonas aeruginosa* as test organisms. There were only minor differences in thin layer chromatography (TLC) chromatograms after terpenoids and flavanoids, possibly the bioactive compounds, were separated. Light fungal infection indicated by small spots on herbarium leaves did not influence the minimum inhibitory concentration (MIC) value or the chromatographic profile, but heavy fungal attack decreased the biological activity of the extracts.

Application of the technique to *Helichrysum pedunculatum* specimens showed that the chemical components apparently did not change over a period of 100 years and that the biological activity, probably due to a fatty acid, did not decrease. There was, however, a 40-fold difference in antibacterial activity and major differences in TLC profiles of *C. erythrophyllum* herbarium specimens collected from across its distribution range. The variation was associated with locality indicating the existance of chemotypes in this species.

Examination of herbarium specimens may therefore be a useful first step to screen plants with stable biologically active components and to identify areas where plants that contain a high concentration of the active compounds may be collected for further chemical work.

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Selection of Combretaceae spp for the isolation of antibacterial compounds based on biological activity, chemical composition and taxonomic information

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Combretum erythrophyllum contains at least 14 antibacterial compounds and some of these have higher activity than currently used antibiotics[1]. Other members of the *Combretaceae* collected from the Lowveld National Botanical Garden were examined to find the best source for isolating antibacterial compounds. Leaves of 27 species of *Combretum, Terminalia, Pteleopsis* and *Quisqualis* were dried, milled and extracted with acetone. The minimal inhibitory concentration (MIC) of extracts was determined by a microplate serial dilution technique using *Staphylococcus aureus, Enterococcus faecalis, Pseudomonas aeruginosa* and *Escherichia coli* as test organisms[2] All extracts inhibited the growth of the four test isolates with MIC values generally between 0.1 to 6.0 mg/ml and an average of 2.01 mg/ml. After storing extracts for six weeks at 7°C there was a slight loss of activity with MIC's increasing from 1.75 mg/ml to 2.24 mg/ml. The Gram-positive strains were slightly more sensitive with an average MIC of 1.80 mg/ml than the Gram-negative strains with a MIC of 2.22 mg/ml.

The chemical composition of the extracts was determined by thin layer chromatography using solvent systems of varying polarity and pH.

In order to select the most promising plants for further work, two things were considered. These were the diversity of antibacterials (as determined by bio-autography)[3] and the existing taxonomic relationship based on morphological characteristics.

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Can extractants be used to selectively enrich antibacterial compounds in complex *Combretum microphyllum* leaf extracts?

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Acetone extracts of *C. microphyllum* (also known as *C. paniculatum*) leaves have shown substantial antibacterial activity as well as strong antiviral activity against HIV-1 and HIV-2. The extracts are however complex and isolating bioactive compounds is challenging. Only a few extractants have generally been used for isolating antimicrobial compounds from plants and a recent review paper concluded that many classes of compounds are commonly obtained in only one solvent. The aim of this study was to investigate whether different solvents could simplify extracts to facilitate the isolation of antibacterial compounds from the complex crude mixture. The solvents were selected to represent a wide range of polarities and selectivity groups.

Intact dried leaves were extracted with acetone and 1 % aqueous sodium bicarbonate and ground leaves were extracted with hexane, carbon tetrachloride, di-isopropylether, ethyl ether, methylene dichloride, tetrahydrofuran, acetone, ethanol, ethyl acetate, methanol and water. The total quantity extracted with each solvent was determined gravimetrically; the complexity of compounds extracted was determined by thin layer chromatography (TLC) using different solvent systems and spray reagents. Antibacterial activity of extracts was determined by a microplate serial dilution method. Total antibacterial activity was calculated by dividing quantity extracted with minimum inhibitory concentration (MIC) values.

In contrast to other *Combretum* spp., sodium bicarbonate gave disappointing results with *C. microphyllum* leaves. The other solvents extracted from 2.6 to 17.4% of the dry weight. Methanol, methylene dichloride and tetrahydrofuran extracted the largest mass. The chemical composition of the different extracts was remarkably similar with the exception of highly polar (water) and non-polar (hexane) extractants, but the minimum inhibitory concentration (MIC) for the different extracts varied from 0.01 to 1.25 mg/ml. The average MIC values for the four test organisms were *Staphylococcus aureus* 0.46 mg/ml *Pseudomonas aeruginosa* 0.30 mg/ml, *Escherichia coli* 0.31 mg/ml and *Enterococcus faecalis* 0.29 mg/ml.

Di-isopropyl ether, ethanol, ethyl ether, acetone and ethyl acetate extracted high antibacterial activity with a lower quantity of other non-active compounds and could be useful for isolating bioactive compounds. The four solvents that extracted the highest total antibacterial activity were: methanol, methylene dichloride, ethanol and acetone. The best solvents belonged to selectivity groups II, III and V, which are good proton-acceptors with a large dipole moment. Because methanol and ethanol extracts also contain highly polar uninteresting compounds, and methylene dichloride is not miscible with water, we decided to use acetone as extractant for isolating antibacterial compounds.

Faculty of Veterinary Science
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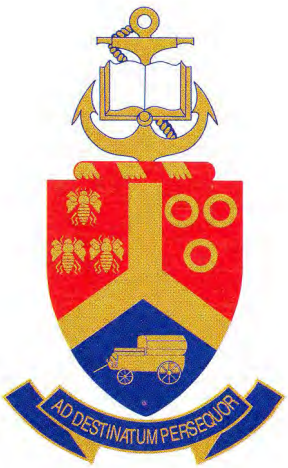
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